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ASPA 21st Congress

Milano, June 9-12, 2015

Book of Abstracts

Guest Editor: Giovanni Savoini

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ASPA 21st Congress

Milano, June 9-12, 2015

Guest Editor

Giovanni Savoini

Table of Contents

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ASPA 21st Congress

Milano, June 9-12, 2015

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Milano, June 9-12, 2015

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ASPA 21st Congress

Milano, June 9-12, 2015

Programme of the Congress

Tuesday, June 9

13.00-15.30	Arrival - Registrations - Welcome coffee
15.30-18.00	Parallel sessions
18.00-19.30	Welcome cocktail

Wednesday, June 10

08.00-09.00	Registration
09.00-10.30	Opening ceremony and main lectures
10.30-11.00	Coffee break and poster session
11.00-13.00	Parallel sessions
13.00-14.00	Lunch and poster session
14.00-15.30	Parallel sessions/Editorial Board Meeting
15.45	Departure to *Polo Universitario Veterinario*, Lodi
16.15-20.00	Main lectures and scientific visit at the facilities of *Polo Universitario Veterinario*, Lodi
20.00	Country dinner at the *Polo Universitario Veterinario*, Lodi

Thursday, June 11

09.00-10.45	Parallel sessions
10.45-11.15	Coffee break and poster session
11.15-13.00	Parallel sessions
13.00-14.00	Lunch and poster session
14.00-16.00	Parallel sessions
16.00-16.30	Coffee break and poster session
16.30-17.15	Reports from study commissions
17.15-19.30	ASPA meeting
20.30	Social dinner

Friday, June 12

System approach for improving the sustainability of animal production, health and welfare
Organized jointly by: Scientific Committee for EXPO, University of Milan, EAAP (European Association of Animal Production), and ASPA (Animal Science and Production Association)

09.00-10.00	Registration and welcome address
10.00-10.30	Keynote speech
10.30-11.45	Session I: Sustainability analysis and life cycle assessment of animal production
11.45-12.15	Coffee break
12.15-13.30	Session II: The impact of animal welfare and health on food quality and safety
13.30-14.30	Lunch
14.30-15.45	Session III: Mitigating the use of antiparasitic and antimicrobial drugs in livestock productions
16.00	Visit Expo sites
20.00	Dinner at Expo

ASPA 21st Congress
Milano, June 9-12, 2015

Italian Journal of Animal Science
2015; volume 14: supplement 1

Agenda for oral presentations

Tuesday, June 9 - Livestock and environment/welfare - Room 211

Chairpersons: Valentina Ferrante - Flaviana Gottardo

15.30-16.00 Elisabetta Canali, Sara Barbieri, Michela Minero, Valentina Ferrante, Silvana Mattiello, Adroaldo Zanella
AWIN project: innovation in animal welfare assessment C-001

16.00-16.15 Susanna Lolli, Inma Estevez, Lorenzo Ferrari, Tatiane Terumi Negrão Watanabe, Sezen Ozkan, Carlo Tremolada, Valentina Ferrante
Protocol development for welfare assessment in commercial turkey farms C-002

16.15-16.30 Monica Battini, Sara Barbieri, Guido Bruni, Giorgio Zanatta, Silvana Mattiello
Testing the feasibility of a prototype welfare assessment protocol in intensive dairy goat farms C-003

16.30-16.45 Katia Parati, Rossana Capoferri, Livia Moscati, Marco Sensi, Guerino Lombardi, Francesca Battioni, Gianpietro Sandri, Carlo Briani, Andrea Galli
The effect of the relaying mode on welfare of the pregnant sow C-004

16.45-17.00 Marta Brscic, Giulia Vida, Annalisa Scollo, Giulio Cozzi, Flaviana Gottardo
Relationship between pig welfare in the pre-slaughter phase and superficial bruises of their carcasses. C-005

17.00-17.15 Maria Lúcia Pereira Lima, João Alberto Negrão, Claudia Cristina Paro de Paz
Corral modification for humane livestock handling can improve cattle behavior and reduce serum cortisol C-006

17.15-17.30 Isabella Lora, Marta Brscic, Luisa Magrin, Clelia Rumor, Flaviana Gottardo
Effects of innovative horizontal fans on beef cattle health, growth and welfare C-007

17.30-17.45 Miriam Iacurto, Roberto Steri, Germana Capitani, Federico Vincenti
Animal welfare in beef cattle: performances and meat quality C-008

Tuesday, June 9 - Genetics and genomics/miscellany - Plenary Room

Chairpersons: Alessio Valentini - Roberta Davoli

15.30-15.45 Emiliano Lasagna, Simona Rimoldi, Simone Ceccobelli, Stefano Paolo Marelli, Maria Cristina Cozzi, Francesca Maria Sarti, Genciana Terova
The genetic of welfare: gene expression and heat stress in chicken C-009

15.45-16.00 Paolo Zambonelli, Martina Zappaterra, Maurizio Mazzoni, Massimiliano Petracci, Francesca Soglia, Federico Sirri, Claudio Cavani, Roberta Davoli
Broiler Pectoralis major muscle affected by emerging abnormalities: histology and gene expression C-010

16.00-16.15 Genciana Terova, Simona Rimoldi, Elena Bossi, Sheenan Harpaz, Marco Saroglia
Transcript quantification of intestinal neutral amino acids and oligopeptides transporters in European sea bass (Dicentrarchus labrax) reared in fresh water and fed fish and plant protein sources. C-011

16.15-16.30 Genciana Terova, Simona Rimoldi, Karolina Kwasek, Marco Saroglia
Dietary methionine in salmonid fish feed alters the expression of genes involved in methionine metabolism C-012

16.30-16.45 Gianluca Sottile, Maria Teresa Sardina, Salvatore Mastrangelo, Marco Tolone, Marcello Chiodi, Baldassare Portolano
Identification of breed informative single nucleotide polymorphisms for assigning individual in Sicilian dairy sheep C-013

16.45-17.00 Maria Grazia Manca, Jessica Serdino, Paolo Urgeghe, Antonio Puledda, Ignazio Ibba, Marino Contu, Nicolò P.P. Macciotta
Multivariate factor analysis of milk composition, coagulation properties and individual cheese yield in Sarda sheep C-014

Tuesday, June 9 - New technologies in animal science - Room 111

Chairpersons: Fabio Luzi - Alessandro Agazzi

Tuesday, June 9 - Animal products and human health/nutrition and meat quality - Medicina Room

Chairpersons: Vincenzo Chiofalo - Oreste Franci

Wednesday, June 10 - Livestock and environment/environment - Room 211

Chairpersons: Giulio Cozzi - Luciana Bava

Wednesday, June 10 - Genetics and genomics/cattle - Plenary Room

Chairpersons: Giovanni Bittante - Paolo Ajmone Marsan

12.15-12.30 Marco Milanesi, Stefano Capomaccio, Yuri Tani Utsunomiya, Lorenzo Bomba, Licia Colli, Elisa Eufemi, Jan Thijs Van Kaam, Katia Cappelli, Stefano Biffani, Ezequiel Luis Nicolazzi, Riccardo Negrini, Josè Fernando Garcia, Carl Rubin, Alessandro Nardone, Nicolò P. P. Macciotta, Alessio Valentini, John L. Williams, Paolo Ajmone Marsan
Combining exome sequencing and high density SNP analysis for the detection of common deleterious recessive mutations segregating in Italian Holstein bulls C-046

12.30-12.45 Andrea Talenti, Marco Milanesi, Ezequiel Luis Nicolazzi, Letizia Nicoloso, Stefano Frattini, Beatrice Coizet, Giulio Pagnacco, John L. Williams, Paolo Ajmone Marsan, Paola Crepaldi
Birth date regression to identify genomic signatures of recent selection in Italian Holstein C-047

12.45-13.00 Antonio Puledda, Marco Milanesi, Giustino Gaspa, Corrado Dimauro, Paolo Ajmone Marsan, Nicolò P. P. Macciotta
Study of genomic prediction accuracy using high density SNP panel in Italian Holstein cattle. C-048

Wednesday, June 10 - Research and development in animal science/ processing and technology transfer part 1 - Room 111

Chairpersons: Biagina Chiofalo - Ambra Rita Di Rosa

11.00-11.30 Giovanni Bernardini, Rosalba Gornati, Genciana Terova, Marco Saroglia
Nanotechnology for animal production C-049

11.30-11.45 Angela Trocino, Alessandra Piccirillo, Marco Birolo, Eirini Filiou, Massimiliano Petracci, Gerolamo Xiccato
Factors affecting growth performance, carcass quality and the occurrence of white striping and wooden breasts in broilers C-050

11.45-12.00 Marco Birolo, Angela Trocino, Marco Tazzoli, Eirini Filiou, Gerolamo Xiccato
Feed restriction programs and slaughter age in growing rabbits C-051

12.00-12.15 Maria Antonietta Colonna, Francesco Giannico, Pasqua Rotondi, Lumturi Papa, Marco Ragni, Prospero Cagnetta, Giuseppe Marsico, Anna Caputi Jambrenghi
Effect of feeds containing durum wheat milling products and age at slaughter on chemical composition and fatty acid profile of meat from Gentile di Puglia lambs C-052

12.15-12.30 Mirco Dalla Bona, Luca Carraro, Stefano Schiavon, Luigi Gallo
Growth and carcass traits of 145 kg body weight pigs fed according to different feeding regimes. C-053

12.30-12.45 Gerolamo Xiccato, Elia Beltrami, Marco Birolo, Nicola Rigo, Angela Trocino
Effect of gender and slaughter age on carcass and meat quality of brown hares (L. europaeus) C-054

12.45-13.00 Alessandra Roncarati, Francesca Mariotti, Alberto Felici, Paolo Melotti
Use of submerged cage and discards as feed for tub gurnard (Chelidonichthys lucerna L.) fattening in the mid Adriatic sea C-055

Wednesday, June 10 - Animal products and human health/ milk and dairy products quality - Medicina Room

Chairpersons: Andrea Summer - Angela Gabriella D'Alessandro

11.00-11.30 Giovanni Niero, Antonio Masi, Martino Cassandro
Preliminary characterization of soluble thiols in bovine milk C-056

11.30-11.45 Manuela Renna, Simone Ravetto Enri, Massimiliano Probo, Carola Lussiana, Paolo Cornale, Alberto Bellio, Sara Astegiano, Lucia Decastelli, Luca Maria Battaglini, Giampiero Lombardi
Production regulations and characteristics of cow Piedmontese Noble Milk C-057

11.45-12.00 Adriana Bonanno, Antonino Di Grigoli, Nicola Francesca, Raimondo Gaglio, Francesca Mazza, Francesca Vitale, Marco Alabiso, Luca Settanni
The effects of the traditional producing system on physicochemical, microbial and sensory properties of Caciocavallo Palermitano cheese C-058

12.00-12.15 Paolo Gottardo, Mauro Penasa, Massimo De Marchi
Fatty acid composition of milk from Holstein-Friesian, Brown Swiss, Simmental and Alpine Grey cattle breeds routinely predicted by mid-infrared spectroscopy C-059

12.15-12.30 Federica Di Frangia, Massimo Malacarne, Piero Franceschi, Paolo Formaggioni, Michele Faccia, Angela Costanzo, Enrico Santus, Attilio Rossoni, Andrea Summer
Influence of milk composition and κ-casein B content on mozzarella cheese yield and cheesemaking losses C-060

12.30-12.45 Massimo Malacarne, Federica Di Frangia, Angela Costanzo, Piero Franceschi, Paolo Formaggioni, Andrea Summer
Influence of milk somatic cell content on cheese yield and cheesemaking losses C-061

Wednesday, June 10 - Physiology and metabolism/microbiome and gastrointestinal tract processing and technology transfer part 1 - Room 113

Chairpersons: Achille Schiavone - Cesare Castellini

11.00-11.15 Paola Cremonesi, Giuseppe Conte, Emanuele Capra, Federica Turri, Marco Severgnini, Luca Rapetti, Stefania Colombini, Stefania Chessa, Marcello Mele, Bianca Castiglioni
Evaluation of the effects of different diets on bacterial diversity and on fatty acid composition in goat rumen C-062

11.15-11.30 Arianna Buccioni, Carlo Viti, Mariano Pauselli, Roberta Pastorelli, Sara Minieri, Grazia Pallara, Valentina Roscini, Luciana Giovannetti, Marcello Mele
Response of rumen microbial ecosystem to diets integrated with chestnut or quebracho tannins in dairy ewes C-063

11.30-11.45 Sara Minieri, Carlo Viti, Stefania Colombini, Arianna Buccioni, Giuseppe Conte, Luca Rapetti, Luca Malagutti, Stefano Rapaccini, Marcello Mele
Effect of dietary starch concentration and fish oil supplementation on fatty acid profile and microbial composition of rumen fluid in lactating dairy cows C-064

11.45-12.00 Maria Pia Franciosini, Patrizia Casagrande-Proietti, Claudio Forte, Daniela Beghelli, Gabriele Acuti, David Ranucci, Alessandro Dal Bosco, Cesare Castellini, Massimo Trabalza-Marinucci
Effects of using an oregano aqueous extract on performance, gut microbiota and immune function in broiler chickens C-065

12.00-12.15 Paolo Bosi, Davide Priori, Michela Colombo, Alfons J.M. Jansman, Sietse Jan Koopmans, Paolo Trevisi
The interaction of early intestinal microbial colonisation with the effects of luminal perfusion with enterotoxigenic E. coli, F4ac fimbria or Lactobacillus amylovorus on the jejunal transcriptome in piglets C-066

12.15-12.30 Maria Grazia Cappai, Franziska Koop, Laura Pistis, Saara J. Sander, Corrado Dimauro, Walter Pinna, Josef Kamphues
Evaluation of Peyer's patch morphological traits as local immune response in relation to different physical forms of one complete diet fed to growing pigs. C-067

12.30-12.45 Riccardo Fortina, Isabel Macchiorlatti Vignat, Frine Eleonora Scaglione, Paola Pregel, Enrico Bollo, Manuela Renna
Performance and rumen papillae development of Friesian calves fed with pelletted starter or total mixed ration C-068

Wednesday, June 10 - Genetics and genomics/swine - Plenary Room

Chairpersons: Paolo Carnier - Bianca Castiglioni

14.00-14.15 Giuseppina Schiavo, Giuliano Galimberti, Daniela Giovanna Calò, Emilio Scotti, Francesca Bertolini, Antonia Bianca Samorè, Vincenzo Russo, Maurizio Gallo, Luca Buttazzoni, Luca Fontanesi
Genome wide analysis of the effects of twenty years of artificial directional selection in the Italian Large White pig breed C-069

14.15-14.30 Anisa Ribani, Francesca Bertolini, Giuseppina Schiavo, Emilio Scotti, Valerio Joe Utzeri, Stefania Dall'Olio, Paolo Trevisi, Paolo Bosi, Luca Fontanesi
Next generation semiconductor based sequencing of bitter taste receptor genes in different pig breeds and populations and association study of identified polymorphisms using a DNA pooling strategy C-070

14.30-14.45 Roberta Rostellato, Valentina Bonfatti, Paolo Carnier
Quantification of boar taint compounds in adipose tissue of intact male pigs at 160 and 220 days of age and estimates of genetic parameterss C-071

14.45-15.00 Roberta Davoli, Gennaro Catillo, Marcello Mele, Andrea Serra, Luca Buttazzoni, Roberto Steri, David Meo Zilio, Paolo Zambonelli, Vincenzo Russo
Study on genetic parameters of subcutaneous fatty acid composition in Italian Large White pig breed C-072

15.00-15.15 Martina Zappaterra, Paolo Zambonelli, Roberta Davoli
Perilipins and the complex gene network of lipid metabolism enzymes in muscle are related to porcine intramuscular fat deposition C-073

Wednesday, June 10 - Research and development in animal science/ processing and technology transfer part 2 - Room 111

Chairpersons: Andrea Formigoni - Mattia Fustini

14.00-14.15 Agnese Arduini, Vincenzo Pace, Veronica Redaelli, Fabio Luzi, Leonardo Nanni Costa
Survey on pre-slaughter handling and skin damages on pig carcasses C-074

14.15-14.30 Marisa Palmeri, Cinzia Cardamone, Massimo Todaro, Luca Settanni, Raimondo Gaglio, Isabella Mancuso, Maria Luisa Scatassa, Onofrio Corona
An investigation of the shelf-life of pdo Vastedda della Valle del Belice cheese subjected to different packaging systems alternative to vacuum C-075

14.30-14.45 Andrea Cabiddu, Mauro Decandia, Giovanni Molle, Michael Lee
Plant secondary metabolites and traceability of dairy products: a key role for livestock production and efficiency in ruminant dairy products C-076

14.45-15.00 Carlotta Giromini, Raffaella Rebucci, Francesca Saccone, Eleonora Fusi, Antonella Baldi
Cytotoxicity, DNA integrity and methylation in mammary and kidney epithelial cell lines exposed to Ochratoxin A C-077

15.00-15.15 Stefano Biffani, Cesare Lubiano, Davide Pravettoni, Antonio Boccardo
Predicting survivors of neonatal calf diarrhea using logistic regression or gradient boosting C-078

15.15-15.30 Eugenio Demartini, Alberto Pirani, Mattia Bertocchi, Maria Elena Marescotti, Anna Gaviglio
The hidden costs of animal health in dairy cattle breeding: evidences from a case study C-079

Wednesday, June 10 - Physiology and metabolism/ feed nutritional quality evaluation - Room 113

Chairpersons: Lucia Bailoni - Umberto Bernabucci

14.00-14.15 Nadia Musco, Federico Infascelli, Raffaella Tudisco, Laura Addi, Raffaella Grazioli, Pietro Lombardi, Giuseppe Vassalotti, Serena Calabrò, Monica Isabella Cutrignelli
Nutritional and functional evaluation of cereals and legumes seeds C-080

14.15-14.30 Achille Schiavone, Michele De Marco, Silvia Martínez, Fuensanta Hernandez, Francesco Gai, Luca Rotolo, Sihem Dabbou, Attawit Kovitvadhi, Laura Gasco
Nutritional value of two different insects meal (Tenebrio Molitor and Hermetia Illucens) for broiler chickens: apparent ileal amino acid digestibility and apparent metabolizable energy C-081

14.30-14.45 Mattia Fustini, Alberto Palmonari, Giorgia Canestrari, Nicola Negri, Andrea Formigoni
Effect of alfalfa hay digestibility and inclusion rate in lactating dairy cows diets on feed intake, digestibility, ruminal pH and productive performance C-082

14.45-15.00 Francesca Martuzzi, Federico Righi, Yarin Mazzoni, Afro Quarantelli
Relationship between horse faecal particle size and forage type/dry matter digestibility C-083

15.00-15.15 Serena Calabrò, Raffaella Tudisco, Valeria Terzi, Monica I. Cutrignelli, Laura Addi, Nadia Musco, Caterina Morcia, Federico Infascelli
In vitro rumen fermentation of barley grain contaminated with Fusarium mycotoxins C-084

15.15-15.30 Alberto Palmonari, Mattia Fustini, Giorgia Canestrari, Antonio Gallo, Alessandro Lotto, Andrea Formigoni
Evaluation of inoculants addition on corn silage nutritional value C-085

Thursday, June 11 - Livestock and environment/feed and food - Room 211

Chairpersons: Stefano Schiavon - G. Matteo Crovetto

9.00-9.15 Aldo Prandini, Samantha Sigolo, Mauro Morlacchini, Gianluca Giuberti, Maurizio Moschini
Pasta by-product as alternative source of starch in diets for finishing pigs C-086

9.15-9.30 Luca Malagutti, Gianluca Galassi, Luca Rapetti, Stefania Colombini, Mauro Spanghero, Diego Capraro, G. Matteo Crovetto
Digestibility and energy balance of heavy pig fed high cut corn silage C-087

9.30-9.45 Luigi Gallo, Luca Carraro, Mirco Dalla Bona, Alessio Cecchinato, Stefano Schiavon

Thursday, June 11 - Genetics and genomics/goat - Plenary Room

Chairpersons: Fabio Pilla - Paola Crepaldi

Thursday, June 11 - Research and development in animal science/feed additives - Room 111

Chairpersons: Giovanni Savoini - Eugenio Demartini

Thursday, June 11 - Animal products and human health/ animal products characterization - Medicina Room

Chairpersons: Agostino Sevi - Adele Meluzzi

9.00-9.30 Antonella della Malva, Marzia Albenzio, Mariangela Caroprese, Antonella Santillo, Agostino Sevi, Rosaria Marino
Factors contributing to meat tenderness: interaction between myofibril structure and proteolytic changes in beef C-102

9.30-9.45 Federico Sirri, Massimiliano Petracci, Claudio Cavani, Adele Meluzzi
Quality characterization of products from Romagnola chicken breed: 2. Eggs C-103

9.45-10.00 Massimiliano Petracci, Francesca Soglia, Federico Sirri, Paolo Zambonelli, Roberta Davoli, Claudio Cavani
Broiler Pectoralis major muscle affected by emerging abnormalities: composition and technological traits C-104

10.00-10.15 Paolo Polidori, Ambra Ariani, Silvia Vincenzetti
Meat and carcass quality in Martina Franca donkey foals C-105

10.15-10.30 Mauro Vasconi, Laura Borella, Fabio Caprino, Federica Bellagamba, Vittorio Maria Moretti
Chemical characterization of European eels (Anguilla anguilla) of different origins collected from Italian market C-106

Thursday, June 11 - Livestock and environment/miscellany - Room 211

Chairpersons: Marco Saroglia - Luca Maria Battaglini

11.15-11.30 Cristina Rossi, Loredana Basiricò, Pier Paolo Danieli, Bruno Ronchi
The β-hexachlorocyclohexane (β-HCH) affects the oxidative status of bovine peripheral blood mononuclear cells in vitro. C-107

11.30-11.45 Luciana Bava, Anna Sandrucci, Stefania Colombini, Milena Brasca, Tiziana Silvetti, Alberto Tamburini, Maddalena Zucali, G. Matteo Crovetto
Milking procedure can affect anaerobic spore-forming bacteria in milk C-108

11.45-12.00 Maddalena Zucali, Luciana Bava, Alberto Tamburini, Anna Sandrucci, Silvana Mattiello, Giovanna Battelli, Milena Brasca
Global milk quality: evaluating tool for dairy farm C-109

12.00-12.15 Giulio Cozzi, Anna Benedet, Paola Prevedello, Dario Tonietto, Ilario Bazzoli, Claudio Valorz, Gianbattista Rigoni Stern
Animal husbandry as a tool for post-war recovery and reconciliation. An update from The Transhumance of the Peace project C-110

12.15-12.30 Alberto Tamburini, Anna Sandrucci, Maddalena Zucali, Luciana Bava, Gabriele Mattachini, Giorgio Provolo
Integrated approach to evaluate primiparous dairy cows performance C-111

12.30-12.45 Luca Parma, Daniela Bertotto, Erika Bonvini, Elena Negrato, Marco Chiumento, Pier Paolo Gatta, Alessio Bonaldo
Light conditions affect development and cortisol response in common sole (Solea solea l.) eggs and larvae: preliminary findings C-112

12.45-13.00 Gloriana Cardinaletti, Tiziana Bongiorno, Matteo Gava, Maria Messina, Massimiliano Bruno, Francesca Tulli, Emilio Tibaldi
Compensatory growth following long term multi-phase cyclic feeding in rainbow trout (Oncorhynchus mykiss) C-113

Thursday, June 11 - Genetics and genomics/cattle - Plenary Room

Chairpersons: Roberta Ciampolini - Baldassare Portolano

Not published C-114

11.15-11.30 Cristina Sartori, Nadia Guzzo, Serena Mazza, Roberto Mantovani
Genetic correlations among selected traits and inbreeding depression in dual purpose Rendena breed C-115

11.30-11.45 Roberta Ciampolini, Sara Casu, Salvatore Mastrangelo, Laurence Flori, Katayoun Moazami-Goudarzi, Tiziana Sechi, Francesca Cecchi, François Casabianca, Anne Lauvie, Mathieu Gautier, Antonello Carta, Baldassare Portolano, Denis Laloë
Genetic diversity of Mediterranean cattle breeds related to geography and climate C-116

11.45-12.00 Salvatore Mastrangelo, Marco Tolone, Maria Teresa Sardina, Rosalia Di Gerlando, Luca Fontanesi, Baldassare Portolano
Genomic inbreeding estimation in small populations: evaluation of runs of homozygosity in local cattle breeds C-117

12.00-12.15 Simone Ceccobelli, Piera Di Lorenzo, Hovirag Lancioni, Irene Cardinali, Licia Colli, Marco Rosario Capodiferro, Luca Ferretti, Paolo Ajmone Marsan, Francesco Panella, Emiliano Lasagna, Alessandro Achilli
The distinctive mitochondrial DNA signature of Podolic cattle in Italy C-118

Thursday, June 11 - Animal products and human health/ nutrition and milk quality - Medicina Room

Chairpersons: Oliviero Olivieri - Federico Infascelli

Thursday, June 11 - Physiology and metabolism/ nutrition and physiology part 1 - Room 113

Chairpersons: Monica Cutrignelli - Luciano Pinotti

Thursday, June 11 - Genetics and genomics/cattle - Plenary Room

Chairpersons: Anna Maria Caroli - Luigi Ramunno

Thursday, June 11 - Research and development in animal science/feed materials - Room 111

Chairpersons: Valentino Bontempo - Luciana Rossi

Thursday, June 11 - Physiology and metabolism/ nutrition and physiology part 2 - Room 113

Chairpersons: Adriana Bonanno - Marcello Mele

14.00-14.15 Andrea Serra, Eleonora Bulleri, Laura Casarosa, Alice Cappucci, Federica Mannelli, Marcello Mele
Effect of different doses of cracked whole soybean on milk fatty acid composition in buffalo C-147

14.15-14.30 Adriano Pilotto, Guido Invernizzi, Antonella Baldi, Giovanni Savoini
Plasma α-tocopherol content and its relationship with milk somatic cells count in Italian commercial herds C-148

14.30-14.45 Achille Schiavone, Michele De Marco, Martina Tarantola, Francesco Gai, Luisa Pozzo, Laura Cavallarin, Stefania Bergagna, Daniela Dezzutto, Maria Silvia Gennero, Luca Rotolo, Manuela Renna, Sihem Dabbou, Alberto Brugiapaglia, Laura Gasco
Mealworm inclusion in Label chicken diet does not affect both performance and welfare parameters. C-149

14.45-15.00 Francesca Petrera, Alessandra Gubbiotti, Fabio Abeni
Haematological, biochemical and serum protein profiles during lactation in Modenese and Italian Friesian cattle breeds C-150

15.00-15.15 Cesare Castellini, Alessandro Dal Bosco, Genciana Terova, Emiliano Lasagna, Lara Macchioni, Simona Mattioli
Evaluation of the gene expression and 6-desaturase activity in different chicken strains C-151

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Agenda for poster presentations

Animal products for human health

Genetics and genomics

Livestock and environment

New technologies in animal sciences

Physiology and metabolism

Research and development in animal science

L-001

Milk and dairy products: dietary partners for life?

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The increasing burdens of obesity and population age and the environmental impact of food production will all shape future food and health policies. Most of these trends will also increase the risk of chronic diseases meaning that diet, a key moderator of chronic disease risk, must play an increasingly important role. The importance of milk in the diet as a source of energy, high quality protein and other key nutrients, including calcium, is broadly accepted, yet many remain uncertain about whether or not these foods contribute to increased risk of cardiovascular and other chronic diseases. Milk is a crucial supplier of calcium, phosphorus and magnesium for bone growth and development in children and it is concerning that due to reduced milk consumption, intake of these nutrients is often sub-optimal, particularly in female children. Such effects in early life increase the risk of osteoporotic fractures in later life, especially in postmenopausal women. Milk is also a key dietary source of iodine and recent studies have identified low iodine status in pregnant women, likely due to reduced milk consumption and one study linked this with poorer child cognitive development. Evidence from most long term prospective cohort studies shows that high milk consumption does not increase cardio-metabolic disease risk in middle aged and older people and indeed may provide benefits via reduced blood pressure and arterial stiffness as well as reduced risk of Type 2 diabetes. The effects of butter, cheese, fat reduced milk and saturated fat reduced milk are less certain. The benefits from dairy products are despite the fact that they are often the major dietary source of saturated fatty acids and there is increasing evidence that interpreting the disease risk needs to account for modifying effects of the food vehicle. For example, studies comparing consumption of equal amounts of dairy fat from cheese or butter have shown fat from the cheese to give lower total and LDL-cholesterol concentrations than from butter. Several of the benefits of dairy products relate to the effects of milk proteins and it is interesting to note that whey protein, widely used in sport to increase muscle mass, may also reduce the rate of muscle loss in the elderly. This in turn reduces the risk of bone breakages and the consequential loss of independence. This paper will review key disease events across the life course and the associated dietary role of dairy products.

L-002

Rumen-protected methyl donors and the genome: beyond nutrigenomics

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Methylation is a critical epigenetic modification influencing metabolism, immune function, and overall health of animals. Dietary deprivation of one or more methyl-group containing nutrients can alter tissue metabolism and immune function at least in part by altering gene expression. As the major methyl donors in the diet, methionine, choline, and folate help maintain the 1-carbon pool in both monogastrics and ruminants. Methyl groups from choline and folate, upon conversion to methionine, lead to synthesis of S-adenosyl methionine (SAM), the global methyl donor. As demonstrated in sheep, the transition from pregnancy to lactation represents a stage where the demand for methyl groups is markedly increased. Although there are no published data with high-producing dairy cattle, the onset of lactation likely leads to a shortfall in the availability of methyl groups as it does for other essential nutrients. The voluntary decrease in feed intake around calving and increased demand by the mammary gland likely give rise to methionine and choline deficiency. Positive effects of supplementing rumen-protected methionine (RPM) have been observed in terms of increased milk production, feed intake, and milk fat yield as well as a better immune-metabolic status. Data revealed that RPM supplementation up-regulates DNA methyl transferases and other metabolic genes in the 1-carbon metabolism pathway during the periparturient period. Clearly, methyl donor supplementation to high-producing dairy cattle could have implications at the molecular level not only in the mother but also the developing calf, for it has been reported that maternal diet during pregnancy induces DNA methylation changes in fetal tissues in sheep. Therefore, besides the well-established role in milk protein synthesis and liver lipid metabolism, the impact of RPM as well as supplementation of other methyl donors could have implications at the genome level, *e.g.* transcriptome and epigenome. The aim of this presentation is to provide an overview of the most-recent data from studies feeding methyl donors to dairy cattle. Emphasis will be placed on nutri-genomic and -epigenomic regulation via dietary methyl donors in dairy cows and calves. Work on enzyme flux through the 1-carbon and methionine cycles also will be discussed. Implications of the proper balance of dietary methyl donors at different stages of the life cycle of dairy cattle for adequate health and productivity are emphasized.

C-001

AWIN project: innovation in animal welfare assessment

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The study of valid reliable indicators is one important objective of welfare research especially for assessing welfare at farm level. In fact, in order to evaluate welfare of animals, it is very important to develop wide variety of indicators, which measures the welfare state of the animals in terms of their health, behaviour and physiology (animal-based indicators). Recently the European Commission stressed the use of science-based animal welfare indicators as a possible means to simplify the legal framework and allow flexibility to improve competitiveness of livestock producers. The Animal Welfare Indicators project – AWIN (www.animal-welfare-indicators.net) – granted under the EU VII Framework program, dealt with integration and dissemination of animal-based welfare indicators including pain, in some commercially important husbandry species: sheep, goats, horses, donkeys and turkeys. The aim of the project was to develop welfare assessment protocols, which can provide a toolbox to assess animal welfare, in order to promote improvements in animal production systems throughout Europe. Animal-based indicators to be include in the welfare assessment protocols have been evaluated for their scientific robustness and classified according to the Five Freedoms, the Four Principles and the 12 Criteria, as they are considered valuable to cover all the aspects of animal welfare (Welfare Quality). One protocol for each species has been generated and tested for validity and feasibility.The protocols offer, as a first level, a quick screening, with a selection of robust animal-based indicators which can be readily and simply applied, a second level with a more comprehensive assessment. For the successful development of the welfare assessment protocols and to assure their acceptability and use at farm level, the perception of different stakeholders (*e.g.* farmers, veterinarians, farmer associations, NGOs) was taken into account through online survey (279 questionnaires filled: horses 40% and sheep 30%) and face-to-face meetings. Digitalized data collection systems were also developed to improve the efficiency and reliability of data collection on-farm, and to allow automatic upload of data. This automatic recording system will allow generating a clear and immediate outcome to the farmer. This outcome will emphasise positive feedback on the welfare conditions of the animals and of the farm, to improve the communication and the attitude towards animal welfare.

C-002

Protocol development for welfare assessment in commercial turkey farms

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The Animal Welfare Indicators (AWIN) project aims to verify the feasibility of the Tansect Walks (TW) method as a potential tool for on-farm welfare assessment in commercial turkeys fattening period. This new approach does not involve bird manipulation; therefore, there is minimal impact on the birds and is less time-consuming and personnel demanding. The age of assessment is crucial as the risk of impair welfare increases at the end of production cycle, when animals are more prone to show welfare problems. Due to the lack of evidence on differences with regard to welfare problems related to gender the aim of this study was to verify how this protocol may emphasize the welfare status of male and female turkey. The protocol was developed and tested on 33 turkey farms (13 male and 20 female) located in Italy and Turkey, with similar management standard procedures. Bird's age ranged from 122 to 138 d for females and 84 to 103 d for males. Each visit included an interview with the farm manager focused on general management practices, biosecurity and some broad farm characteristics. The incidence of birds showing any of the defined indicators was collected using the TW method. The used animal based indicators were: aggression towards mate, dead, sick, terminal, dirty, featherless, locomotory (immobility and lameness), interactions with human, mating, small, wounds. The effect of gender was evaluated by using ANOVA. The results showed clear differences across male and females farms ($P<0.0001$), for the incidence of locomotory, wounds, small and interactions with human. Male birds showed higher incidence of locomotory (2.1% *vs.* 0.8%) and wounds (0.8% *vs.* 0.3%) than female flocks, respectively. This method allowed to emphasize the difference in welfare status between male and females moreover the TW method was proven to be feasible to assess the welfare and health status of turkey flocks on farm, as well as being acceptable in term of time requirements (3h) and personnel demands.

Acknowledgments

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C-003

Testing the feasibility of a prototype welfare assessment protocol in intensive dairy goat farms

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A prototype was tested during February-July 2014 in 30 Italian farms, previously classified according to their size (small:<50 goats; medium: 51-100; large:101-500). The assessment was conducted at group level from outside the pen (queuing at feeding and at drinking, improper disbudding, hair coat condition, kneeling, thermal stress, isolated animals, abnormal lying, Qualitative Behaviour Assessment), from inside the pen (severe lameness, kneeling, latency to first contact test, avoidance distance test), and at individual level (3-level BCS, faecal soiling, discharges, udder conformation, cleanliness, lesions, abscesses, claw overgrowth, knee calluses). Time needed to apply the prototype was recorded. ANOVA was used to compare the time required to apply the prototype in farms of different size. The average time required was 144.0±9.6 minutes (min:37, max:272): the group assessment took 81.0±9.6 minutes; the individual assessment took 63.0±5.8 minutes (min:10, max:146, depending on farm size: small *vs.* medium: $P<0.05$; small *vs.* large: $P<0.01$), ranging from 131.4±15.9 seconds/per goat if goats were manually restrained in the pen to 119.3±10.9 seconds if goats were locked at the feeding rack. The assessment at individual level showed many constraints, mainly due to the difficulties in restraining goats. Some indicators (*e.g.* abscesses, lesions) were separately recorded for different body regions, but this resulted too time-consuming and not particularly informative. Feasibility constraints were found at group level for avoidance distance test, mainly due to the difficulties in identifying individual animals. Qualitative Behaviour Assessment was welcomed by farmers, being a non-invasive method of observation. Hair coat condition was the most prevalent problem (24.1%±2.8), followed by improper disbudding (12.7%±3.0) and queuing at feeding (7.2%±0.7), whereas low prevalence was recorded for kneeling and abnormal lying (less than 0.5%). At individual level, the most frequent problems were claw overgrowth (47.2%±6.0), faecal soiling (16.7%±4.7), too thin (14.5%±2.5) and too fat goats (5.1%±1.3). The prototype seems suitable to be used in farms of different size. Farm routine was not altered and only slight disturbance was caused to the farmers. Some adjustments are required for improving the feasibility of the protocol, considering the constraints identified, the possibility of reducing disturbance to farmers and animals, and the low prevalence of some indicators.

C-004

The effect of the relaying mode on welfare of the pregnant sow

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Animal welfare, particularly in pig, is a constraint for animal breeding. In this regard the Italian D.Lgs 53/2004 established that from 2013 sows and gilts shall be kept in groups during the four weeks after the service to one week before the expected farrowing time. Although its relevance is conceptually and extensively recognised, animal welfare is not yet fully characterized at an objective level and a protocol based on parameters for objectively evaluating animal welfare is still not available. The aim of this study was to evaluate a series of approaches in order to define informative markers associated with animal welfare, which allow an objective assessment of this status in the farm. Eight hundred pregnant sows reared in single and group-housing systems in a local farm were compared at different levels of investigation before and after the changeover imposed by law. Ethological, clinical, reproductive, metabolic and immunological measures were detected for each of the eight weeks of gestation (4 and 8-14). At the same time transcriptomic analysis of blood cells was conducted by OpenArray System on 224 genes involved in immunological response. The informativeness of the variables was studied by factor analysis and the effect of the housing system and the gestational week were studied by GLM. The odds ratio was calculated with the random component. Results showed that the group-housing system, contrary to the single-housing one, presented no stereotypy but significant increase in the frequency of lameness ($P<0.001$) and a decrease in fertility parameters (non-return to estrus 56-day post AI: 83% *vs.* 92%; farrowing rate: 78% *vs.* 88%, respectively) ($P<0.05$). The group-housing system revealed differences in the hematological picture for some parameters indicator of the metabolic status (ameliorative values for albumin, OR=4.4 and ALP OR=1.5 and pejorative for the AST, OR=0.6 and bilirubin, OR=0.4) when compared to the single, while at immunological level showed ameliorative values for bactericidal (OR=3.2) and complement (OR=24.3) and pejorative for lysozyme (OR=0.3) and C-reactive protein ($P<0.001$). At the functional genomics level, out of the 224

genes analyzed, 88 were under-expressed (Log FoldChange ≤ 1.5; $P<0.05$) in group-housing with respect to the single-housing system, within all the weeks and for all the gestational groups. Overall, the results of this multidisciplinary study provide potential candidates markers of sows housing relating stress.

C-005

Relationship between pig welfare in the pre-slaughter phase and superficial bruises of their carcasses

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Pre-slaughter stress represents a problem for both, pig welfare and product quality particularly in the case of high quality ham production. The aim of this study was, therefore, to assess the level of welfare in the pre-slaughter phase of 1079 heavy pigs destined to the production of PDO San Daniele ham in a commercial slaughterhouse in Friuli Venezia Giulia, Northeastern Italy. Twelve batches of pigs coming from 5 farms and transported by 8 truck drivers were considered in this study. The journeys from farm to slaughter were always shorter than 100 km and lasted from 15 to 90 minutes. At the slaughter, a trained assessor recorded the number of pigs slipping, fell down, reluctant, backing, vocalizing, lame, sick, or shivering during the unloading. Truck drivers were interviewed to gather data regarding characteristics of each batch (number of pigs, average body weight, age, sex), qualitative descriptors of pigs behavior during uploading at the farm (speed, reluctance, aggressiveness), and information on the transport (animal density, use of devises for uploading, distance and duration of the journey). At the slaughterline, the assessor scored pig carcasses in 3 areas (front, thorax, and back) for superficial bruises using a five point-scale (0 = no lesion; 4 = at least 1 lesion >15 cm). A total carcass score was calculated by adding the scores of the 3 areas. Results showed positive correlations between similar behavior descriptors at uploading and at unloading ($r>0.58$; $P<0.05$). Direct observation during unloading revealed prevalence below 1% of problematic events (2 pigs panting and 1 lame) and no animal sick, shivering or dead at arrival. Only 16% of the carcasses were scored 0 (no lesion in any area) and 63% of them had a total score above 6 (indicating at least 1 large and/or several to medium bruises). Carcass lesions were more prevalent on the thorax and this was probably due to both, the fact that it is a part of the body more exposed to hits and to the feasibility of observation by the assessor since this portion is easier to see in the slaughter-line compared to the extreme sides of the carcass. The risk of having bruised carcasses calculated according to the driver and to the farm effect showed which drivers and farms provided the best batches with the lowest lesion scores pointing out the importance of the welfare level achieved also during handling and loading at the farm.

C-006

Corral modification for humane livestock handling can improve cattle behavior and reduce serum cortisol

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The handling of cattle in corral facilities can be an important source of stress and can induce behavioral changes in the animals. This experiment was conducted to evaluate whether corral modifications designed to transform them from a traditional into a humane livestock handling system influence cattle behavior, cortisol levels, and working time. The corral modifications consisted of blocking vision when the worker stands inside the animal's flight zone, eliminating contrast of light and dark or shadows, and keeping the workers calm at work, not allowing them to scream or hit the animals during handling. Electric cattle prods were never used. A total of 141 Nellore cows from two different farms were studied. First, the behavior of the animals in a traditional corral was evaluated. After modification of the corral, the same animals returned (6 days later) for a second behavioral assessment. The cows were evaluated using visual scores. Entry behaviour (EB) into the restraint device was evaluated by observing whether the cows walked, trotted or ran into the chute. Chute temperament (CT) was assessed by considering whether the animal was calm, agitated or struggled to escape, and exit speed (ES) by observing whether the animal walked, trotted or ran. Blood samples were collected from the jugular vein for analysis of serum cortisol. The time spent (TS) on blood sample collection during restraint in the chute was also recorded. After corral modification, the cows exhibited lower EB ($P=0.049$) and ES ($P<0.0001$) and a higher proportion of animals was calm (CT-$P=0.002$). The proportion of cows that walked, trotter or ran was, respectively, 65.9, 27.0 and 7.1% for EB and 44.0, 32.6 and 23.4% for ES before corral modification, and 73.0, 23.4 and 3.6% for EB and 68.1, 27.0 and 4.9% for ES after corral modification. For CT, the proportion of calm animals, agitated animals and animals struggling to escape was 51.8, 25.5 and 22.7% before corral modification, respectively, and 66.0, 24.1 and 9.9% after corral modification. Serum cortisol levels were significantly lower after corral modification ($P=0.019$). Mean serum cortisol was 6.3 mg/dL before corral modification and 4.1 mg/dL after corral modification. There was no difference in the TS on blood collection before and after modification ($P=0.576$). The mean TS collecting blood was 1:42 minutes before and 1:04 minutes after corral modification. Changes in corral facilities and good handling practices reduce stress in cattle

C-007

Effects of innovative horizontal fans on beef cattle health, growth and welfare

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Air quality and microclimate conditions on farms are factors of great importance to ensure a good state of health and welfare of animals. The topic saw a growing interest for its relationship with pollution and in particular for its effects on climate and on presence of fine particles. The air quality control and the adoption of cooling systems in order to mitigate thermal stress are studied in pig and poultry sectors, and in dairy cows, while they are still neglected in beef cattle farms. This lack of interest is likely due to the fact that beef cattle farmers have no feasible tools to assess the impairment caused to the animals if environmental conditions are inadequate. Aim of the research was to evaluate the effects of horizontal fans on shed environmental conditions and state of health and welfare of beef cattle. The study was carried out from June to August 2014. It involved 72 Charolaise beef bulls (BW 689±39.5) housed in 12 pens (6 bulls/pen). Six pens were provided with horizontal fans (F) while the remaining without fans were used as control (C). Data collection considered: growth performance (weight, average daily gain, feed intake), health (cleanliness, nasal and ocular discharge, altered respiration, altered walking), behaviour (lying, rumination, feeding and feed selection, explorative and social activities). Health and behaviour were recorded 4 times during the experimental period. Behavioural observations lasted 8 hours from 08:30 to 16:30. At each observation session ammonia concentrations inside the shed were measured by a Drv§ger X-am 7000. Temperature and humidity were recorded continuously for the entire experimental period using a delta ohm 2101.2 data logger. Final live weight and average daily gain were similar between treatments, while cleanliness and respiration were improved by the presence of fans especially at the assessments carried out with the highest temperature. Bulls of the F treatment displayed a lower frequency of lying behaviour and the highest interest in exploration and grooming activities. Mount behaviour was observed more frequently in F bulls likely because the deep litter was drier and cleaner reducing therefore floor slipperiness which allowed animals to move easily.

In conclusion, the presence of the fans kept litter drier and animals cleaner, determined a reduction of ammonia especially under conditions of high temperature. Moreover, bulls in F pens were more active and showed more explorative behaviours.

C-008

Animal welfare in beef cattle: performances and meat quality

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The aim of this study was to evaluate the effects of animal welfare on productive performances and meat quality. Sixty-seven young bulls were used: 40 Charolaise (CH) and 27 Limousine (LM), reared in 4 farms. Welfare evaluation was performed in two times: the first when the animals arrived at the farm (A); the second two month before slaughtering (R).The following parameters were registered: vitality, mounting, fighting, stool type, respiratory disease, coughing, nasal discharge, hair coat, skin and lesions. For each box, the following production performances were calculated: initial body weight (IBW); final body weight (FBW); average daily gain (ADG) and dressing percentage (DP). For each individual: slaughter age, carcass weight (CW), meat quality parameters: pH; colour; drip (DL) and cooking losses (CL) were assessed on Longissimus Thoracis at different times after slaughtering. Data were processed with Multivariate Factor Analysis (MFA), a method of dimension reduction which explores the covariance structure of many complex traits, and allows the detection of latent factors that can be used as new phenotypes. Four factors were able to explain 69% of total original variability (27, 17, 14 and 11%, respectively).The first extracted factor included genetic type, as well as growth and slaughter performances (IBW, FBW, age, ADG and CW), some meat quality traits (CL at 3 days and pH at 48h) and stool type at time R and could be considered a genetic type index; the second factor included welfare parameters (vitality, mounting and fighting at time R, stool type and coughing at time A) and could be considered a welfare index; the third factor included only some colorimetric traits (Lightness, Hue and Chroma) and could be considered a colorimetric index; the last factor included farm effects together with growth and slaughter performances (ADG and DP), and meat quality traits (DL and Chroma). The association between genetic type and stool type depends on the two used breeds: in the formulation of feedstuff for LM the farmers use high levels of starches, so explaining the occurrence of loose stools. On the contrary, more attention is paid to the formulation of feeding for CH. This study gave evidence that some welfare parameters are associated to meat quality, although previous authors tended to ascribe their effect to productive performances. The farm affected only productive performances, but not animal welfare, as assessed in this study.

C-009

The genetic of welfare: gene expression and heat stress in chicken

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High environmental temperature is one of the most important stressors associated with economic losses to the poultry industry. It causes poor growth performance, immunosuppression, and high mortality, contributing thus to a production decrease. Broilers are in general more sensitive to high environmental temperatures than other domestic animals but the responses to heat differ between different chicken genetic backgrounds. We used One Step Taqman real time RT-PCR technology to evaluate the effect of heat stress on the expression of six genes codifying for the antioxidative enzymes (SOD and CAT), heat shock protein (HSP) 70 and HSP90, glucocorticoid receptor (NR3C1), and Caspase 6 (CASP6) in the liver of two chicken strains: Red JA Cou Nu Hubbard (CN) and Ross 508 Aviagen (RO). A total of 120 RO and 120 CN broilers, 4 weeks of age, were divided into 4 groups of 60 animals, which were then reared for 4 weeks at two different environmental temperatures: 34°C and 22°C. Corticosterone (CORT) concentration was measured in blood samples using enzyme immunoassay method. We also analyzed the association between transcriptomic response and specific SNPs in each genetic strain of chickens. The results demonstrated that there was both a genotype and a temperature effect on the chickens' growth, given that a different trend was seen in the four combinations genetic strain-environmental temperature from the 6th week to the end of the experiment. Chronic heat stress caused a significant increase in CORT levels due to activation of the hypothalamic-pituitary-adrenal axis in both genetic strains. Carcass yield at slaughter were similar in all groups, ranging from 86.5 to 88.6%, whereas carcass weight was negatively influenced by heat stress in both breeds. Heat stress affected the expression of quite all target genes and the differences found in the mRNA copies of two (CASP6 and HSP70) out of the six genes could be partly explained by SNPs. In particular, we found three SNPs for the HSP70 gene (HSP70-3, -7, -9) with different allelic frequencies between the two target breeds, and a SNP polymorphism in the CASP6 gene (CASP6-9). The evidence given in this study, on gene expression and genome polymorphisms will be useful in the future marker assisted selection of chickens more tolerant to heat stress.

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C-010

Broiler *Pectoralis major* muscle affected by emerging abnormalities: histology and gene expression

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In the last few years, modern chicken hybrids selected for an increased breast muscle weight exhibited new breast muscle myopathies, termed "wooden breast" and "white-striping", which impair product appearance and quality properties. This study is aimed to preliminarily analyse the genomic basis of wooden breast and white striping (WB/WS) abnormalities using Affymetrix expression array. For this purpose, 10 normal (NORM) and 10 WB/WS Pectoralis major muscles were obtained from the same flock of heavy broilers (males, slaughter weight of 3.8kg) slaughtered on a single day under commercial conditions. Furthermore, a second sample was collected for histological analysis to be stained with Masson's trichrome. Comparing the expression profiles obtained for WB/WS and NORM samples, 226 differentially expressed genes (DEG) were found, 114 up- and 112 down-regulated. A functional classification was carried out using DAVID tools. The significant ($P<0.05$, Benjamini corrected) functional categories represented by the up-regulated genes are: regulation of organismal growth and cellular developmental processes related to skeletal muscle, activation of polysaccharide metabolism (extra cellular matrix and collagen), calcium and sodium metabolism. The down-regulated genes did not show any specific clustering. The histological analysis revealed the presence on WB/WS samples of an intense proliferation of the perimysial collagen (fibrosis) in respect to the muscular fibers that appear decreased both in number and in diameter and, in some cases, degenerate (necrosis). In many cases, these degenerative lesions were accompanied by attempts of muscle tissue to replace the necrotic fibers with adipose tissue manifested with an abnormal increase of intramuscular fat. On the whole, these functions seem to indicate the alteration of several cellular

processes and/or activation/regulation of actions aimed to attempt to repair the enormous damage of the muscles. However, these attempts cannot prevent the disaggregation of the muscle tissue because our analyses revealed a strong compromise of the component of the extracellular matrix. Furthermore, the analysis of the DEG reveals an alteration of the ion homeostasis, in particular calcium and sodium balance. These results are in agreement with chemical composition modifications found on the same muscle samples as described by Petracci et al. in a parallel abstract.

C-011

Transcript quantification of intestinal neutral amino acids and oligopeptide transporters in European sea bass (*Dicentrarchus labrax*) reared in fresh water and fed fish and plant protein sources

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Oligopeptides and free amino acids are absorbed along the intestinal tract by specialized membrane transporter proteins. Di- and tri-peptides are transported via the H^+/coupled peptide transporter (PEPT1), which is located in the brush border membrane of intestinal epithelial cells. Free amino acids are absorbed by a variety of Na^+-dependent and independent membrane transporters, frequently referred to as "systems". Among the amino acid transporting proteins, the neutral amino acid transporter *SLC6A19,* also called system B(0) neutral amino acid transporter AT1 (B0AT1), is an integral plasma membrane protein responsible for the uptake of a broad range of neutral amino acids across the apical membrane of enterocytes and renal cells. In humans, the B0 system is associated with a severe neutral aminoaciduria known as Hartnup disorder. The objective of the present study was to examine the effect of diets with descending fish meal (FM) inclusion levels and the addition of salt to the diet containing the lowest FM level on growth performances, food conversion ratio, and intestinal SLC6A19 and PEPT1 transcript levels, in freshwater adapted European sea bass (*Dicentrarchus labrax*). We have firstly isolated by molecular cloning and sequencing a full-length cDNA representing the neutral aminoacid transporter SLC6A19 in sea bass. The cDNA sequence was deposited in GenBank database (accession no. KC812315). The 12 transmembrane domains and the *de novo* prediction of the three-dimensional structure of SLC6A19 protein (634 amino acids) are presented. We then analysed diet-induced changes in the mRNA copies of SLC6A19 and PEPT1 genes in different portions of sea bass intestine using real-time RT-PCR. Sea bass were fed for 6 weeks on different diets, with ascending levels of fat or descending levels of FM, which was replaced with vegetable meal. The salt-enriched diet was prepared by adding 3% NaCl to the diet containing 10% FM. SLC6A19 mRNAs in anterior and posterior intestine of sea bass were not modulated by dietary protein sources and salt supplementation. Conversely, including salt in the diet containing low FM percentage, upregulated the mRNA copies of PEPT1 in hindgut. Fish growth correlated positively with the content of FM in the diets. Interestingly, the addition of salt to the diet containing 10% FM improved food intake, as well as specific growth rate and food conversion ratio.

C-012

Dietary methionine in salmonid fish feed alters the expression of genes involved in methionine metabolism

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Methionine (Met) is a source of sulphur required for the synthesis of cysteine and taurine as well as an essential methyl-donor in cellular metabolism. The objective of the present study was to investigate the effect of dietary Met concentration and alternating feeding strategies in Met delivery, on the mRNA transcript levels of genes involved in Met resynthesis (betaine-homocysteine methyltransferase, BHMT; S-adenosylhomocysteine hydrolase, SAHH) and net Met loss (taurine synthesis) (cystathionine beta-synthase, CBS) in Atlantic salmon (Salmo salar) liver. Salmon alevins (265±3 mg) were distributed into 24 tanks (50 fish per tank; 3 replicates). The experimental diets were supplemented with L-Met at 0, 1.9, 5.8, and 17.4 g/kg (M0, M1/3, M1, and M3, respectively). The M3 diet without glycine was prepared to examine Met toxicity (M3-G). These diets were provided via 'mono-feeding strategy' meaning fish were fed a designated single diet. This experiment also included alternative feeding groups with 'duo-feeding' strategy: AF1 (fish fed M0 for 2 days followed by M1 for 1 day), AF2 (fish fed M0 for 2 days followed by M3 for 1 day), and AF3 (fish fed two meals of M0 followed by one meal of M3). Salmon fed M0 diet had smaller weight compared to all other groups. There was no effect of alternative feeding on the growth except for the M0 group. The highest expression of CBS gene was found in the M0, M1/3, and AF-1 groups compared to M3 and M3-G groups. The expression of CBS gene in the M1 group was lower compared to the AF-1 group. The expression of SAHH gene was the highest in the M3 group compared to M1, M1/3, and AF-3 groups. The highest expression of BHMT gene was found in the M0, M1/3, and AF-1 groups indicating enhanced re-methylation of homocysteine by betaine to Met. The lowest BHMT expression in M1, M3, M3-G, AF-2, and AF-3 groups compared to other treatments can be indicative of downregulation of remethylation in the liver. In conclusion, the present study found that dietary Met can directly affect the expression of genes involved in its hepatic metabolism. Although the alternate provision of Met in a form of combination of low and high Met containing diets seems to support its efficient utilization in the salmon liver, there is no evidence that this strategy will be compensating enough in the case of inadequate Met levels.

C-013

Identification of breed informative single nucleotide polymorphisms for assigning individual in Sicilian dairy sheep

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Assignment tests using genetic information to establish population membership of individuals, provide the most direct methods to determine the population of origin of unknown individuals. The identification of the breed or population of origin of individuals potentially offers unbiased tools in livestock and is useful in a variety of biological contexts. The aim of this study was to identify the minimum number of informative SNPs from high-throughput genotyping data in Sicilian dairy sheep breeds, and to investigate their usefulness for breed assignment purposes. Individuals of Valle del Belice (48), Comisana (48) and Pinzirita (53) sheep breeds were genotyped using Illumina OvineSNP50K Genotyping BeadChip. We used as input data the normalized log R Ratios, instead of the genotypic data, in order to work with numerical variables, analytically more treatable. A mixed strategy, using Regularized K-means, Penalized Multinomial Regression and Linear Discriminant Analysis, was applied. The method of Regularized K-means reduced the number of useful SNPs at 1,738. On a reduced set of SNPs, the Penalized Multinomial Regression has been performed, with a selection of 100 SNPs. Further, a classical Linear Discriminant Analysis has been applied to reduce again the number of SNPs, obtaining a set of 40 markers. The study demonstrated that the combination of these methods was able to efficiently distinguish the individuals of the breeds involved. In fact, using a leave-one-out cross validation technique, this reduced set of SNPs classified correctly 100% of individuals to their breeds of origin. Regularized K-means and Penalized Multinomial Regression perform selection through an L1 penalization, while the third step, Linear Discriminant Analysis, provides a further selection with classical techniques. The results laid the basis for the potential use of these selected SNPs to generate a panel that may be used for assignment of parentage or within an industrial setting for tracing the origin of animal products derived from the three breeds involved in the study.

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C-014

Multivariate factor analysis of milk composition, coagulation properties and individual cheese yield in Sarda sheep

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Sarda dairy breed represents about 60% of the Italian dairy sheep stock. All the milk produced is used for cheese making. Milk technological properties are therefore of great interest as potential breeding goals. Several variables contribute to define the technological properties of milk and their interpretation is difficult due to the high correlation among them. Milk coagulation properties (MCP) are considered valid indicators of cheese yield and are measured as rennet coagulation time (RCT, min), curd firming time (k20, min) and curd firmness (a30, mm). Cheese making ability can be assessed also by individual cheese micro-manufacturing experiments (ILCY). The aim of this work is to study the relationship pattern between milk composition, MCP, and ILCY with multivariate factor analysis in order to derive new indices of milk technological properties. Individual milk samples were collected from 1018 Sarda ewes farmed 47 flocks in the period April to July 2014. Chemical composition were determined by MilkoScan, MCP were measured using Formagraph, and cheese yield was assessed by ILCY. Factor analysis extracted five latent factors able to explain about 75% of the original variance. The first, which explained about 30% of the total covariance, was associated with ILCY, fat and protein content, casein content, fatty acid composition. It could be defined as “cheese yield” indicator. The second latent factor explained about 20% of the variance and it was associated with fatty acids and somatic cells and could be defined as “quality”. The third showed the largest loadings for RCT and a30; it can be interpreted as a “coagulation” indicator. Lactose and cryoscopy index were associated with the fourth common factor, that has been interpreted as an index of “diluition”. Finally, the last factor was termed "syneresis" because it was linked with k20 and NaCl. Multivariate factor analysis was able to decipher the complex relationship pattern that exists among milk quality variables and MCP. Factors with a clear technical meaning have been extracted. These new variables could be used as novel phenotypes of milk technological properties for management and breeding purposes.

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C-015

Polymorphisms at β-defensin genes and mastitis in Valle del Belice dairy sheep: a case control study

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Mastitis is the most common and costly pathology affecting dairy animals. The identification of genetic markers that allow the inclusion of mastitis resistance in selection programs would help to reduce the costs due to the disease and the use of antibiotics. In recent years, β-defensin genes have been studied in several domestic species due to their important role in the innate immune response. The aim of this study was to determine whether β-defensin genes variability, β-defensin 1 (SBD1) and β-defensin 2 (SBD2), could distinguish between infected cases and healthy controls in Valle del Belice dairy sheep. Somatic cell count (SCC) is a powerful tool to identify animals with intramammary infection. Ewes were classified as affected by mastitis due to major (MAJP) and minor pathogens (MINP) within a lactation, based on having more than 645x103 or 2138x103 somatic cells, respectively, as reported in a previous study. Two single nucleotide polymorphisms (SNPs) in the SBD1 and five SNPs in the SBD2 genes were genotyped in 300 individuals by PCR and Single Nucleotide Primer Extension assay. All ewes had SCC test day registration. Association analysis were performed using the Case Control procedure in SAS 9.2, with the overall association with genotype based on the Armitage trend test and odd ratios (OR) based on allele counts, reflecting additive effects. Frequency differences indicated an association of the SNPs with the disease. On the basis of minor allele frequency for control group, both SNPs of SBD1 (A/G 1747 and T/C 1757) were associated with MAJP ($P<0.05$, OR=1.26, CI=1.08-1.48 and $P<0.05$, OR=0.79, CI=0.68-0.92, respectively) and MINP ($P<0.001$, OR=1.45, CI=1.28-1.64 and $P<0.001$, OR=0.69, CI=0.61-0.78, respectively). Only one SNP in SBD2 (G/A 1659) was simultaneously associated with MAJP ($P<0.001$, OR=0.79, CI=0.71-0.88) and MINP ($P<0.001$, OR=0.81, CI=0.74-0.89). Moreover, one SNP in SBD2 (G/A 1761) was associated with MINP ($P<0.05$, OR=0.81, CI=0.69-0.95). Association analysis between SBD1 and SBD2 genotypes and SCC showed that the investigated SNPs were associated with increased risk or resistance to intramammary infection.

C-016

Strategies to improve full genome sequences for livestock species

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In recent years, technological advances have made it possible to construct the entire genome sequences of mammalian species with relatively low cost. The two major advances that have facilitated this were both sequencers capable of producing several Giga bases per day and bioinformatic tools, mostly based on De Brujin graphs, that allow the joining short sequences of around 100 bp into larger contigs and even larger scaffolds. However, the process often results in thousands of scaffolds and are not easily assigned to specific chromosomes. In livestock species availability of genome sequences has produced spectacular advances, and in particular the development of Genomic Selection as seen for the larger dairy breeds. The use of SNP chips is often seen as soon as the first draft of the genome is completed for a species, and when the genome is still a set of un-annotated scaffolds. Here, we present a strategy to take advantage of SNP chip data to improve the genome sequence, by increasing the size of the draft scaffolding. In addition we use other tools to assign a large part of the genome to chromosomes. We applied this strategy to the buffalo genome sequence. The draft assembly of the buffalo genome was created from 70X Illumina mate-paired sequence and 1.5X Roche 454 long paired end sequence assembled using the MaSuRCA genome assembler. This draft sequence is 2,836

Mbp fragmented into 366,983 contigs with N50 of 1,412,388. Resequencing of diverse breeds of river and swamp buffalo has identified over 16M SNPs and a 90K SNP genotyping panel has been designed and tested on more than 1,000 Italian mediterranean river buffalo. In order to anchor the buffalo scaffolds on the respective chromosomes, 2621 probes typed on a 5000 Rad buffalo/hamster panel were BLASTed on the 366K scaffold set. Afterwards, to build a comparative map, the same scaffolds were BLASTed against the Bos taurus UMD 3.1 genome sequence. The mapped scaffolds were joined with neighbouring scaffolds that overlapped and had 50 overlapping bases. The relative positions of scaffolds that abutted on the bovine sequence, but without overlap was verified by computed the linkage disequilibrium of each SNP versus each other, retaining the pairs with correlation greater than 0.6. Following this strategy we were able to assign further scaffolds to specific buffalo chromosomes. Such that we were able to assign much more than half of the sequenced genome to specific chromosomes with a reasonable position.

C-017

Patterns of diversity in swamp and river buffalo as revealed by SNP molecular markers

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After sequencing the Buffalo genome, the International Buffalo Consortium has used the derived 90K Affymetrix Axiom® Buffalo Genotyping Array to characterize a set of river buffaloes from Pakistan, Iran, Turkey, Egypt, Romania, Bulgaria, Italy, Mozambique, Brazil and Colombia, and swamp buffaloes from China, Thailand, Philippines, Indonesia and Brazil. SNP genotype data have been analyzed with Bayesian clustering algorithms (ADMIXTURE software), multivariate statistics (Multi-Dimensional Scaling plots) and graph tools highlighting population splits and migration events (TREEMIX software) to estimate the levels of molecular diversity, population structure and the historical relationships among populations. When placed in the geographical context, the patterns of diversity confirmed archeozoological evidence for the domestication of river and swamp buffalo in the Indian subcontinent and in Southeast Asia, respectively, and provided hints on buffalo migrations and history.

Admixture analysis revealed the presence of three main gene pools in pure river buffalo populations: one characterizes the breeds from the Indian subcontinent (Pakistan) and those recently exported from there to Bulgaria, Brazil and Colombia; the second includes breeds that, from the domestication center, spread westwards to Iran, Egypt and Turkey; the third includes the Italian Mediterranean buffalo, sampled both in Italy and in Mozambique where it has recently been exported. Among the breeds analysed, Mediterranean buffalo represents the most differentiated river buffalo gene pool, which is most likely due to genetic bottlenecks, isolation, selection and possible genetic contributions from breeds not included in our sampling. Four gene pools have been identified in pure swamp buffalo populations: the first from China; the second in Indonesian islands, other than Sumatra; the third in the Philippines and the fourth in Thailand and Sumatra. Some level of admixture is seen between river and swamp buffalo in the Philippines and in Brazil. TREEMIX software analyses confirmed the gene flows identified by Bayesian population structure analysis including those from the river buffalo gene pool to the admixed swamp populations and, within river buffaloes, from the Mediterranean to the breeds from Colombia and Brazil. Furthermore, these analyses revealed some unexpected migration patterns, which suggest that the westward spread of domestic buffaloes may have followed alternative migration routes.

C-018

Lipid metabolism related gene expression in European sea bass (*Dicentrarchus labrax*): effects of fasting and refeeding

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European sea bass (*Dicentrarchus labrax*) is the second most cultured fish species in the Mediterranean area and there is a great interest to gain better knowledge on nutritional requirements for this species, by exploring not only new feed ingredients and feeding strategies, but also, by understanding in depth the molecular mechanisms regulating feed digestion, utilization and metabolism of nutrients. In this context, transcriptomic analysis represents a useful nutrigenomic discovery tool for identifying the molecular basis of biological responses to nutrition and novel nutritional biomarkers in fish. The objective of the present study was to evaluate how the transcriptional activity of genes controlling lipid metabolism in sea bass are modulated in a tissue-specific manner in response to 15 days of fasting, followed by refeeding. The study focused on liver, white skeletal muscle, brain and mesenteric adipose tissue and included a panel of 30 genes including desaturases, elongases, triacylglycerol lipases, fatty acid binding proteins, β-oxidation and oxidative phosphorylation enzymes, phospholipid-related enzymes and lipid transcription factors. Fasting increased the expression of lipolytic proteins, in particular that of LPL-like and adipose triglyceride lipase (ATGL) in liver and muscle of sea bass. Markers of lipogenesis, such as SCD1b, showed a down-regulation in liver and adipose tissue in response to fasting. Contrariwise, genes involved in synthesis and remodeling of phospholipids (LPCAT2, PEMT, PLA2G12B) were upregulated in liver and muscle of fasted sea bass. The up-regulation of CTP1A and SDHC in response to fasting in both, muscle and liver may indicate an increase mitochondrial β-oxidation of of fatty acids in order to provide energy during fasting. Ten days of refeeding were sufficient to reverse the expression of most part of key genes. In conclusion, the present study clearly showed in European sea bass a tissue-specific regulation of lipid-related genes according to the different metabolic capabilities of each tissue and their vital functions. Our data revealed that brain is highly refractory at the transcriptional level to changes in nutrient and energy availability. In contrast, liver is clearly the most reactive tissue, with changes in gene expression affecting not only the biosynthetic, but also the oxidative and lipolytic machinery.

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C-019

Innovative technologies for the future of animal production

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There is general agreement that the next decades will be characterized by an increasing demand for food for human consumption, caused by a relentless population growth, which can not be met by current levels of productivity and /or by an expansion of arable land. Significant climate changes will also expect and their effects appear not easily predictable. Moreover, the increased demand for food will be differentiated. The progressive urbanization of people living in areas most affected by demographic development and the expected increase of income for large sectors of these populations will lead to an increase in demand for animal products. Therefore, not only the agricultural production systems but also livestock production will inevitably increase both efficiency and environmental sustainability. Three key areas are more expected to be involved in innovation processes: animal breeding, animal nutrition, and innovative technologies applied on livestock farming. Animal breeding can offer a relevant contribution in the development of adaptability, biodiversity and quality of animal products . In animal nutrition area, a reduction of chemicals and additives with proven negative impacts on human health is expected. Regarding the innovative technologies, they can be adapted to the livestock farming in order to achieve a continuous automated real-time monitoring of production, reproduction, health, welfare and environmental impact. In the near future, there will be also the need to new models for spreading innovation in order to ensure high levels of awareness and adoption. Who will manage this change ? In the last years, an increasing number of young people showed interest to study livestock science and its role will became really important in the future for managing innovation.

C-020

A new strategy to increase sheep meat production in Sardinia

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Dairy sheep is a milestone of livestock production in Sardinia. In the traditional farming system, ewes are mated at May-June. On each July-September, i.e. at the end of lactation, about 600-650.000 ewes are culled when they are on the first gestation months. A shift of culling in early winter after the lambing could represent a management strategy for improving the outcome of dairy sheep farms by increasing meat production. A survey has been carried out on farmer's attitude towards this possible management option. Interviews and questionnaires have been carried out in 60 farms between June and October 2014. The questionnaire had different sections regarding information about the general flock management and specific questions on breeding program for culled ewes. Questionnaire data were analyzed to understand the awareness and the perception of the critical issues by farmers. Moreover, 386 culled ewes from 19 farms were inspected after slaughtering by a skilled veterinary and the pregnancy status was determined. Number and age of foetuses were determined by visual assessment and palpation of the exposed uterus and then confirmed by its dissection. Results of questionnaire data indicated that already in about 45% of farms the unproductive ewes are sacrificed in winter along with their last suckling lambs. Moreover, about 1/3 of those farmers are interested in using rams of meat breeds to inseminate their ewes in order to improve lamb meat production. In about 30% of farms ewes, that are going to be culled are not mated, whereas the remaining farmers do not adopt a specific management for culled ewes in breeding season. Data of post mortem inspection revealed that approximately 80% of ewes are pregnant when slaughtered. Of the pregnant ewes, 40% have foetuses of less than two months, whereas in remaining animals the foetuses are between three and four months old. In conclusion, results of this survey confirm that an increase of lamb meat production can be achieved in traditional dairy sheep systems by a proper management of culled ewes for a further lambing.

C-021

The effect of chemical dehairing of pig carcass on sanitation and meat quality

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The aim of the study was to test if chemical dehairing improves hygienic quality of pig carcass without either adverse effects on removal of bristles or on the meat quality. System was applied in a separate area of a commercial abattoir, in an immersion tank for dehair solution, and spray-cabinet for neutralization and water-wash. Conventional dehairing system (scalding at 63°C and gas-flame singe), was used as control. On five different days, the test system was applied on a total of 21 carcasses from pigs selected randomly in a batch delivered by one farm. On each day, at least one carcass from the same batch was collected on the slaughter-line as the control. The treated carcasses were immersed for 5 minutes in a tank holding a solution of sodium hydroxide (Depil B2/C9, Slaughtering, Italy). Tank had a warming system for tests ranging from 35°C to 60°C. Total bacterial, enterobacteriaceae and Escherichia C. counts and level of *Salmonella* were evaluated by swab-samples collected after water washing for the test carcasses and after gas singe for the control group. Dehairing was subjectively assessed by a abattoir expert using a 4 classes scale: unacceptable, slightly acceptable, acceptable and good. After carcass dissection, pH and colour (Lab) were measured at 1 h post mortem on semimembranosus muscle of left ham. Counts data were processed after log transformation by ANOVA. Total bacterial and Enterobacteriaceae counts were affected by the dehairing treatment. Carcasses chemically dehaired showed lower total bacterial counts ($P<0.05$) compared to the control (61 *vs.* 3400 CFU/cm^2). Entrobacteriacee showed a similar trend (<1 *vs.* 14 CFU/cm^2) while E. Coli was < 1 CFU/cm^2 in both carcass groups. Only one occurrence of *S. Thiphimurium* in the control and one of *S. Livingstone* in treated carcasses were recorded. Optimal dehair efficacy was affected by the solution's temperature: at >55°C dehairing evaluated as "good", with improved whiteness of skin colour. Carcasses conventionally dehaired were classified "good". No differences between treated and control carcasses were found for pH and colour measurements. These data indicate that chemical dehairing could be an effective treatment for sanitizing pig carcass and removing bristles without affecting the quality of meat.

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C-022

Image analysis for characterization of fishmeal material in aquafeed

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Most of the methods developed and implemented for processed animal proteins (PAPs) identification have been focused on the feed ban for terrestrial animal, and therefore fish meal characterisation especially in microscopy was limited and mainly narrative. Furthermore, the recent revision of the feedban rules (EC Reg. N 56/2013), which re-authorized PAPs from non-ruminants as feed ingredient in aquaculture, represents a further challenge for feed sector. In this respect, the aim of this study was to investigate the use of microscopy in combination with image analysis (IA) measurements for the characterization of fish bone lacunae in aquafeed-extracted material. For this study, 6 samples of commercial aquafeed containing fish meal were analyzed by the microscopic method (EC Reg. N 152/2009). Sediment fractions of each sample were observed with a compound microscope at X40 (Olympus BX41, Germany). Two hundred fifty nine bone fragment lacunae images were recorded and processed through an IA software. Accordingly, on each lacunae 30 geometric variables grouped in two main families, i.e. size descriptors and derived shape descriptors, have been obtained and measured as previously described. Data obtained have been analyzed by PROC MEANS and BOX-PLOT procedures using SAS statistic software 9.3. A further step in the experiment was to compare data obtained in fish material with avian and mammalian PAP materials, in order to assess possible bone lacunae markers useful in distinguishing these materials. Results obtained indicated that the analyzed samples were indicatively homogenous in term of mean value for all variables/descriptors measured. For this reason 5 descriptors has been selected as representative variables. Within size descriptors, lacunae Area, Axis Major, Axis Minor ranged from 85.79 μm^2 to 107.11 μm^2, 28.85 to 35.02 μm and 3.86 to 5.20 μm, respectively. Aspect and Roundness 2, both derived shape descriptors, ranged from 6.18 to 8.11, and 0.11 to 0.16 respectively. In comparison with avian and mammalian bone lacunae, fish material has shown different absolute bone lacunae values for several size and derived shape descriptors. Therefore, even though a larger dataset is needed for an exhaustive evaluation, it can be concluded that: i) fish material in aquafeed appears quite homogenous in term of bone features; ii) fish meals can be distinguished selecting specific descriptors, from avian and mammalian materials.

C-023

In vitro characterization of proliferation, differentiation and pluripotency markers in hen oviduct epithelial cells which are potential model to study expression of exogenes delivered by nucleofection

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Hens are predicted bioreactors of pharmaceuticals and the oviduct epithelial cells (OEC) serve as model to develop efficient expression vectors of human therapeutic genes. As every system dedicated to routine use, OEC require full biological evaluation.

Here, OEC were assessed in terms of their proliferation, clonogenicity, differentiation and expression of pluripotency markers *in vitro*. Furthermore, the nucleofection was optimized as non–viral method of stable transfection of OEC. TetraSL laying hens (n=6, age 36 wks) were donors of tissue for *in vitro* assays. After passage, OEC were seeded at density of 0,5-2×10^4 on 6-well plates (clonogenicity) and 12-well plates (differentiation) and at density of 1×10^5 on 24-well plates (nucleofection). Clonogenicity and differentiation were assessed after incubation of OEC in DMEM:F12+10% FBS and Promocell mesenchymal stem cell media for 14d. Proliferation and expression of markers CD34,44,45,90 were measured by XCelligence system and FC. To optimize nucleofection, OEC were transfected with pmaxGFP in P1 and P3 Nucleofection solutions (Lonza) and treated with 7 different Nucleofector programs +control (no program) in 16-well strips in Nucleofector X-Unit device. As a result nor the cells sourced from infundibulum of oviduct, neither from distal or proximal sections of magnum exhibited clonogenic potential or stem cell markers. Proliferation rate of OEC was characterized by a conspicuous adaptive phase (30-40h), long proliferation phase (100- 200h) and short plateau (20h) followed by descent of proliferation. Highly different viability and transfection efficiency were noted at 6-72h post nucleofection depending on the P1 or P3 solution and program used. The highest transfection rate 58-90% combined with >70% viability was obtained with P3 solution and program EL-110 or EC-104. High transfection rate of 69% was also noted for P1 solution however it was at expense of low 31% viability. In summary, OEC sourced from distal 5 cm section of oviduct, cultured up to first passage are suitable for routine testing of expression vectors. The recommended program to further test of human expression vectors in hen oviduct cells is EC-104 or EL-110 with P3, which is also recommended for nucleofection of Human small airway and mammary epithelial cells. Hen oviduct cells show no mesenchymal character, however it was confirmed for the respective distal sections of fallopian tube in mammal studies.

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C-024

A human stunning and killing method applied to marine aquatic species

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In recent years, public opinion has devoted increasing attention to welfare issues in the fields of fisheries and fish production. Furthermore, several public institutions (EFSA, FAO) have so far highlighted the possible negative effects of traditional killing methods on fish and crustacean welfare and quality parameters. The aim of the study was to compare the effects of traditional killing and new (stunning and killing) methods in two marine species (Sea bass, Dicentrarcus labrax and American lobster, Homarus americanus). All the experiments were carried out in the facilities of the Department of Food Science, University of Udine. In the first experiment, 27 adult Homarus americanus males (initial mean weight: 537 g) were reared for four weeks in four tanks (0.56 m^2) in a recirculating system. Animals were slaughtered using three different methods (nine animals for each group): 1– Splitting: cutting though the animals' midline; 2–Chilling in ice for 20 minutes and cutting though the animals' midline; 3 – Electric stunning: animals were subjected to a electric current (50 V, 50 Hz, direct current; Fishkill EG100, Scubla s.r.l., Remanzacco, Italy) for 10s in marine water (18 ‰ salinity) and cutting though the animals' midline. From each animal about 50g of muscle was sampled for pH (pH meter) and proximate analysis (AOAC). Homarus americanus treated with the electric stunning method showed the lower pH muscle values compared to the other two methods. American lobster can be effectively stunned by the electric current and than cut though the animals' midline. In the second experiment, sixty European sea bass (*Dicentrarchus labrax*) (344±57 g) were maintained in cubic fibreglass tanks (200-L), in a recirculating system, before the start of the experiment. Fish were fed daily with a commercial diet and feed was withdrawn 24h before the start of the stunning/slaughtering experiment. Before slaughtering, all fish were exposed to an electric current (50 V, 50 Hz, direct current; Fishkill EG100, Scubla s.r.l., Remanzacco, Italy) for 5s in marine water (30‰ salinity). Two slaughtering methods were compared: immersion in water/ice slurry (ratio 1:1) and spiking (Ikigun, Adept Ltd, New Zealand) (nine fish for each treatment). All fillets were measured for pre rigor and rigor mortis, pH, color and texture properties for 72h. Apparent viscosity and water muscle holding capacity were lower in sea bass slaughtered in water/ice compared with the spiking treatment. pH values were not significantly different between the two procedures. Sea bass can be effectively stunned by the electric current (immediate loss of consciousness) and than killed by spiking.

C-025

Cheese chemical and physical properties predicted by Vis-NIR reflectance and NIR transmittance spectrometry

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Italian dairy industry is characterized by high-quality cheese productions appreciated worldwide. The interest of cheese manufacturers is focused on monitoring the products quality by using cheap, rapid, non-destructive techniques. The aim of this study was to compare visible and near infrared (Vis-NIR, 350-1830 nm) reflectance and NIR (850-1050 nm) transmittance spectrometry on predictive performance for cheese chemical and physical traits. A total of 685 samples of different cheeses belonging to 36 categories were collected at the 2012 and 2013 *Caseus Veneti* contest. A spectral acquisition was carried out by using a portable Vis-NIR (LabSpec 2500, ASD Inc., Boulder, CO, USA) device and a lab instrument (FoodScan, Foss Electric A/S, Hillerv√∏d, Denmark). Physical traits (color: L*, a*, b*, C* and h; maximum shear force) and pH were measured on all samples, while chemical components (moisture, protein, fat, ash and soluble nitrogen) were analysed following the official reference methods on 197 cheeses selected according to their spectra variability. A bayesian approach was implemented to develop calibration models by using the BGLR (Bayesian Generalized Linear Regression) package of R software (R Core Team, 2013). Three different calibration procedures were adopted: i) the entire data set and the full spectrum were used, without any external validation; ii) the full spectrum and a validation procedure, in which 80% of the samples was used for calibration and the remaining 20% for validation, was used; iii) procedure ii) was applied on the following portion of the Vis-NIR spectra: 350-850 nm, 850-1050 nm and 1050-1830 nm. Moreover, a separate validation set was established using only Grana Padano cheeses. The NIR transmittance spectrometry (R2VAL 0.01 to 0.88 for different traits) was always less efficient than Vis-NIR reflectance using the whole spectrum (from 0.48 to 0.96), and also using the tested portions of the spectrum (from 0.03 to 0.97). In the case of calibrations using the entire spectrum, correlations between measured *vs.* predicted values in validation resulted: near or higher than 95% for chemical components, excluded ash and soluble nitrogen; higher than 84% and 70% for colour and shear force traits, respectively, while pH showed lower values (<70%). Similar results were obtained on validations referred specifically to Grana Padano cheese. These results showed the feasibility of using Vis-NIR spectrometry as an efficient non-destructive, inexpensive technique to monitor cheese quality directly in the dairies on cut cheese surface without sample up taking.

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C-026

Influence of multiple injections of vitamin E on carcass characteristics and meat quality of Laticauda lambs

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The effect of intramuscular (i.m.) injections of vitamin E on carcass characteristics and meat quality were investigated on 24 Laticauda male lambs. Lambs were divided into 2 groups of 12 animals homogeneous for weight: control (C) or vitamin E-treated (V). From the beginning of the study (15 days of age) until day 57 of age, lambs of the V group received, weekly, i.m. injections of DL-α-tocopheryl acetate (left gluteus) in aqueous solution (Vitalene® E, Fatro, Bologna) for a total dose of 1,500 IU. C group received injections of physiological saline. Lambs received maternal milk and a supplementation of hay and commercial concentrate (from the 28th day of age to slaughter) and were housed in 6 pens (4 animals/pen). To calculate weight gain, lambs were individually weighed at 15 days of age and at slaughter (64 days of age). Hot and cold carcass weights were recorded and dressing percentages were calculated after dressing and chilling at 2–4°C for 24 h. Carcass shrink losses were expressed as a percentage of hot carcass weight. From the right side of the carcass, the pelvic limb was removed and its percentage was calculated based on cold carcass weight. *Longissimus Dorsi* (LD) muscle was removed for the determination of the fatty acid (FA) composition, cholesterol and α-tocopherol content. Data were analysed by one-way analysis of variance (ANOVA). Vitamin E did not affect significantly slaughter weight, carcass weights, dressing percentages and carcass shrink losses, while increased pelvic limb percentage ($P<0.05$). The α-tocopherol content of LD muscle was higher for V lambs ($P<0.01$). SFA and MUFA contents were similar between groups, PUPA was higher ($P<0.01$) in V group. For the proportion of single SFA, only heptadecanoic acid (C17:0) was lower ($P<0.05$) in V group. Among the PUFA, lambs from V group had higher α-linolenic acid (C18:3 n-3), docosadienoic acid (C22:2) ($P<0.01$), eicosapentaenoic acid (EPA, C20:5 n-3), docosapentaenoic acid (DPA, C22:5 n-3) ($P<0.05$) and docosahexaenoic acid (DHA, C22:6 n-3) ($P<0.06$). The V group showed a higher ($P<0.05$) total n-3 FA content and lower ($P<0.01$) n-6/n-3 FA ratio and thrombogenic index. The cholesterol content was similar between groups. This study has shown that DL-α-tocopheryl acetate increases pelvic limb percentage and muscle α-tocopherol content, and affects positively the FA acid profile.

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C-027

Organic Bilberry pomace in rabbit nutrition: effect on meat quality and fatty acid profile

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To evaluate the effect of Bilberry pomace (BP) diet supplementation on rabbit meat quality, 144 Grimaud weaned rabbits were divided into 4 groups of 36 animals and fed *ad libitum* with a basal diet (BP0, 177g CP and 17.9 MJ/kg GE) tested against three assay diets developed by substituting 50, 100 and 150 g/kg of the BP0 diet with BP (BP5, BP10 and BP15, respectively). At 84 days of age, 12 rabbits per diet were chosen and slaughtered without fasting. Proximate composition, colour, cooking losses and fatty acid (FA) profile of *Longissimus Dorsi* (LD) muscle were evaluated. Meat lipid oxidation was studied at 1, 4 and 7 days of refrigerated storage and at 30 and 60 days of frozen storage, by Thiobarbituric Acid-Reactive Substances (TBARS) assay. All data were analyzed by One-way ANOVA and differences of means by Duncan's test. Significance was set at $P \leq 0.05$. Chemical composition and meat quality traits were not affected by BP supplementation in diet with the exception of redness for BP10 group which reported the lowest ($P=0.001$) value (-0.41). Regarding FA profile, α linolenic acid (C18:3n3) content significantly ($P<0.001$) increased with the increase of BP inclusion level (from 2.16% to 8.79%). Dietary treatment significantly ($P=0.02$) affected TBARS values of refrigerated meat with the highest value reported for BP15 group. No significant effects were reported for frozen meat. In conclusion, the results suggest that BP supplementation improve the fatty acid profile of rabbit meat with a negative effect reported in LD oxidation for BP supplementation level higher than 10 %.

C-028

Effects of diets containing linseed oil and supplemented with grain amaranth (*Amaranthus caudatus*) on growth performances, oxidative status and serum biochemical parameters in broilers

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The high fat content of the diet contributes to the pathogenesis of the most common human chronic diseases. The major nutritional causes of these metabolic alterations and damage to the body have always been considered the saturated fatty acids. Therefore, nutritionists suggest to replace in part the saturated fats with polyunsaturated fatty acids (PUFAs). However, PUFAs are subject to lipid peroxidation, in the absence of antioxidant protection. The objective of this work was to evaluate the effects of supplementing with different levels of grain amaranth (*Amaranthus caudatus*) on the growth performances, oxidative status and serum biochemical parameters in broilers fed diets rich in linseed oil. A total of 132 female broilers were randomly divided into three equal groups (44 broilers/group, 11 broilers/cage, 4 cages/treatment). Animals were fed for 5 weeks with three diets containing 5% linseed oil and supplemented with 0%, 5% and 10% of amaranth grain, respectively. To allow the assessment of oxidative status was measured the level of serum lipid peroxidation and the level of serum antioxidant capacity using LP-cholox test and anti-ROMs test (Diacron, s.r.l., Grosseto, Italy), respectively. The following biochemical serum parameters were also determined: triglycerides, cholesterol, alanine amino transferase (ALT) and albumin. Our results showed a decrease on broilers growth performances with increasing ($P<0.05$) amaranth inclusion level. Broilers fed diets containing 10% and 5% amaranth grain had a significantly higher serum antioxidant power compared to the control group (250 and 219 μequivalents/l against 177 μequivalents/l, respectively), and a lower level of serum lipid peroxidation compared to the control group (262 and 419 μmoles/l against 700 μmoles/l, respectively). Furthermore, the amaranth grain supplementation had a significant decrease ($P<0.05$) on cholesterol and triglycerides levels in comparison to broilers fed diet without amaranth grain. No differences were found for ALT and albumin levels among the three groups. In conclusion, a diet rich in PUFAs supplemented with amaranth grain determines a reduction of growth performances, but it may be effective in decreasing serum lipid peroxidation and in improving antioxidant defenses and some serum biochemical parameters in broilers.

C-029

Carcass traits and meat quality in Italian Heavy Draught Horse foals fed two diets differing in protein levels and slaughtered at 13 or 18 months

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The present study aimed at evaluating carcass traits and meat quality in Italian Heavy Draught Horse (IHDH) foals, fed 2 isoenergetic diets differing in crude protein (CP) levels up to the 2 typical slaughter ages (SA). Forty-one foals were grouped in 8 pens according to sex, age, and BW up to 13 (1st period) or 18 mo. of age (2nd period). Eighteen foals were fed a low protein (LP) diet (10.6 and 11.2% CP on DM in 1st and 2nd period, resp.) and slaughtered at 13 (3 F and 7 M) or 18 mo. (3 F and 5 M). Other 23 foals were fed a high protein (HP) diet (13.2 and 14.7% CP on DMin 1st and 2nd period, resp.) and slaughtered at 13 (4 F and 8 M) or 18 mo. (3 F and 8 M). The final BW of foals slaughtered at 13 and 18 mo. was 505±15 and 599±16 kg, resp. After slaughter, the 7th-8th ribs were cut from the left carcass side and *Longissimus Dorsi* (LD) dissected for further analyses. Drip losses were quantified on dissected cuts and physical parameters and chemical compositions were determined on LD. Data were analysed via GLM including diet (D), slaughter age (SA), sex (S) and their interaction effects. Results showed no significant effects of the diet on carcass traits. Conversely, the SA affected significantly cold carcass weight (302 *vs.* 375 kg; $P<0.01$) and dressing percentage (60.7 *vs.* 62.5%; $P\leq0.01$). Among carcass traits, only tight thickness was affected by S ($P<0.05$). As expected, the effect that exerted greater magnitude on meat quality was the SA. At a SA of 18 mo., meat resulted less bright than at 13 mo. (34.2 *vs.* 31.0, resp.; $P<0.01$), and a* and b* indexes resulted increased with age (11.5 *vs.* 19.7; $P<0.01$ and 12.2 *vs.* 18.9; $P<0.01$, for SA of 13 and 18 mo., resp.). Accordingly, the heme iron in meat was greater in older than in younger foals (1.70 *vs.* 1.25 mg/100g; $P<0.01$), but cholesterol was greater in foals slaughtered at 13 than at 18 mo. (70.4 *vs.* 43.0 mg/100g, resp.; $P<0.001$). Significant differences in LD measures between F and M were found in the cooking losses (32.3% *vs.* 33.6%; $P<0.01$), in shear force (3.02 *vs.* 3.94 kg/cm^2; $P<0.01$), and in the fat content (6.07 *vs.* 4.57 g/100g; $P<0.05$). Small interaction effects were detected for all analysed variables. In conclusion, a moderate reduction of CP in the diet of IHDH foals produced no differences in carcass traits and in the meat quality, either considering different age at slaughter or different sex.

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C-030

Sensory acceptability of rabbit meat patties manufactured with increasing levels of rooibos (*Aspalathus linearis*) tea extract

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Rooibos (*Aspalathus linearis*) tea extract is a South African leguminous shrub. Recently, it exhibited interesting potential to increase the shelf-life of meat and meat products mainly by lowering their lipid oxidation rate. However, sensory acceptability of the final product still needs to be evaluated. The study tested the sensory attributes and acceptability of rabbit meat patties manufactured with increasing levels of fermented rooibos tea extract. With this purpose 16 kg of rabbit meat were used. Meat was divided in four batches, and manually mixed with different rooibos inclusion levels: Control (0%), R1 (0.5%), R2 (1%) and R3 (2%). Subsequently, meat patties were stored under refrigerated conditions (+4°C) until cooking in a water bath for 25 min at 80 °C (n=20 patties/treatment). Afterwards, an eight members trained panel carried out a ranking test during which each sample was evaluated by two different assessors. Ranked sensory attributes data were computed with a Friedman's two-way non-parametric ANOVA. The type and frequency of perceived off-flavours were evaluated by a chi-square test. As expected, increasing levels of rooibos extract determined a greater coloration of rabbit meat patties ($P<0.001$) which adversely affected general visual acceptability of the product when rooibos inclusion level exceeded 1% (13.6 and 15.1 *vs.* 8.7 and 5.5 for C, R1, R2 and R3, respectively; $P<0.001$). Higher rooibos percentages raised aroma intensity as well as rooibos aroma perception and lowered rabbit aroma (6.5 *vs.* 17.5 for R1 and R3, respectively; $P<0.0001$). The 1% and 2% rooibos inclusion level also reduced aroma general acceptability (14.7 and 15.2 *vs.* 9.3 and 3.8 for C, R1, R2 and R3, respectively; $P<0.001$) with rooibos presence being identified as an off-flavour. This was confirmed also by the frequency of perceived off-flavours which exhibited greater percentages for R2 and R3 treatments compared to C and R1 groups which showed similar scores. This was particularly true for rancid ($P<0.001$), rooibos ($P<0.0001$) and acid ($P<0.05$) off-flavours. Rooibos inclusion reduced the juiciness of rabbit meat patties when its incorporation exceeded 0.5% ($P<0.001$). Interestingly, a rooibos inclusion of 0.5% guaranteed the same general product acceptability of the C rabbit meat patties, thus confirming the potentiality of this plant to be successfully used also in the meat sector as a natural additive.

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C-031

No detection of transgenic DNA (tDNA) in tissues from rabbits fed with genetically modified soy-bean meal

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Several studies investigated the presence of DNA from transgenic plants in different animal species but very controversial results are reported. Some studies did not identify any tDNA in tissues from animals fed with transgenic plants, in others tDNA has been found in tissues from different species. The study was aimed at detecting the presence of tDNA in tissues from rabbits fed with genetically modified (GM) soybean meal (RRS, line GTS-40-3-2). Ten New Zealand White male rabbits, coming from a herd where GM soybean is commonly used, were utilized. The animals were slaughtered at 80±5 d of age and at 3.0±0.2 kg of body weight in a slaughterhouse. Blood was withdrawn before slaughtering and samples of liver, kidney, heart, stomach, intestine, muscle and adipose tissue were collected from each rabbit. Samples were collected avoiding environmental contamination. Samples of hair and stomach content were collected to evaluate the possible presence of tDNA. Samples of mixed feed containing GM soybean were collected as positive control. From rabbit tissues, the genomic DNA was extracted by using the Isolate II Genomic DNA Kit (Bioline). Genomic DNA from feeds, stomach content and hair, was extracted by using the Plant/Fungi DNA isolation Kit (Norgen Biotek. Corp). A Picogreen dsDNA Kit (Life technologies) was utilized to quantify dsDNA following purification. All samples were monitored, by using the real-time polymerase chain reaction (rt PCR), for oligonucleotide primers and probes specific for the inserts of RRS event GTS 40-3-2 and for the lectin gene reference to generate amplicon sizes of <100 bp. The β-actin gene was used as positive control for rabbit tissues, tDNA of soybean was not detected in all rabbit tissue samples except in the samples of feed, stomach content and hair. Similar results were obtained for the gene reference of lectin. The β-actin gene was detected in all rabbit tissue samples. The absence of soybean tDNA in tissues from rabbits found in the present study represents an important result indicating that feeding animals with GM soy is not risky for animal and human health. The detection of soybean tDNA in rabbit hair, compatible with an environmental contamination, suggests the need to do the maximum attention during samples collection to avoid wrong findings.

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C-032

Antioxidant mixture supplementation in the medium-heavy pigs: effects on performances and shelf life of *Longissimus Dorsi* muscle

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The improvement of quality and shelf life of meat has received great attention in the production chain. Moreover, the relationship between diet and health has modified in consumer habits, demanding healthier products. Due to concerns about the synthetic antioxidants safety, meat products containing natural antioxidants are needed to improve the consumers' acceptability. A previous study showed that natural antioxidant supplementation in pig enhanced meat vitamin E content. The aim of the study was to investigate the effects of dietary supplementation with antioxidant mixture in medium-heavy swine on growth performances, oxidative status and shelf life of *Longissimus Dorsi* (LD) muscle. Seventy-four pigs were assigned to two experimental groups: one was fed a commercial diet (CTR) and the second one the same diet with the addition of an antioxidant mixture containing vitamin E and verbascoside (AOX) from Verbenaceae extract. The antioxidant mixture was administered to pigs 45 days before slaughter. Growth performances were recorded and oxidative status was assessed by KRL test. At slaughter, the LD muscle was sampled from 10 pigs per treatment and packaged under modified atmosphere (MAP). Physical, chemical, and microbiological parameters were evaluated during 15 days (T0; T6; T12; T15) of storage at 4°C and sensory analyses was performed using a difference test. Blood total antiradicalic activity was higher ($P<0.05$) in AOX group than control (94.4±2.8 HT50 CON *vs.* 124.8±3.7 HT50 AOX). Dietary treatment positively affected ($P<0.05$) carcass dressing percentage (79±0.27 % AOX *vs.* 80±0.26 % CON). Oxidative stability and colour indices were significantly affected ($P<0.05$) by dietary treatment and storage time. No differences in total viable count was observed in relation to dietary treatment. Sensory analysis revealed a loss of colour during storage along with the appearance of off odours. The shelf life of LD under MAP, based on sensory data, was established at 15 days. The present data shows that dietary supplementation with antioxidant mixture improve total antioxidant status and carcass dressing percentage in medium-heavy pigs. The oxidative and colour stability in LD muscle was improved by antioxidant supplementation during storage under modified atmosphere.

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C-033

Effects of breed and a different lipid supplement on beef quality in heifers: performances, meat quality and fatty acid composition of *Longissimus dorsi* muscle

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The present study is part of a larger project aimed to evaluate both the most suitable breed and the most appropriate nutritional strategies to improve healthy properties (ω-3 fatty acids concentration in intramuscular fat) and to increase marbling in muscle of beef. Sixty four heifers of two crossbreed Bleu Belge x Frisian (I) and Charollais x Aubrac (F), after an adaption period of forty days, were divided into 2 homogenous groups that differ for dietary lipid source used: group C (control) received a basal diet composed of ground ear corn (4.5 kg), wheat bran (1.5 kg), wheat straw (1 kg), commercial feed (3.8 kg) containing rumen-protected vegetable fats (6.3% of feed); group T (treated) received the basal diet supplemented with a different commercial feed containing extruded flaxseed (0.8 kg/head/day). Animals were subdivided in 8 boxes (4 thesis, 16 animal per thesis, 8 heifers per box), diets were offered *ad libitum* and weekly daily consumption of each boxes was recorded as a difference between feed delivered and refusal, all diets were isoproteic and isoenergetic. Slaughtering took place every week after 10 months of rearing; carcasses were chilled for 24 h before sampling. Samples of *Longissimus Dorsi* (LD) from 56 heifers were collected for meat quality analysis (composition %, Warner–Bratzler shear force, instrumental color parameters and cooking loss) and fatty acid content. Results were analyzed by two-way ANOVA, with breed and diet as main effect, using Student's t-test and Newman-Keuls as post-hoc test. As regards zootechnical parameters and slaughtering performances statistical differences were observed and Charollais x Aubrac (F) groups shown better results; conversely marbling score improved in I groups (Bleu Belge x Frisian), due both to breed and interaction between breed and dietary treatment. No differences were observed on meat quality and composition. Concerning fatty acid profile of LD muscle, statistical differences were observed in MUFA and α-linolenic acid content in IT groups, due both to diet and to interaction between breed and dietary treatment. In conclusion animals deriving from FT groups seem to have better growth and slaughtering indexes (conformation and fattening) and ω-6/ω-3 ratio in intramuscular fat, but IT groups have a better marbling score and a high level of α-linolenic acid content in LD muscle. The crossbreed Bleu Belge x Frisian (I) appears to be more interesting for our purposes.

C-034

Caprylic acid and its effect on *Campylobacter jejuni* in broiler chickens

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The study aimed to estimate the effect of caprylic acid (CA) on Campylobacter jejuni in chickens, using two alternatives: a dietary supplementation, or the surface-treatment of chilled chicken carcasses. To determine the dietary effect of CA on *C. jejuni*, individually housed broiler chickens (n =48) were divided into 4 groups (positive control, negative control, treatment I: 2.5 g/kg of CA in the feed and treatment II: 5 g/kg of CA in the feed). On the 21st and 35th day of chickens' life, all groups, except the negative control, were infected with *C. jejuni* VFU612 (107 CFU/bird). Each 2- 3 days, *C. jejuni* counts were determined in chicken faeces to assess the effect of dietary CA on the dynamics of *C. jejuni* shedding. In the second part of the study, the surface-treatment of chilled chicken carcasses with CA was used. Chicken skin was treated by CA for 1 min at 0, 1.25 and 2.5 g/l concentration. After the surface-treatment, chicken carcasses were stored at 4°C for 3 days. Each day, counts of *C. jejuni* on chicken surface treated with CA were determined using selective agar plates and compared to the control. Dietary CA significantly decreased the number of *C. jejuni* in faeces ($P<0.05$). However, the effect only lasted for 3-7 days after the artificial infection of chickens. The number of *C. jejuni* shed by the positive control birds reached its maximum on the 37th day of the birds' life (2 days after the second infection), while at the same day, both treatment I and treatment II groups shed significantly lower ($P<0.05$) numbers of *C. jejuni* (by 0.8 and 1.8 orders of magnitude, respectively). After euthanasia at the 42nd day of chickens' life, no differences in *C. jejuni* couts in the crop, gizzard, ileum and cecum were found between the positive control and treated groups ($P<0.05$). Surface-treatment of chilled chicken halves with CA significantly reduced *C. jejuni* contamination of chicken skin ($P<0.05$). The chicken skin was also artificially contaminated with *C. jejuni* before the experiment. All data were statistically analyzed, using Sheffe's test of the SAS programme. In conclusion, dietary CA affected numbers of *C. jejuni* in the gastrointestinal contents of chickens, whereas surface-treatment reduced or eliminated *C. jejuni* contamination in processed chicken carcass.

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C-035

Effect of in ovo prebiotic administration on growth, carcass characteristics and meat quality of broiler chickens

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The effect of in ovo injection of prebiotic on growth, carcass characteristics and meat quality of broiler chickens were evaluated. At the 12th day of incubation, 160 fertilized eggs (Ross 308) were randomly divided into 4 groups treated with different prebiotics, in ovo injected: C, Control with physiological saline; T1, DiNovo® (commercial algae extract, 0.88 mg/embryo); T2, Bi^{2}tos® (Galactooligosaccharides GOs, commercially available, 3.50 mg/embryo); T3, Lupin oligoscacharrides (RFOs, raffinose family oligosaccharides, in-house extracted from seeds of lupin, 1.9 mg/embryo). Among the hatched chickens, 48 males were randomly allotted to different cages and were grown up to 42 day of age. FCR was calculated on a cage base. At slaughter, broilers were individually weighed, hot carcass weight was recorded, and carcass yield was calculated. The pectoral muscle (PM) was removed from carcass, weighed and its percentage was calculated based on hot carcass weight. The right PM was taken for histological analyses. pH and color were measured at 24 h. Water holding capacity (WHC), cholesterol content, intramuscular collagen (collagen amount and crosslinking) and intramuscular fat of PM were examined. Data were evaluated by ANOVA. Compared with the C group, the treatment groups had higher final body weight ($P<0.05$); T3 group showed higher body weight ($P<0.05$) compared with T1 and T2 groups. The FCR was similar among C, T2 and T3 groups; however, FCR was higher in T1 compared with C and T3 groups ($P<0.05$). Carcass weight, carcass yield, PM percentage, intramuscular fat, collagen content and crosslinking were not influenced by prebiotics. The PM weight was higher in T1 group than in C group ($P<0.05$), with intermediate values for T2 and T3 groups. pH and WHC values were found to be similar among the groups. The meat of C group was more red than that of the other groups, however significantly different a* values were found only between T2 and C groups ($P<0.05$). In general, the prebiotic treatment increased ($P=0.045$) PM fiber diameter, but differences were found only between T3 and C groups ($P<0.05$). In ovo prebiotic administration had a positive effect on growth of birds, as well as on both weight and fiber diameter of PM, while it had a little effect on most of the investigated meat quality traits. DiNovo® affected negatively the FCR.

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C-036

A survey on environmental footprint of intensive beef herds based on farm data: gate-to-gate LCA approach

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Livestock has been recognized as an important contributor to environmental impact of human activity. The beef sector of Veneto Region (Italy) represents a main player in the national beef production, but knowledge on its overall impact lacks. This study aimed to survey the environmental footprint of intensive beef sector in Veneto Region through a gate-to-gate Life Cycle Assessment (LCA) approach. The study considered 1 kg of body weight (BW) as functional unit and the batch (a group of animals homogenous for breed, diet, fattening period, and finishing herd) as system boundary. Greenhouse gas (GHG) emission (kgCO2-eq), acidification (gSO2-eq) and eutrophication (gPO4-eq) were taken as impact categories. The study involved 328 batches and 16 specialized beef fattening herds. Information about breed (mainly Charolaise, Limousine and French and Irish crosses), sex, fattening period (226±17 d), initial and final BW (365±47, 686±82 kg, respectively), composition of Total Mixed Ration (TMR), dry matter intake (10.2±1.2 kg DM/d) and growth rate (1.43±0.20 kg/d) were recorded or computed for each batch. Data on materials used (plastic, fuel, electricity), and dedicated-to-feed-herd crop production were collected for the year 2013 in each herd. Off-farm emissions have been acquired from literature data and software (Simapro 7.3.3 – Ecoinvent database v.2.2). Different methods were used to assess the impact categories resulting in a range of possible values. The impact on GHG emissions category ranged from 7.3±1.2 to 8.7±1.4 kg CO2eq/kg BW, on acidification was 206±52 g SO2eq/kg BW, while on eutrophication 41.2±10.5 g PO4eq/kg BW. Impact categories were analysed according to a mixed model with breed, arrival season and BW, neutral detergent fibre (NDF), and average daily gain (ADG) classes (calculated as mean±0.5 SD within each breed) as fixed effects and herd as random effect. Breed, ADG and NDF classes influenced ($P<0.01$) all impact categories, while arrival season influenced only GHG emission and eutrophication categories. The environmental footprint of this specialized system seems lower than values reported for other beef fattening systems. However, high variation was observed among estimates obtained using different methods, suggesting the need to standardize the approaches used for LCA analysis. Moreover, further research is needed to extend the system boundary for including the production of stock calves in the country of origin.

C-037

Effects of five additives with antimicrobial properties on *in vitro* rumen fermentation and methane production of a commercial diet for dairy cows

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This study explored effects of four plant extracts (allyl-sulfyde, AS; cinnamaldehyde, CI; eugenol, EU; limonene, LI) and one synthetic compound (monensin, MO), all with antimicrobial properties, on *in vitro* rumen fermentation and methane (CH4) production of a diet for dairy cows (NDF=37.0%; CP=15.2%; lipids=3.3%, on DM). Four incubations at 24 h were carried out using a gas production (GP) system made up of bottles wireless-connected to a PC. Bottles (317 ml) were filled with 1.0±0.010 g of diet, additive, and 150 mL of buffered rumen fluid, and incubated at 39±0.4°C. Two dosages of each additive were tested: 3 or 30 mg/g diet for AS, CI, EU, and LI; 0.015 or 0.030 mg/g diet for MO. For each bottle GP was recorded every minute. Gas was vented when the pressure into the bottles reached 6.8 kPa. The experimental design was: 4 incubations×5 additives×2 dosages×3 replications, plus 24 bottles as control (without additive; 6/run) and 12 as blanks (without diet and additive; 3/run), for a total of 156 bottles. At the end of each incubation, gas (10 ml) was sampled from headspace of each bottle and analyzed for CH4 by GC. Fermentation fluids were treated with neutral detergent solution to compute degradability of NDF (NDFd, %) and of true DM (TDMd, %). Data were submitted to ANOVA considering the treatment (n=11; 5 additives×2 dosages, plus control) and incubation (n=4) as variation sources. High dosage of LI strongly depressed ($P<0.001$) both NDFd (-68%) and TDMd (-14%) compared to the control; less marked ($P<0.05$) reductions were noted for high dosage of MO (-16% and -3%, for NDFd and TDMd, resp.). No effects emerged for other additives, irrespective by dosage. Compared to the control, high dosage of AS, CI, LI, and MO reduced ($P<0.001$): i) *in vitro* GP (ml/g DM), with decrements from -12% (for MO) to -35% (for LI); ii) *in vitro* CH4 production (ml/g DM), with decrements from -27% (for MO) to -48% (for AS); iii) *in vitro* CH4 proportion (% CH4 on total GP), with decrements from -17% (for MO) to -40% (for AS). On the contrary, EU never affected gas emissions. In conclusion, all additives, except EU, reduced gas and CH4 production, but only at high dosage. The most promising results emerged for AS and CI, as CH4 depression was not accompanied by a reduction of *in vitro* degradability.

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C-038

Sustainability of conventional and organic dairy farms in mountain areas

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Traditional and organic mountain farms appear unsustainable from an environmental point of view when emissions are allocated only on the quantity of milk produced. However, mountain farms deliver to the community also co-products and ecosystem services (ES) that need to be taken in to account. Animal welfare could be considered an additional service provided by mountain farming systems to urban consumers through more ethical foods. The aim of this work is a multicriteria evaluation of 16 transhumant alpine dairy farms, 8 organic (ORG) and 8 conventional (CON). Different functional units and allocations were considered in a Life Cycle Assessment (LCA). In particular on-farm emissions were allocated on the co-product meat and on ES performing an economic allocation on the basis of agri-environment payments: the services recognized and paid to the farms object of this study were the maintenance of local breed (Rendena) and the management of pastures. In addition Welfare Quality® assessment protocol and Animal Needs Index score (ANI 35L) were used to score dairy cow welfare. Comparing ORG and CON farms for kg of Fat and Protein Corrected Milk (FPCM), emissions were always higher for the ORG, although only the difference concerning the warming potential (1.03 *vs.* 0.92 kg CO_2 eq) was statistically significant. Effects on acidification (17.42 g SO_2 eq/kg FPCM) was higher than the most intensive farms of the plain, while effects on eutrophication (2.43 g PO43-eq/kg FPCM), was instead rather low. However, when the environmental burden is allocated also on meat and ES, greenhouse gas emissions attributed to milk are reduced by 34% (0.75 kg CO_2 eq/kg FPCM). When considering welfare, all farms resulted in 2 welfare categories (acceptable and enhanced) according to the Welfare Quality®. Similarly, according to the ANI 35L score, medium-high values were recorded (25.8 and 26.9 for ORG and CON respectively). The findings of the survey show that welfare scores are flatten to medium values even when different management practices, housing systems, herd sizes are taken into consideration. The capability of LCA and welfare assessment to capture the beneficial effects of multifunctional mountain farms is limited. Agreed and integrated methodologies are needed in order to properly assess environmental sustainability of mountain farms.

C-039

Green house gas emissions in the production of heavy pig

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Meat is one of the food products with the biggest impact on the environment. This is a consequence of the low efficiency of the animals in converting feed into meat, as a large part of the energy ingested is necessary for maintenance or lost as excretions and emissions. Italian pig production is characterized by a very high slaughtering weight in comparison with European standards: a minimum of 9 months age and an average final live weight of about 160 kg are requested to comply with the rules of the Consortia for the production of Parma and San Daniele dry-cured PDO hams. Advancing livestock age and increasing fat deposition negatively affect feed conversion ratio, which is one of the main determinants of meat production environmental impact. Only few studies analysed the global warming potential of pork meat production, while none focused on the production of heavy pigs. A cradle to farm gate Life Cycle Assessment was performed to evaluate the greenhouse gas emission of meat production of heavy pigs in 6 farms located in the Po valley. Key parameters concerning on-farm activities (feed production, fuel and electricity consumptions, manure and livestock management), off-farm activities (production of fertilizers, pesticides, purchased feed, replacement animals, electricity, fuel) and transportation were collected through personal interviews to the farmers to calculate the related emissions. The functional unit was 1 kg of live weight leaving the farm gate. Among the six swine farms, four were farrow-to-finish while two were grow-to-finish farms. Moreover, two of the 6 farms were very large (more than 15,000 heavy pigs sold yearly) while the other four were small (less than 5,000 heavy pigs/year). The average slaughtering weight was 168.7 kg (±33.3). The global warming potential per kg live weight was on average 4.25 kg CO_2 eq from a minimum of 2.69 to a maximum of 5.81, consistent with the results of previous studies carried out on lighter pigs. The major contributor to the emission of greenhouse gases was represented by purchased feed (58.1±10.5%); enteric and manure emissions from housing and storage loaded for the 17%. On one of the closed cycle farms the contributions of the different stages of reproduction, rearing and fattening were calculated. The phase of heavy pig from 100 kg to slaughtering had the biggest impact, with a contribution of 27.5% to global warming potential per kg of live weight.

C-040

Global warming potential of broiler chicken production system using a life cycle assessment

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The livestock sector contributes to environmental problems, such as air and climate change, water and land pollution, land degradation and biodiversity loss. The animal production is responsible for 14.5% of greenhouse gas emissions measured in CO(2) equivalent; poultry meat and eggs contribute 8% to the sector's emissions. Chicken meat represents one of the most consumed food products in the world, data about environmental impacts of life cycle of poultry production are few in comparison to those of other species. The aim of this trial was to study the impact of broiler chicken production system from cradle to slaughterhouse gate on Global Warming Potential (GWP) performing throughout Life Cycle Assessment. Data were collected in an integrated poultry farm located in Northern Italy that control all stages of production (feedstuff manufacture, broiler breeder production, eggs incubation, chicken rearing and slaughterhouse). GWP (kg CO_2eq) was quantified according to LEAP (2014) and Simapro software (8.0.3.) was used to calculate this impact category. Direct Land Use Change for soya bean production was included. The functional unit was 1 kg of broiler chicken meat. The results of this study showed value of 5.85 kg CO_2 eq/kg broiler meat of GWP. Other authors presented results for GWP ranging from 5.48 to 4.41 kg CO_2 eq/kg broiler meat.The largest contribution to GWP came from production of male broiler with 66.8%, while female rearing represents 25.7% of GWP. This difference is due to the length of the production cycle (male was slaughtered at 53 d, while female at 34 d) and, consequently, to the different feed consumption and efficiency. Additionally, more than 75% of the GWP (78.7 and 75.5% respectively for male and female broiler production) derived from the production of protein feeds (soybean meal and oil). Broiler breeder production and eggs incubation, instead, had the lowest impact for GWP (4.94% and 0.39% respectively). As reported by other authors also the contribution of the slaughterhouse stage to the GWP is low (2.64%) and mostly due to electricity consumption. These data confirmed that the crop-production stage, the feed conversion ratio and carcass yield have a strong influence on GWP. Improving feed efficiency and animal productivity could be considered the main solutions to mitigate emission intensity in poultry production.

C-041

Assessment of factors Influencing *in vitro* gas and methane production by meta-analysis

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The analysis of specific factors affecting *in vitro* gas production (GP; ml/g DM), methane (CH(4); ml/g DM), and CH(4)/GP was carried out by a meta-analysis approach using 274 results from 39 papers published over 12 years. Only feeds or diets incubated without additives were considered. The common factors accounted were: the pressure in the GP equipment used (PR; constant or increasing), the incubation time (IT; 24 or ≥48 h), the collection of rumen fluid (CRF; from fasted or fed animals), the donor species (DS; sheep or cattle), and the nitrogen (N) in the buffer solution (presence or absence). In addition to these dichotomous factors (0 or 1), the NDF content (g/kg), the rumen fluid/buffer ratio (ml/ml), the rumen fluid/feed sample ratio (ml/g DM), the buffer/feed sample ratio (ml/g DM), and the buffered rumen fluid/feed sample ratio (BRF/FS, ml/g DM) were considered. However, with the exception of NDF (391.6±175.3 g/kg), only the BRF/FS (129.7±61.4 ml/g DM) was considered as potential factor to be accounted after a preliminary analysis, being the most important source of variation. A final stepwise analysis was carried out using the 5 dichotomous factors, NDF and BRF/FS contents to assess the relative magnitude of each factor on GP, CH(4) and CH(4)/GP. The most relevant factors analysed were NDF and IT for GP and CH(4) (sum of partial R^2 of 0.44 and 0.35, resp.), followed by CRF and BRF/FS (sum of partial R^2 of 0.06 and 0.09, resp.). The increasing PR was the fifth factor entering the model (partial R^2 of 0.01), but affected only CH(4). The relative magnitude of factors affecting the GP/CH(4) was in order: CRF, BRF/FS, PR, and IT (R^2=0.18). The analysis carried out showed that DS and N in the buffer did not influence the analysed variables. As expected, the increase of IT and the reduction of NDF enhanced the GP and CH(4). Looking at methodological factors, rumen fluid from fed animals showed greater GP and CH(4) (+37.7 and +10.6 ml/g DM, resp.) than using fluid from fasted animals. For a unit increase in BRF/FS (ml/g DM), a corresponding increase of 0.32 and 0.14 ml/g DM in GP and CH(4), resp., was observed. The increasing PR enhances CH(4) production of 4.5 ml/g DM, as compared to constant PR in the GP equipment. Results of this meta-analysis suggest that a strong standardization of methodological procedures (accumulation of gas at constant or increasing PR, CRF, IT, and BRF/FS) is needed to compare results of different trials.needed to compare results of different trials.

C-042

Genomics: an essential tool to improve limited and novel phenotypes in dairy cattle breeding

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Sophisticated statistical methods are used to predict the genetic merit of individuals using information from daughters, parents and other relatives. These methods are able to separate genetic and environmental effects. Their application has been very successful in dairy cattle for the improvement of easily measured traits, such as milk yield. Milk yield per cow has increased dramatically over the past 50 years. As a consequence, a smaller number of dairy cows meet a growing demand for dairy products. The success of this approach is mainly due to the feasibility of recording traits of interest from thousands of herds. However, this strategy is not feasible for traits that are limited and costly to measure. The advent of genomics and its swift uptake by the dairy industry provide an exceptional opportunity to genetically improve the national herd for those novel traits that are more costly to record. Provided enough genotypes and phenotypes can be collected to create a reference population of sufficient size, marker effects for novel and expensive phenotypes can be calculated and genomic predictions made for all animals genotyped in a population, including young animals without known phenotypes. Industry breeding strategies can then effectively incorporate these two traits. An evolving trend in livestock farming is increased demand for the production of healthier, higher quality food, while decreasing the environmental impact and improving animal welfare. Milk products are an important part of a healthy diet. Milk contains components that provide critical nutritive elements, immunological protection, and biologically active substances for both neonates and adults. Furthermore, improvements in the efficiency of feed utilization by dairy cattle have become increasingly important given the rise of input costs in dairy farms. Genomic selection for feed efficiency will lower the amount of feed required for achieving the same production, and reduce manure and methane emissions, which will have a cumulative and permanent beneficial impact on the environment. Similarly, genomic selection for immune response, disease resistance and hoof health will lead to more disease resistant animals, thus decreasing the usage of drugs while improving significantly the welfare of the animals. The presentation summarizes several research initiatives underway in Canada addressing the growing demand for healthier, higher nutraceutical milk through sustainable low-impact dairying.

C-043

A comprehensive strategy for detecting genomic regions associated with traits of economic interest using information generated in genomic selection programmes

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The availability of high throughput SNP platforms has allowed the genotyping of large number of animals involved in Genomic Selection (GS) programmes. Genotypic and phenotypic data can be processed differently for obtaining measures of inbreeding, genetic relationships and breeding value either genome or chromosome-wide. For example, SNP effects estimated for GS can be used to predict values both at genome-wide (DGV) and chromosomal (DCV) level. As a consequence, genome-wide (G_GW) or chromosomal (G_CHR) correlation matrices between genomic predictions for different traits can be calculated. In this work, an approach for detecting genomic regions associated to traits of economic interest based on different information deriving from genomic data is presented. The study was carried out on a sample of 460 Italian Simmental bulls genotyped with 50K Illumina beadchip within the SELMOL and INNOVAGEN projects. DGV and DCV values were calculated for 10 dairy and functional, and 3 beef traits using a BLUP model in a two-step approach. G_GEN and G_CHR were analysed using multivariate factor analysis. Distribution of Runs of Homozygosity (ROH) was also assessed. Results showed differences between G_GEN and G_CHR values in BTAs 14 and 17. In particular, in the chromosome wise analysis of BTA17, milk protein percentage (MPP) and average daily gain (ADG) tended to aggregate in the same latent factor, whereas they remained separated at genome-wide level. SNP effects for BTA17 revealed peaks at approximately 55 Mb and 61 Mb for ADG and MPP respectively. Moreover, a high occurrence of ROH was observed in this region. Putative candidate genes are ABCB9 and MDL13. In BTA14, a latent factor associated with calving ease (CE) was obtained from the analysis of G_CHR, whereas no relevant loadings were observed in factors extracted from G_GEN for this trait. A peak of SNP effects was detected at approximately 24.5 Mb, where also a large number of ROH occurreds. In this region, a QTL affecting calving ease has been reported in German Fleckvieh. A putative candidate gene is TGS1. The joint use of different information that are generated from GS data could be proposed as a preliminary scan for detecting genomic regions harbouring genes that affect traits of economic interest.

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C-044

Genome-wide gene-based analysis reveals new associations between SNP and production and morphological traits in Italian Holstein

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Genome wide association studies (GWAS) have been widely used to disentangle the genetic variation in complex phenotypes, such as production and morphological traits in dairy cattle. While procedures to identify trait–marker association signals are now well established, there is less agreement on the downstream analysis strategies. Arbitrary choices in the steps to move from significant SNPs to functional units (i.e. genes) may end up in distorted conclusions. Gene-based methods have been proposed as a solution to jump from association to annotation by combining the effects of all SNPs in a gene and correcting for linkage disequilibrium. These approaches have the advantage that they i) reduce problem of GWAS multiple-testing by more than one order of magnitude (i.e. from 800,000 markers to ~20,000 genes), ii) potentially boost low association signals that are usually discarded by common filtering procedures and iii) provide a non subjective list of genes for network or pathway analyses. We developed an analysis suite that calculates a gene-based p-value starting from single marker GWA results, with the aim of facilitating post-GWAS analysis. We used this suite with a dataset of 2917 Holstein bulls composed of 1009 subjects genotyped with Illumina BovineHD and 1908 genotyped with Illumina BovineSNP50, imputed to the higher density with BEAGLE software. Single-SNP regression was performed on 32 EBVs (Estimated Breeding Value), comprising production and morphological traits. Genome-wide significance threshold was overcome in 17 out of 32 traits with classical GWA analysis. The gene-based association analysis identified significant signals for 25 traits, showing that this approach has the potential to identify novel associations between genes and traits of interest, missed using the classical approach. Network and pathway analysis revealed the key metabolic pathways in which the candidate genes are involved.

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C-045

Genome-wide association study to identify chromosomal regions associated with coagulation properties, curd firmness and syneresis modeling, and acidity in milk from Italian Brown Swiss cows

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A genome-wide association study (GWAS) was performed for the traditional milk coagulation properties (MCP) together with predicted curd firming (CF) and syneresis traits. Milk acidity (pH) and milk protein percentage (Prot%) were also considered. The first set of phenotypes regarded 3 traditional single point lactodynamographic properties (RCT: rennet coagulation time, min; k(20): time to a CF of 20 mm, min; a(30): CF 30 min after rennet addition) and pH. The second set of phenotypes grouped 4 parameters modeling 360 CF data recorded over 90 min time period (1 every 15 sec) for each milk sample (CF(P): potential asymptotic CF at infinite time, mm; kCF: curd firming instant rate constant, %×min^{-1}; k(SR): syneresis instant rate constant, %×min^{-1}; RCT(P): RCT obtained from modeling individual samples), 2 traits calculated from individual equations (CF(max): maximum CF, mm; t(max): time at CF(max), min) and Prot%. Milk and blood samples were collected from 1,264 Italian Brown Swiss cows (85 herds). Animals were genotyped with the Illumina SNP50 Beadchip v.2. Single marker regression GWAS (GRAMMAR-GC approach) was used. In total, 66 significant single nucleotide polymorphisms (SNP) were identified in twelve chromosomes with some being in common among the two set of phenotypes (i.e., the traditional MCP and the modeled CF and syneresis traits). On BTA6 sharp peaks were found. For MCP significant SNP were located on BTA6, 13, 15 and 19, while for the CF and syneresis traits on BTA1, 6, 9, 11, 16, 20, 23, 26 and 28. All but BTA6, BTA11 and BTA28 chromosomes were associated to only one trait. CF(max) and CF(P) had the largest number of significant associations (34 and 17 SNP, respectively). No significant association was found for milk acidity. The importance of BTA6 for milk technological traits has been confirmed in this study. Partition of milk coagulation and CF in 11 related phenotypes resulted in genomic associations with 12 chromosomes. The new CF and syneresis traits identified 8 extra significant chromosomes in addition to those depicted from MCP. Genomic regions found to be significantly associated with milk technological traits in this study will further contribute to the mapping of corresponding quantitative trait loci and identification of the genes regulating those traits in dairy cattle.

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C-046

Combining exome sequencing and high density SNP analysis for the detection of common deleterious recessive mutations segregating in Italian Holstein bulls

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In a free breeding population the long-term destiny of deleterious recessive mutations is to decrease in frequency due to reduced fitness and eventually disappear because of genetic drift. However, deleterious variants may be maintained in livestock populations when in linkage with favourable alleles for traits under selection or if they are present in the genome of sires having high genetic value. In this investigation, the presence of deleterious mutations in Italian Holstein was assessed by examining: i) High Density (HD) SNP genotypes of 1009 progeny tested Italian Holstein (IH) bulls, ii) exome sequence data from 20 IH bulls, sampled from opposite tails of the male and female fertility EBV distribution, and iii) evolutionary conservation based on sequence data of vertebrate species in public databases. Deleterious mutations are expected to induce a change in protein structure and function, and hence to occur in protein sites subjected to evolutionary constraints. Haplotypes carrying such mutations would be expected to occur at lower frequency than predicted from HW equilibrium and in extreme cases not to be found in the homozygous state. In the bull population genotyped with the HD panel, 48,443 haplotypes lacked one of the homozygous classes. Most haplotypes falling in this group were rare, but 2479 were at medium frequency and expected in the homozygous state at least in 92 bulls. These were classified as candidate deleterious haplotypes. Exome analysis detected 164,277 variants (bi-allelic SNPs in autosomes). Among these, 4,389 were classified as deleterious (*e.g.* non synonymous mutations altering protein physico-chemical characteristics, premature stop codons, etc) when annotated using the SIFT algorithm and ANNOVAR software. A total of 224 deleterious variants co-mapped with the deleterious haplotypes. The evolutionary conservation of these 224 mutations was examined in 100 vertebrate species using phastCons and phyloP methods from the PHAST package. Mutations identified in the 12 loci most conserved across vertebrates are in genes either expressed in testicles, and implicated in sperm quality and fertility, or responsible for genetic defects in human and mouse. Two of the genes cause embryonic lethality when knocked-out in mice. These variants are candidate deleterious recessive mutations still segregating in the Holstein population.

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C-047

Birth date regression to identify genomic signatures of recent selection in Italian Holstein

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Genomic signatures of recent selection were identified in 2918 Italian Holstein bulls born between 1987 and 2007 using a birth date regression on EBVs, and the analysis of changes in allele frequencies. Under strong directional selection, allele frequencies rapidly change and permit the identification of genomic regions that carry genes controlling production, functional or type traits. Genotype data from SELMOL, PROZOO and INNOVAGEN projects were used along with EBVs (Estimated Breeding Value) for 32 production and morphological traits of the genotyped animals, provided by the Italian Holstein association (ANAFI). Bulls were genotyped with BovineSNP50 and BovineHD SNPchips. Imputation using SNPchiMp v.1 and BEAGLE (v.3) was used to obtain HD genotypes for all individuals. A total of 2918 animals and 613,956 SNPs were included in the working dataset, after quality control. Birth date regressed Protein Yield EBVs, show a strong positive trend in the birth date interval analyzed. To detect genomic regions involved, we first identified animals with outlier PLUS- and MINUS-variant EBVs, over the total range of birth years (164 bulls, group 1) and in each birth year (159 bulls, group 2). Then, allele frequencies were obtained for each SNP, in PLUS and MINUS variants pools. Finally, we calculated the absolute allele frequency difference between PLUS and MINUS pools within each group and identified genomic regions with high values by overlapping sliding windows of 50 SNPs. Comparing the information from the plus and minus pool identified 0.53% shared windows in genomic regions under recent selection. A ~1.2 Mb region on BTA13 (from position 23.2 to 24.4Mb) had the highest absolute mean difference across datasets. This birth date based analysis is a novel and potentially powerful approach to identify regions under recent selection associated with production, type and functional traits.

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C-048

Study of genomic prediction accuracy using high density SNP panel in Italian Holstein cattle

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High throughput genotyping technologies have increased the amount of molecular information available for breeding together with computational requirements for data intrepretation. Principal Component analysis (PCA) can be used to reduce the amount of information to be processed. In this study, the effect of PCA reduction on the accuracy of direct genomic breeding values (DGV) using either medium (MD) or high (HD) density panels was investigated. Data were from 916 Italian Holstein bulls genotyped with the 800K BovineHD BeadChip (Illumina). After edits, 897 animals and 561,580 SNPs were retained. The MD panel was simulated by extracting markers (34,685) in common with the BovineSNP50v2. PCA was carried out both genome (MD_GW) and chromosome (MD_CHR) wide for the MD panel, only by chromosome (HD_CHR) with HD panel. For each approach, three scenarios were created according to the variance accounted for by PC (80-90-99%). PCs retained for each variance threshold were 409, 577, 834 (MD_GW), 2,066, 3,633, 10,723 (MD_CHR) and 2,193, 3,945, 12,610 (HD_CHR). DGVs were obtained using PC scores as predictors with a BLUP model. Phenotypes were deregressed proofs (DRPF) for productive, functional and conformation traits. Accuracy (rDGV) was computed as correlation between DGVs and DRPF. Average rDGV across traits for each variance threshold were 0.26, 0.24, 0.19 (MD_GW), 0.28, 0.30, 0.32 (HD_CHR) and 0.33, 0.35, 0.38 (MD_CHR). rDGV increased passing from MD_GW to HD_CHR to MD_CHR panel, probably due to the reduced asymmetry between the number of predictors and observations. An increase of rDGV according to the variance threshold used was observed within HD_CHR and MD_CHR, whereas an opposite trend was observed for MD_GW. Despite the different SNP panel size, the total variance was likely coerced in a quite similar number of PCs in the chromosome-wide approach. To try to explain these results, the HD_CHR panel was split at a sub chromosomal level (8 subsets x chromosome - HD_SPLIT). PCA extraction provided 208,104 PCs, and 4,350, 7,402, 27,075 PCs were retained for the three variance thresholds. The average rDGV were 0.30, 0.31, 0.34, respectively. These figures suggest that splitting the HD markers into blocks may allows for a more balanced repartition of the original variance in PCs extraction and, particularly for small populations, an improved DGV accuracy.

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C-049

Nanotechnology for animal production

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Nanotechnology, one of the most promising technologies, is well known for its pervasiveness in several diverse fields, including agro-food industry. With this presentation, we will show how nanotechnology is peeping in the animal production world and the improvements that will originate from this liaison.
Nanotechnology focuses currently on the use of nanomaterials. It is generally accepted that a nanomaterial is a material having at least one dimension on the scale of 100 nanometers or less. Nanomaterials can be nanoscale in one dimension (*e.g.* films), two dimensions (*e.g.* fibers and tubes) or three dimensions (*e.g.* particles). These nanomaterials will help in the management of diseases with antibacterial nanostructured surfaces that will control pathogens, or with the nanodelivery of veterinary products (drugs and vaccines) to the farmed animals. Bio-functionalized magnetic nanoparticles will allow sperm selection to generate the desired gender. Water will be filtered more efficiently with nanostructured filters or it will be treated with nanoparticles that are capable to selectively remove toxicants, and pathogens in the water will be detected by nanosensors. Nanotechnology can be used also for feed supplementation with the idea that nanosized components could be more bioavailable than their bulk counterparts. The agro-food industry has recently developed several of such systems for the delivery of nutrients, but their application to aquaculture is still scarce. In this context, we have evaluated diets supplemented with nanoparticles of essential metals such as iron zinc and selenium and observed an influence in survival rate and grow performance as well as an effect on the expression of genes involved in trace mineral intestinal absorptions, bone formation and oxidative stress response. In spite of the potential benefits that nanotechnology offers to the animal production industry and more in general to the agri-food sector, little is known on the safety aspects of this technology. Therefore, studies aimed at the improvement and implementation of nanotechnological applications should be paralleled by studies on risk assessment.

C-050

Factors affecting growth performance, carcass quality and the occurrence of white striping and wooden breasts in broilers

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The present study aimed at evaluating whether growth performance, carcass and meat quality, and the occurrence of white striping and wooden breast in broiler chickens could be affected by genotype (standard *vs.* high breast yield hybrid, S *vs.* H), sex, and feeding regime (*ad libitum vs.* restricted rate, 80% from 13 to 21 d of age). 768 one-day-old chicks were housed in 32 pens and slaughtered at 46 d of age. The S broilers showed higher final live weight (3,270 *vs.* 3,139 g; $P<0.001$) and lower feed conversion (1.56 *vs.* 1.61; $P<0.001$) compared to the H genotype. The S broilers exhibited higher thigh yield (18.3 *vs.* 17.7%; $P<0.01$) and pHu (5.89 *vs.* 5.85; $P<0.05$), lower lightness (L^*, 45.3 *vs.* 46.2; $P<0.05$), and higher thawing losses (10.5 *vs.* 9.4%; $P<0.05$) of the P. major muscle compared to the H genotype. Males showed higher ($P<0.001$) final live weight (3,492 *vs.* 2,845 g), daily weight gain (77.3 *vs.* 62.8 g/d) and feed intake (119 *vs.* 102 g/d), and lower feed conversion (1.54 *vs.* 1.63) compared to females, besides heavier carcasses, higher dressing percentage and hind leg yield at slaughter. The breast had higher pHu (5.89 *vs.* 5.85; $P<0.01$) and was less yellow (b^*, 13.4 *vs.* 14.2; $P<0.05$) in males than females. Feed restriction impaired final live weight (3,142 *vs.* 3,194 kg; $P<0.01$), despite the compensatory growth (95.4 *vs.* 91.5 g/d; $P<0.001$) measured during the second period (22 to 46 d) in the previously restricted chickens compared to those always fed *ad libitum*, and improved feed conversion (1.57 *vs.* 1.60; $P<0.01$). At slaughter, the restricted broilers showed lower carcass weight, dressing percentage (73.5 *vs.* 73.9%; $P<0.05$), breast yield (39.5 *vs.* 40.6%; $P<0.10$), and higher thigh yield (18.3 *vs.* 17.7%; $P<0.05$) compared to the birds always fed *ad libitum*, besides higher breast pHu (5.89 *vs.* 5.85; $P<0.01$); otherwise the restricted birds tended to have a higher proportion of white-striped breasts compared to the birds fed *ad libitum* (79.5 *vs.* 69.5%; $P<0.10$). Wooden breast occurrence averaged 12.2% and was significantly lower in females than males (8.0 *vs.* 16.3%; $P<0.05$). In conclusion, genotype had a moderate effect on growth performance as well as carcass and meat quality but it did not modify the occurrence of breast muscle abnormalities, whereas gender and feed restriction affected growth performance and abnormality incidence.

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C-051

Feed restriction programs and slaughter age in growing rabbits

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The present study aimed at evaluating the effects of the feeding system (AL: *ad libitum vs.* D: day-by-day restriction *vs.* W: week-by-week restriction) and the slaughter age (73 *vs.* 80 d) on health status, growth performance, carcass and meat quality, and material balance of growing rabbits. A total of 300 commercial crossbred rabbits were housed in individual cages from weaning (36 d of age) to slaughter. The feed restriction was based on the administration of a restricted amount of the diet varying from 80% of the *ad libitum* intake at the beginning of the trial to 100% of the *ad libitum* intake at the beginning of the 4th week. The restriction level (about 90% on average) was obtained by two restriction curves: a day-by-day curve with small daily increments (+4 g/d; D group) and a week-by-week intake curve with large weekly increments (+23 g/week on average; W group). Mortality was significantly higher in the AL group compared to the daily and weekly restricted groups (20.7% *vs.* 11.0% and 6.5%; $P<0.05$). Final live weight (on average 2749 g), feed conversion (3.07), cold dressing percentage (59.9%) and other carcass and meat quality traits were not affected by the feeding system. However, the restricted rabbits evidenced increased empty body fat and energy gains ($P<0.01$) as a consequence of the compensatory growth during the second half of the trial. The delay of slaughter from 73 to 80 d of age significantly increased final live weight (2,647 *vs.* 2,847 g; $P<0.001$), reduced daily weight gain (47.3 *vs.* 44.3 g/d; $P<0.001$), increased feed intake, and impaired feed conversion (2.92 *vs.* 3.22; $P<0.001$). Besides, lipid and energy body content raised in the older rabbits ($P<0.001$). No substantial interaction between feeding regime and slaughter age was found. In conclusion, feed restriction improved rabbit health status and did not affect either growth performance or carcass and meat quality, whereas increased the body fat and energy deposition compared to the *ad libitum* feeding. A progressive day-by-day restriction curve appeared to be less risky and more respectful of the feeding behaviour of growing rabbits in comparison with a discontinuous week-by-week increment of feed administration and permitted to obtain a restriction level nearer to what expected (91% in D group *vs.* 93% in W group). The increase of slaughter age was much more effective in modifying growth and slaughter performance as well as body composition of rabbits than the feeding system.

C-052

Effect of feeds containing durum wheat milling products and age at slaughter on chemical composition and fatty acid profile of meat from *Gentile di Puglia* lambs

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By-products from the durum wheat industry in South Italy areas such as bran, middlings and wheat flour, are valid ingredients for ruminant feeding due to their suitable chemical composition. The exploitation of local cereal resources may contribute to reduce animal feeding costs. Bran and middlings have a similar content of protein (14.8; 15.4%), fat (3.5; 5.2%) and fiber (14.1; 12.7%), respectively. Wheat flour may be used as a basic ingredient for its energy value, good content of starch (55%) and poor amount of fiber (0.7%). The current study aimed to evaluate the effect of feedstuffs all containing wheat flour (10%), in association with bran (10%) or middlings (10%), on the chemical composition and fatty acid profile of the Longissisum lumborum muscle in lambs slaughtered at 75 or 90 days. The trial was carried out on 30 Gentile di Puglia breed male lambs born as singletons. Lambs were weaned at 42 d of age by a commercial weaning feed administered for a week. They were then divided into 3 homogeneous groups and fed *ad libitum* with a control feed or with feedstuffs containing alternatively bran (10%) or middlings (10%). Homogeneous subgroups of 5 lambs per each feeding treatment were slaughtered at 75 d of age while the remainder continued the feeding treatment and were slaughtered at 90 d. Diet did not affect the chemical composition of the muscle regardless of the age at slaughter. Slaughtering at 90 d markedly ($P<0.05$) lowered the protein content in meat samples from all the three feeding treatments (on average 74.49 *vs.* 72.93%), in turn of a slight increase of the lipid fraction (on average 16.99 *vs.* 17.83%). The same trend was recorded for N-free extracts, that markedly ($P<0.05$) increased with the age at slaughter (on average 2.57 *vs.* 3.97%). As for the fatty acid profile of meat, the feeding treatment had no influence on neither of the fatty acid classes. The age at slaughter, adversely, affected the MUFA content of meat, that increased from 46.85 to 48.15%, respectively from 75 to 90 d, with a contemporary decrease of the PUFA content from 4.84 to 4.07%. The results of the present research show that by-products of wheat milling industry may be successfully used for lamb feeding with no effects on meat quality. Meat nutritional properties of Gentile di Puglia breed lambs were more satisfactory in terms of fatty acid profile when slaughtering was performed at 75 instead of 90 d of age.

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C-053

Growth and carcass traits of 145 kg body weight pigs fed according to different feeding regimes

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This study aimed to investigate the effect of feeding regimen on growth and carcass traits and estimated energy balance of pigs slaughtered around 145 kg body weight (BW) and addressed to high quality baked ham production. At the beginning of the trial, 96 crossbred barrows (Topigs Talent) averaging 30.4±4.1 kg BW were randomly allotted into 8 pens (12 pigs per pen) equipped with electronic feeding stations. All animals were provided with the same feeds containing on average 13.6 ME MJ/kg and 161 g CP/kg as-fed. Within each pen, 4 pigs were assigned to an *ad libitum* (AL), 4 pigs to a low-high restricted and 4 pigs to a high-low restricted feeding regime. Individual feed intake was recorded daily, pigs were weighed weekly, and backfat depth (P2) was measured with an ultrasound device every 3 weeks, till the end of the experiment, when the pigs averaged 142.6±7.8 kg BW. Measurements of BW and P2 were used to estimate changes of body composition over the time, from which ME requirements for protein and lipid growth were derived. Estimated ME for growth and the measured ME intake were used to estimate the ME used for maintenance. All pigs were slaughtered in the same day in a commercial abattoir, and carcass and major cuts weights were recorded. Samples of *Longissimus Dorsi* were collected for physical and chemical analysis. After 24 h of chilling thighs were deboned and weighed. Traits were analysed according to a mixed model which accounted for the effects of pen (random) and of feeding regime. Pigs fed AL showed ($P<0.01$) higher growth rate and final BW (966 *vs.* 832 g/d, and 145 *vs.* 141 kg, respectively), lower gain:feed and higher estimated amount of ME for maintenance than restricted fed pigs (0.368 *vs.* 0.383, and 0.98 *vs.* 0.89 MJ/kg BW0.60, respectively), whereas carcass and meat quality traits were similar among feeding regimes. Pigs fed different restricted feeding regimes gave similar growth and carcass performances. In conclusion, the AL feeding regime allowed to attain the target 145 BW some days earlier than restricted feeding regimes, but growth rate was not greater enough to compensate increased feed intake, thus penalising feed efficiency of AL fed pigs.

C-054

Effect of gender and slaughter age on carcass and meat quality of brown hares (*L. europaeus*)

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The present study aimed at evaluating slaughter and carcass traits and meat quality in 48 farmed brown hares (L. europaeus) of both genders slaughtered at two ages (sub-adult hares, on average 73 d, *vs.* adult reproducing hares, on average 17 months) and then dissected following standardized procedures for rabbits. At slaughter, the females were heavier than the males (2783 *vs.* 2629 g; $P<0.05$), showed higher full gut incidence (13.7 *vs.* 12.9%; $P<0.05$), lower skin and distal fore and hind leg incidence (13.3 *vs.* 14.0%; $P<0.01$), and lower dressing percentage (66.7 *vs.* 67.6%; $P=0.06$). The carcasses of females had lower dissectible fat (1.34 *vs.* 2.17%; $P<0.05$) and higher proportion of both l. lumborum (14.3 *vs.* 13.8%; $P<0.05$) and hind legs (37.2 *vs.* 36.4%; $P=0.06$). When age increased, slaughter live weight (2022 to 3391 g) and carcass weight increased ($P<0.001$), but dressing percentage did not vary (67.2% on average); the gut incidence decreased (14.5 to 12.1%; $P<0.001$), whereas skin and distal leg proportion increased (12.9 to 14.5%; $P<0.001$). When age increased, head, liver and thoracic organ proportions decreased ($P<0.001$); dissectible fat (1.34 to 2.17%; $P<0.05$) and l. lumborum (13.5 to 14.5%; $P<0.001$) increased; moreover, hind leg proportion decreased (37.3 to 36.3%; $P=0.01$) but the muscle to bone ratio of hind legs increased (5.11 to 6.23; $P<0.001$). Meat quality did not vary with gender, whereas sub-adults significantly differed ($P<0.001$) from adults, showing lower meat pH, higher lightness index, higher redness index for l. lumborum (3.03 to 1.46) and lower redness index for b. femoris (4.07 to 5.76); besides they had higher meat thawing losses and lower shear force measured on the hind leg muscles (2.97 to 4.02 kg/g; $P<0.001$). In conclusions, in hares, slaughter and carcass traits differed according to both gender and age, whereas meat quality was affected only by age.

Acknowledgments
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C-055

Use of submerged cage and discards as feed for tub gurnard (*Chelidonichthys lucerna* L.) fattening in the mid Adriatic sea

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Tub gurnard (*Chelidonichthys lucerna* L.) is a fish species of great socio-economic importance for the coastal people communities of the mid Adriatic sea because allows the recovery and enhancement of traditional activities. Demand for this species is continuously increasing because, in this area there is a strong tradition of consuming this fish appreciated for its meat. The commonest weight captured and commercialized is less than 150 g but the largest size, which represents a small part of captures, would be more appreciated and get high price. In order to obtain larger gurnard, some fishermen's cooperatives are involved in promotion and management of marine protected areas and in experimental aquaculture for this species. This paper refers about a research concerning the suitability of tub gurnard juveniles to be reared in cylindrical bottom cages. A fattening trial was performed in order to evaluate the growth performance and survival rate of wild juveniles, fed on discards obtained by fishery. The trial involved 300 juveniles (mean body weight 72.9±11 g, mean length 18±1 cm), subdivided in four circular submerged cages with a 35.3 m^3 volume. Two different stocking densities were tested, corresponding at 60 fish per cage (1.7 fish m^3) in two cages (CG-L) and at 90 fish per cage (2.5 fish m^3) in the other two (CG-H). Cages nets were routinely cleaned by a diver. Different types of food, obtained by catches of fishing vessels that were not targeted, were used to feed the juveniles, represented by fish as sardine, chub mackerel, goldline and common crab. After 240 days, the final mean body weight did not show significant differences ranging between 346±39 g (CG-L) and 385.5±52 g (CG-H). Body length had not notable differences sizing 35.6±1 cm (CG-L) and 34.8±1.1 cm (CG-H). The condition index K did not differ between CG-L (0.85) and CG-H (0.82). The final stocking density was included between 0.59 kg m^3 in the CG-L group, and 0.77 kg m^3 in the CG-L group. The survival rate was similar in the two groups (CG-L=91.0%; CG-H=90%). The feed conversion ratio was more favorable in CG-H (3.92) than CG-L cage (4.95) The fattening trial of tub gurnard in submerged cages showed interesting results and suggested that growth rates is possible using cages placed on the seabed, respecting the benthonic behavior of the species.

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C-056

Preliminary characterization of soluble thiols in bovine milk

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Free radicals (FR) are unstable and reactive molecules with adverse effects on animal and human cells: lipid peroxidation, alterations of proteins and DNA cleavage. For this reason antioxidants, acting against FR, are essential for cell homeostasis maintenance. Antioxidants are defined as substances able to compete with other oxidizable substrates and, thus, to inhibit the oxidation of the substrates themselves. This definition includes antioxidant enzymes and molecules acting as antioxidants, such as α-tocopherol, β-carotene, ascorbate, and glutathione (GSH). In particular GSH belongs to the class of low molecular weight (LMW) thiols, highly reactive molecules involved in the maintenance of cellular redox homeostasis. Other LMW thiols, biosynthetically connected with GSH, are cysteine (CYS), cysteine glicine (CYS GLY) and γ-glutamylcysteine (γ-GC). GSH and LMW thiols are involved in FR scavenge with the formation of disulphide bound (GS-SG). In scientific literature only few studies have investigated thiols content in products of animal origins, therefore this study aimed to characterize LMW thiols of bovine milk. Twenty-four individual milk samples from each of 4 dairy cattle breeds, Brown Swiss (BS), Holstein-Friesian (HF), Alpine Grey (AG) and Simmental (SI), were collected in 8 herds and stored at -20 °C. Low molecular weight thiols were detected using the soluble fraction of milk sample and applying a derivatization protocol for marking thiols with a fluorescent die. Detection and quantification were performed in RP-HPLC. Six thiols species were detected and only two of them were identified. The mean (standard deviation) of CYS GLY and GSH were 0.087 (0.068) and 0.067 (0.064) μM, respectively. Average concentration of CYS GLY was greater than that of GSH across all breeds. Milk from dual purpose breeds (SI and AG) was richer in CYS GLY and GSH concentration than milk from HF and BS cows. Great variation of both thiols was found among herds. Cysteine glicine exhibited a moderately low Pearson correlation with protein (0.290; $P<0.001$) and casein (0.271; $P<0.001$), while GSH did not show any correlation with protein and casein. Glutathione and CYS GLY, closely linked in biosynthesis pathway, were quite strongly correlated (0.653; $P<0.0001$). Negligible effects of days in milk and parity were detected. This study allowed a preliminary characterization of thiols in bovine milk from different cattle breeds.

C-057

Production regulations and characteristics of cow *Piedmontese Noble Milk*

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The need to combine the quality of animal products with the human health as well as to revitalize the milk chain has recently led to the development of a new model based on milk and dairy products characteristics and specificity. The model, called *Latte Nobile* (Noble Milk), was conceived in South Italy in 2011, and it is now fast spreading in other parts of the country. In Piedmont (NW Italy) a two-year project, developed on different research lines (agronomy, animal nutrition, chemical and microbiological features of the product, heat treatment processes, shelf-life and traceability), aimed to adapt the *Noble Milk* model to the environmental and productive conditions of this region. Thanks to the results of the project, *Piedmontese Noble Milk* (PNM) production regulations have been proposed and the product is expected to enter the milk market in 2015. According to the regulations, the PNM is obtained from animals yielding maximum 6000 kg of milk per lactation and fed fresh grass and/or hay from local mixed grasslands (minimum 70% of daily dry matter intake). Silages and genetically modified feedstuffs are forbidden and only pastures or meadows with at least four dominant species are allowed for animal feeding. Pastures and meadows must also constitute at least the 50% of the farm forage system. Cows must graze for at least 150 days per year under a stocking rate not exceeding 1.5 animal unit ha^{-1} $year^{-1}$. Animal welfare has to be guaranteed in accordance with the Welfare Quality standards. PNM milk contains >0.25g 100 g^{-1} fat of total conjugated linoleic acid (CLA) and >0.50g 100 g^{-1} fat of total omega-3 fatty acids (n3 FA). According to the results of the project, typical values range from 0.29 (winter) to 1.71 (summer) g 100 g^{-1} fat and from 0.60 (winter) to 1.99 (summer) g 100 g^{-1} fat for CLA and n3 FA, respectively. The linoleic/alpha-linolenic acids ratio must be lower than 4 all year round. The PNM can be sold raw or pasteurized; the findings of the heat treatment trials suggest that, to preserve the best chemical, nutritional and organoleptic characteristics of the product, PNM should be pasteurized at 72°C for at least one minute. A specific software has been implemented to guarantee the traceability of all the PNM production process, from the forage system to the derived *Piedmontese Noble Milk* chemical and microbiological characteristics.

C-058

The effects of the traditional producing system on physicochemical, microbial and sensory properties of Caciocavallo Palermitano cheese

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Caciocavallo Palermitano (CP) is a traditional cheese made with raw milk from cows of Sicilian local breeds fed pasture-based diets in extensive farms (EXT), processed by an artisanal technology (ART) based on wooden tools and the action of native microflora. CP is obtained also in intensive farms (INT) where milk from cows of specialized breeds fed dry diets is transformed by advanced procedures (ADV) using a stainless steel equipment and lactic acid starter cultures. This research was planned to investigate the changes in cheese properties due to production system and ripening., Milk was collected 3 times from an EXT and an INT farm and processed in ART and ADV conditions. The 12 produced CP were evaluated at 30, 60 and 120d. EXT CP showed higher yield and protein, lower fat, NaCl and soluble N, a less yellow color, a smoother paste, and a sweeter, less bitter and salty, and more acidic taste than INT CP. The pasture led to EXT CP with a beneficial FA profile to human health, richer in PUFA, n-3 FA and CLA. ART CP showed lower yield, higher fat and color indexes, lower NaCl and soluble N, a harder and more compact paste, with less holes, and at taste was less bitter, salty and acidic, and more piquant than ADV CP. During ripening, the soluble N, the yellow color and the consistency of paste increased. In ART CP, the proteolysis was slower, and the increasing of yellow color was more intense. The microbial analysis of ART CP showed that the wooden vat is a reservoir of lactic acid bacteria (LAB) inoculating milk, among which strains of Streptococcus thermophilus were dominant and acted as a starter culture, while undesired microorganisms were absent or very low. During ripening, all LAB decreased except enterococci. The canonical discriminant analysis of physicochemical and microbial data was able to separate CP from different productions and ripening time. Among dominant LAB, 10 species were genetically identified at strain level and some of them showed antibacterial activity. In ART CP, the comparison of polymorphic profiles of LAB strains isolated from the wooden vat with those of strains collected during maturation showed the persistence of 3 Enterococcus spp. strains, among which E. faecalis was found at dominant level till 120d. The absence of these strains in ADV CP evidenced the contribution of the wooden vat LAB during ripening. EXT and ART CP showed the best features in terms of hygienic safety, health benefits and typical sensory properties.

C-059

Fatty acid composition of milk from Holstein-Friesian, Brown Swiss, Simmental and Alpine Grey cattle breeds routinely predicted by mid-infrared spectroscopy

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Fat is an important and variable milk component influencing many physical and organoleptic properties of dairy products. Lasting years several studies have been carried out to investigate the role of fatty acids (FA) on human health and results highlighted both positive and negative associations between FA intake and the risk of cardiovascular diseases. The aim of this study was to investigate the variation of groups and major individual FA routinely predicted in milk of four cattle breeds reared in an alpine area. Individual samples (n=153,801) of 14,301 Holstein-Friesian (HF), Brown Swiss (BS), Simmental (SI) and Alpine Grey (AG) cows from 1000 single-breed herds were analysed for traditional quality traits (fat, protein, casein and lactose contents, urea and somatic cell count) and groups and major individual FA through mid-infrared spectroscopy (MIRS). Data were analysed using a linear mixed model including fixed effects of breed, month of sampling, year of sampling, stage of lactation (DIM), parity and first order interactions between the main effects and the random factors of herd nested within breed, cow nested within breed and the residual. Breed and herd effects were the most important sources of variation for FA composition of milk, followed by DIM. Saturated FA, C14:0 and C16:0 content increased from calving until 120 DIM, while unsaturated FA and C18:1 decreased, indicating that the release of saturated FA inhibits the formation of *de novo* FA in the mammary gland during the negative energy status of the cow. The month of sampling was highly significant in explaining the variation of milk FA across seasons; in particular, the greatest content of unsaturated FA and C18:1 was found during summer, whereas the content of saturated FA, C14:0, C16:0 and C18:0 decreased in summer and increased in winter. Milk from AG cows presented the lowest content of saturated FA (68.7%), C16:0 (31.5%) and C18:0 (10.2%), and the highest content of unsaturated FA (31.3%). Milk from HF and SI was intermediate between that of AG and BS for all the FA except for C18:1, which was higher in HF (22.9%). Results from this study indicated that milk from AG exhibited better FA profile than milk from HF, BS and SI breeds.

C-060

Influence of milk composition and κ-casein B content on mozzarella cheese yield and cheesemaking losses

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The profitability of a cheese factory industry is based on cheese yield capacity of milk. The presence of the B variant of κ-casein (k-Cn) in milk is associated with smaller micelles size, increased casein content and, therefore, improved cheese-making properties and cheese yielding efficiency. The aim of this research was to study the effect of different 2 levels of milk k-Cn B content on cheese yield and cheesemaking losses in the manufacture of Mozzarella cheese, under field conditions. The study was carried out following the production of 8 Mozzarella cheese batches in 4 commercial cheese factories. In each trial, vat milk (V-milk) and cheese produced were weighed and actual cheese yield was calculated (cheese weight x100/ milk weight). For each cheesemaking, samples of V-milk and cheese whey (C-whey) were collected and analyzed to assess the level of cheese-making losses for a milk component (C-whey value x 100/ V-milk value). As the overall mean value of k-Cn B content was 0.1 g/100 mL, two classes were established: class 1, up to 0.1 g/100 mL (LKB) and class 2, over 0.1 g/100 mL (HKB). Both the actual yield (15.51 *vs.* 12.86 kg/100kg; $P<0.001$) and 60% moisture adjusted yield (17.68 *vs.* 14.04 kg/100kg; $P<0.05$) were higher in HKB milk compared to the LKB one. Losses of dry matter (51.39 *vs.* 55.99 %; $P<0.01$), crude protein (23.27 *vs.* 28.05 %; $P<0.05$), casein (1.75 *vs.* 2.61 %; $P<0.05$), and ash (74.89 *vs.* 77.45 %; $P<0.05$) were lower in HKB milk than LKB one. In conclusion, increasing the content of k-Cn B leads to improved cheese yield, mainly because of higher casein content and reduce cheesemaking losses.

C-061

Influence of milk somatic cell content on cheese yield and cheesemaking losses

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The milk quality products implementing programs in EU countries aimed at reducing the milk somatic cell content and to maintain this level under the 400,000 cells/ml (i.e. 853/2004 CE Regulation). The aim of this research was to study the influence of this threshold value of milk somatic cell count on vat milk chemical composition, actual cheese yield (ACY) and cheesemaking losses (concentration in the cheese whey*100/concentration in vat milk) in the manufacture of Parmigiano-Reggiano cheese. Ten comparative cheesemaking trials were performed on Parmigiano-Reggiano cheese. In each trial, two cheesemakings were carried out in parallel: one characterized by a somatic cell count, determined on whole evening milk, below to 400,000 cells/ml (LCC) and the other over 400,000 cells/mL (HCC). In each cheesemaking a sample of whole evening milk, vat milk (partially skimmed evening milk + full cream morning milk, in the ratio, w:w, 1:1), and the residual cheese whey were collected and analysed. ACYs after 24 h, 18 and 24 months of ripening were calculated. Compared to LCC vat milk, the HCC one was characterised by lower values ($P<0.05$) of casein (2.43 *vs.* 2.57 g/100 g), casein number (77.03 *vs.* 77.80 %;), P (88.37 *vs.* 92.46 mg/100 g), titratable acidity (3.16 *vs.* 3.34 °SH/50 mL), and by a higher content of chloride (111.88 *vs.* 104.12 mg/100 g) and pH value (6.77 *vs.* 6.71). The ACY resulted lower for HCC than LCC vat milk, after 24 hours (8.02 *vs.* 8.68 kg/100 kg; $P<0.01$), 18 (7.04 *vs.* 7.64 kg/100 kg; $P<0.05$) and months 24 of ripening (6.74 *vs.* 7.39 kg/100 kg; $P<0.05$). Finally, HCC milk was characterized by higher fat losses in the cheese whey (20.16 *vs.* 16.13% $P<0.05$) compared to the LCC one. In conclusion, the processing of milk with SCC over 400,000 cell/mL lead to significant decrease in cheese yield, with negative repercussion for the profit of the cheese factory. This was related to reduced milk casein level and low fat recovery in cheese.

C-062

Evaluation of the effects of different diets on bacterial diversity and on fatty acid composition in goat rumen

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The equilibrium of ruminal microbiota depends on diet, which carries fermentation substrates and strongly affects the efficiency of ruminal microbiota. High-starch diets can lead to a reduced efficiency of fibre digestion, decreasing the number of fibrolytic bacteria and also diets supplemented with unsaturated fats improving the profile of milk fatty acids negatively affect the fibrolytic activity of ruminal microbiota. The objective of this study was to assess the effects of linseed or hemp seeds diet supplementation on bacterial diversity and on fatty acid profile in the rumen content of goats using the Next Generation Sequencing and gas chromatography techniques, respectively. In nine pluriparous Alpine goats fed the same pre-treatment diet for 40 days, ruminal fluid samples were collected before feeding using an aesophageal polyethylene probe. After this pre-treatment period, the goats were arranged to three dietary treatments consisting of control diet (C), linseed (L) and hemp (H) seeds supplementation. The chemical composition of the administered diets, in terms of neutral detergent fibre (NDF), starch, and ether extract (EE), were as follows, for C, L and H, respectively: NDF: 41.6, 42.2 and 43.3 % DM; starch: 17.2, 13.2 and 13.2 % DM; EE: 2.66, 6.3 and 5.6 % DM. Ninety days later, the same ruminal sample collection procedure was repeated. The bacterial DNA was extracted using a specific protocol and 16S rRNA gene amplicons on V3-V4 region analyzed by Miseq (Illumina). Contemporary, for the same ruminal samples, the fatty acid (FA) composition was determined by using a gas chromatograph equipped with a flame-ionization detector and a high polar fused-silica capillary column. Data of ruminal fluid samples were analysed with a mixed linear model and treatment effects were declared significant at $P<0.05$. In the three dietary treatment groups, bacterial community was dominated by Bacteroidetes and Firmicutes with a high abundance of Prevotellaceae, Porphyromonadaceae and Veillonellaceae and a low presence of Ruminococcaceae and Lachnospiraceae. A tendency towards a separation of the samples in the three diet groups was observed evaluating the principal components of the inter-sample variability. Linseed and hemp seeds supplementation determined a significantly increase of C18:0, C18:1 t6-10, C18:1c9 and c15. Linseed supplementation, moreover, increased C18:2 t11c15, one of the α-linolenic acid biohydrogenation products.

C-063

Response of rumen microbial ecosystem to diets integrated with chestnut or quebracho tannins in dairy ewes

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The aim of this study was to evaluate the response of rumen microbial ecosystem to diets integrated with chestnut or quebracho tannins in dairy ewes. The experiment was conducted as 3 X 3 Latin square design and the trial was repeated 2 times. Three fistulated ewes fed 3 diets based on chopped grass hay *ad libitum* administered and on 800 g/head/day of 3 experimental concentrates containing 84.5 g of soybean oil/kg of DM and 52.8 g/kg DM of bentonite (CON) or chestnut tannin extract (CHT) or quebracho tannin extract (QUE). At the end of each experimental period rumen liquor was analysed for fatty acid and microbial profiles. On the basis of the molar stoichiometric relations between rumen volatile fatty acid and CH4 production (CH4=0.45 x acetate-0.275 x propionate + 0.4 x butyrate), the CH4 emission was also predicted for each diet. A canonical correspondence analysis (CCA) was performed in order to find potential connection between microbial community, fatty acid composition of rumen liquor and potential CH4 emission, and how these connections are influenced by the different diets. DGGE bands were used as "species" data, while fatty acids and potential CH4 emission as "environmental" variables. The microbial profile was affected by the presence of tannins. The bacterial community of QUE and CHT samples of rumen liquor was positively correlated to vaccenic acid, conjugated linoleic acid and C18:2 trans-11 cis-15. Moreover, the bacterial communities as affected by CHT resulted mainly positively correlated to butyric acid, acetic acid and with potential CH4 emission. In contrast, the bacterial communities as affected by CON resulted mainly correlated positively to C18:2 cis-9 cis-12 and C18:0 production.

C-064

Effect of dietary starch concentration and fish oil supplementation on fatty acid profile and microbial composition of rumen fluid in lactating dairy cows

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The aim of this study was to evaluate the effects of diets with different starch concentration and fish oil (FO) supplementation on fatty acid (FA) profile and changes in microbial population of rumen liquor in lactating dairy cows. The experiment was conducted as 4 X 4 Latin square design with a 2 x 2 factorial arrangement: i) 2 concentration of dietary starch (low *vs.* high: 23.7 and 27.7% on DM) basis; ii) the presence or absence of FO (0.80% on a DM basis); and their interaction has been evaluated. Four Italian Friesian cows, in 4 consecutive 26-d period, were fed the following diets: i) low starch (LS); ii) low starch plus FO (LSFO); iii) high starch (HS); iv) high starch plus FO (HSFO). At the end of each experimental period the rumen liquor was sampled and analyzed for FA profile and microbial composition. The percentages of C16:1 cis9 (P=0,0483), C18:1 trans6-8 (P=0,0060), C18:1 trans11 (P=0,0219) and C22:6 n-3 (P=0,0183) were significantly increased in the rumen liquor by the FO supplementation while the total saturated FA percentage decreased. Butyrivibrio fibrisolvens, Prevotella spp. (involved in the biohydrogenation pathway (BH) of polyunsaturated FA), Ruminococcus albus, Ruminobacter flavefaciens and total protozoa content were not affected neither by starch level neither by FO inclusion in the diets. In contrast Streptococcus bovis, a pectin fermenting species, increased with LS diets (P=0,0491), according to a higher content of neutral detergent fiber and a lower starch content of the diets. At the dosage tested, FO did not induce perturbation in the rumen microbial community, but improved the concentration of several functional FA (C18:1 trans11 and C22:6 n-3) without promoting the shift of the BH toward the C18:1 trans10 production, as previously reported.

C-065

Effects of using an oregano aqueous extract on performance, gut microbiota and immune function in broiler chickens

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A total of 150, 1-day-old female broilers (Ross 308) were divided into 2 groups of 75 birds each and fed a starter diet from 1 to 21 days and a grower-finisher diet from 22 to 56 days. The two experimental diets were based on a standard commercial feed used as control (C group) which was supplemented with 2 g/kg oregano aqueous extract (OAE) (O group). Individual body weight (BW) and feed conversion efficiency (FCE) were measured. Blood samples from 5 chickens/group were collected for IgG titration at 22 (T1) and 57 days (T2). At the same time intervals, 8 samples were collected for intestinal microflora examination. Physico-chemical parameters were determined on 10 breast meat samples of both groups. The OAE supplementation affected the BW of birds (P<0.01) up to 22 d of age, while no effects were observed at 36 d and at the end of the experiment. A numerically lower FCE was observed at 36 and 57 d in birds fed the OAE diet. Although the dietary treatment did not appear to affect mean serum IgG values in terms of absolute numbers, their increases (expressed in percentages) from T1 to T2 revealed a significant (P<0.05) dietary effect with the highest value in the O group. Regarding gut microbiota data, coliforms were lower (P<0.001) in the cecum of the O group. The Enterococcus spp. resulted higher in the OAE group (P<0.001), particularly in the caecum tract, and its value increased at T2, supporting the hypothesis of a time-dependent effect. Birds in the C group showed the highest values for Lactobacilli in the ileum and cecum tracts at T1. Values increased (P<0.001) in the O group at T2, showing a potential time-dependent effect. No differences in the physico-chemical parameters of meat were recorded between groups, except for the drip loss value (1.46% in the C group *vs.* 1.17% in the O group, P=0.03). The OAE supplementation reduced meat oxidation as confirmed by TBARs values (0.150 *vs.* 0.246 for O and C groups, respectively; P=0.001). Considering the obtained results, OAE could be able to improve chicken performance and immune response, and balance gut microbiota. Although these results are in agreement with others (Garrido *et al.*, 2004; Cross *et al.*, 2007), further studies are needed to ensure the actual effectiveness of aqueous extracts on the performance and welfare of broiler chickens.

C-066

The interaction of early intestinal microbial colonisation with the effects of luminal perfusion with enterotoxigenic *E. coli*, F4ac fimbria or *Lactobacillus amylovorus* on the jejunal transcriptome in piglets

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An early settlement of a complex gut microbiota can protect against gastro-intestinal dysbiosis, but the effects of neonatal microbiota colonization on the gut barrier upon the further encounter of favorable bacteria or not, are largely unknown. The jejunal transcriptome of differently perfused intestinal loops of 12 caesarian-derived pigs previously associated with microbiota of different complexity was studied. Pigs received pasteurized sow colostrum at birth (d0), 2 mL of starter microbiota (10^7 CFU of each Lactob. amylovorus (LAM), Clostr. glycolicum, and Parabacteroides spp.) on d1-d3 of age and either a placebo inoculant (simple association, SA) or an inoculant consisting of diluted feces of an adult sow (complex association, CA) on d3-d4 of age. On days 26-37 of age, jejunal loops were perfused for 8 h with either enterotoxigenic E. coli F4 (ETEC), F4 fimbriae (F4), LAM or saline (CTRL) and jejunal samples were obtained from each piglet immediately afterwards. Total RNA expression was analyzed by Affymetrix Porcine Gene 1.1_ST array strips. Exploratory functional analysis of gene expression values was performed by Gene Set Enrichment Analysis. Compared to CTRL, 184 and 74, 2 and 139, 2 and 48 gene sets, were up-regulated and down-regulated by ETEC, F4 and LAM, respectively. ETEC up-regulated several nodes related to inflammatory response and cytokine activation, immune response, RNA and micro RNA processing, nuclear chromosome and cell cycling. There was a limited overlap in up-regulated gene sets as affected by ETEC and F4. Nodes down-regulated by LAM were related to inflammatory and immune response, and to cellular compound metabolic processing. Compared to SA, 57 gene sets were up-regulated by CA (*e.g.* related to regulation/activation of lymphocytes), while 73 were down-regulated (related to various metabolic processes and metabolism of amino acids and lipids). CA up-regulated gene sets related to regulation/activation of lymphocytes, and to cellular defence responses in ETEC and CTRL loops. Genes for chemokine and cytokine activity, and in general for response to external stimuli were down-regulated in ETEC perfused loops obtained from CA pigs, compared to SA pigs. In pigs, associated with a complex microbiota, the activation of genes related to chemokine and cytokine activity, and in general for response to external stimuli, induced by ETEC is reduced compared with ETEC effect in piglets associated with a simple microbiota at young age.

C-067

Evaluation of Peyer's patch morphological traits as local immune response in relation to different physical forms of one complete diet fed to growing pigs

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The antigenic stimulation by the gastrointestinal (GIT) content is controlled by balanced immune responses of the host. The composition of digesta (including gut microflora) can be affected by the physical form of the diet, but the local immune response needs to be explored. The Peyer's patch (PP) in the terminal ileum (TI), considered as an immunological sensor of the GIT, could display different morphological traits as expression of the stimulation level. A feeding trial was carried out to screen out the morphology and function of the PP in a total of 32 growing pigs (initial BW: 8.30±0.83 kg) fed with four experimental diets, identical for botanical and chemical composition, but different grinding intensities and compaction (FP – pellet, fine, dMEAN: 0.463 mm; CM – meal, coarse, dMEAN: 0.880 mm; CP –pellet coarse, dMEAN: 0.836; CE – extrudate, coarse, dMEAN: 0.659). After four weeks, the last 3 cm of the TI were removed and all PP analyzed for the follicle associated epithelium height (FAE, μm), germinal centers count (n GC/mm^2), parenchyma to connective tissue ratio (P/Ct). The PP showed a lower stimulation level in the CM diet group, statistically differing from the CP ($P=0.033$), the CE diet ($P=0.025$) and the FP diet ($P=0.011$). Different physical forms of one diet were associated with morphometric and functional changes (FAE, GC and P/Ct) of the PP. In conclusion, the level of stimulation of the PP outlines the role of the diet's physical form in the predisposition of different antigenic potentials.

C-068

Performance and rumen papillae development of Friesian calves fed with pelletted starter or total mixed ration

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The growth of the rumen microflora, the production of volatile fatty acids and the development of rumen papillae greatly depend on the type and composition of solid ration administered to calves during weaning. Aim of this trial was to compare the effects of two solid feeds, a starter and a dry total mixed ration (TMR), on intake, growth characteristics of 24 Friesian weaning calves (20 females and 4 males) and rumen papillae development in males. Calves were divided into two homogeneous groups (10 females and 2 males) and fed milk replacer (from day 4 to day 40: 2×2 L/d; from day 41 to day 70: 1×3 L/d) and pelletted starter (group S) or TMR (group T). Solid feeds differed for ingredients, nutrient composition and physical structure. The starter was higher in crude protein (CP: 21% *vs.* 14% dry matter) and lower in neutral detergent fiber (NDF: 30% *vs.* 46% dry matter) than TMR; non structural carbohydrates were similar in both solid feeds (NSC: about 30% dry matter). For each calf, solid feed intake was measured every 3 days from birth to 70 d of age, while body weight and height at withers were recorded at weekly intervals. The males were euthanized at 70 days of age and tissue from their rumen was sampled from 9 different districts, to measure papillae length. Total solid feed intake was subjected to an independent samples t test and a repeated measure ANOVA was used for the statistical evaluation of calves' growth characteristics; a non parametric Kruskal-Wallis test was used to analyze papillae length. Results showed that total solid feed intake was significantly higher in group S than group T (57.5 *vs.* 50.2 kg DM head-1; $P<0.001$), but no significant differences were observed in the final weight and height at withers of the calves. The length of the rumen papillae was variable; only in the caudoventral blind sac (district 9) of group T the papillae were significantly longer than in group S. This difference might be attributable to the different weight and specific gravity of feeds. Future programmed trials will verify whether these 20 females, now entering into production, will perform similarly or not, and if any difference in rumen functionality can be attributed to the feeding system adopted during weaning.

C-069

Genome wide analysis of the effects of twenty years of artificial directional selection in the Italian Large White pig breed

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In this study, we analysed, at the population genomic level, the effects of twenty years of artificial directional selection of the genetic program, based on a classical BLUP animal model, that shaped the Italian Large White pig breed. All boars of this breed born from 1992 to 2012 with estimated breeding value (EBV) reliability >0.85 (n=200) were genotyped with markers in five major genes (IGF2 intron3-g.3072G>A, MC4R p.D298N, VTNR PRE1 insertion, PRKAG3 p.I199V and FTO g.276T>G) and with about 60,000 single nucleotide polymorphisms (SNPs) of the Illumina PorcineSNP60 BeadChip. Genotyping data were analysed grouping boars in several classes according to their year of birth. Then we evaluated the influence of the time on allele frequencies for all analysed markers using a multinomial logit regression model. The markers in the IGF2, MC4R, VTNR and FTO genes showed significant changes of allele frequencies over the last 20 years, according to the directional selection program in this breed. In addition, other 493 SNPs changed significantly allele frequencies over the considered period ($P<0.10$, Bonferroni corrected). These SNPs were not evenly spread along all autosomes, in particular there were windows enriched in significant SNPs on chromosomes 2 , 7 and 8. Haplotype analysis was carried out including these SNPs. In addition, runs of homozygosity (ROH) identified in the Italian Large White boar population reported other selection signatures and provided a genomic measure of inbreeding over the considered time window. Most of these markers and genomic regions were very close or included annotated genes providing potential information on the functional effects of these allele shifts and fixations. These results indicated that selection carried out during the last two decades modified substantially the genome of the Italian Large White pig breed.

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C-070

Next generation semiconductor based sequencing of bitter taste receptor genes in different pig breeds and populations and association study of identified polymorphisms using a DNA pooling strategy

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Taste perception has been shown to affect feed preference and feed intake. In particular, bitter taste perception in animals evolved as a key mechanism for the protection against toxic and harmful foods. Taste 2 receptors (TAS2Rs) have been identified as the members of the bitter taste receptor family. These receptors are G-coupled receptors (GPCRs) that activate G proteins linked to the trasductional bitter perception signal. In this work we amplified and sequenced 10 TAS2R genes (TAS2R1, TAS2R3, TAS2R4, TAS2R7, TAS2R9, TAS2R10, TAS2R16, TAS2R20, TAS2R38 and TAS2R39) in six pig breeds (Italian Large White, Italian Duroc, Italian Landrace, Pietrain, Casertana and Meishan) and in wild boars using the next generation semiconductor sequencing platform Ion Torrent PGM. The experimental design was based on a target PCR amplification step that was carried out on equimolar DNA pools from animals of the same breed or population. Moreover, two pools were prepared including DNA each from 50 Italian Large White pigs having extreme and divergent estimated breeding values for back fat thickness (BFT), as polymorphisms in TAS2R genes are associated with obesity and fat deposition traits in humans and other species. The other breed specific pools were prepared each with DNA from 5-15 animals. After barcoding of the different breed/population specific amplicons, the prepared library was sequenced using the Ion Torrent PGM sequencer. A total of 2,513,009 reads was obtained and after quality control and read alignment, a total of 130 single nucleotide polymorphisms (SNPs) were identified in coding and non coding regions of the target genes. Some of these SNPs were identified in one or a few breeds. Estimation of allele frequencies from read counts within each barcode was validated by PCR-RFLP genotyping of four SNPs. Comparing estimated allele frequencies from the two extreme DNA pools for BFT, it was possible to identify several SNPs associated ($P<0.05$, Fisher exact or chi square tests) with this trait. This approach can, at the same time, directly discover SNPs and analyse their allele frequencies for the association analysis, speeding up the identification of markers affecting production traits. Polymorphisms identified in TAS2R genes will be used to evaluate feed preferences of pigs with different genotypes to develop nutrigenomics applications.

Acknowledgments

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C-071

Quantification of boar taint compounds in adipose tissue of intact male pigs at 160 and 220 days of age and estimates of genetic parameters

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Boar taint (BT) is mainly due to accumulation in adipose tissue of intact male pigs of three compounds: androstenone (AND), skatole (SKA) and indole (IND). In order to investigate effectiveness of selective breeding as a tool to reduce the risk of BT in intact males, aims of this study were to assess the variability of BT compounds content in adipose tissue of intact male pigs at 160 and 220 d of age and to estimate genetic parameters for those traits. A backfat biopsy sample was collected from the neck area of 500 intact male pigs of the C21 boar line (Gorzagri, Fonzaso) at 160 (106±9 kg) and 220 d of age (154±14 kg). In addition, a sample of subcutaneous fat was collected at slaughter from the left ham of 100 of the investigated animals. Quantification of AND, SKA and IND in fat was performed by HPLC with fluorescence detection. Estimates of genetic parameters were obtained through univariate and bivariate Bayesian analyses. Contents of BT compounds measured at 220 d were higher than those at 160 d of age. At 220 d the percentage of samples exceeding sensory thresholds discriminating tainted from untainted pork was twice as high as the one at 160 d. Hence, prevalence of BT is expected to be greater in mature and heavy pigs than in young and light pigs. Phenotypic correlations between contents of BT compounds measured in backfat and ham subcutaneous fat were 0.76, 0.88 and 0.70, for AND, SKA and IND, respectively, indicating that concentrations of BT compounds in hams were consistent with those in backfat. Medium-high heritabilities for BT compounds were estimated at both ages. In particular, at 220 d heritabilities were 0.58, 0.60 and 0.69 for AND, SKA and IND, respectively. Genetic correlations between the contents of BT compounds measured at the two different ages were 0.67 for AND, 0.47 for SKA and 0.74 for IND, suggesting that concentrations of BT compounds assessed at 160 and 220 d cannot be treated as repeated measures of the same trait. In addition, moderate Spearman's correlations indicated that animals ranked differently when using measures of BT compounds at the different ages. In conclusion, the results suggest that selective breeding seems to be a suitable tool to reduce BT in pigs slaughtered at heavy weight and it might be exploited as an alternative to surgical castration of piglets.

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C-072

Study on genetic parameters of subcutaneous fatty acid composition in Italian Large White pig breed

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The fat content and fatty acid composition of porcine meat and carcass influence nutritional value of meat and various technological aspects of meat and carcass quality. Variation in lipid composition has an important effect on firmness of the fat in meat, especially the subcutaneous and intermuscular (carcass fats) but also the intramuscular (marbling) fat. Moreover, as a major source of energy and as structural components of membranes, fatty acids are essential for life. The aim of the study was to estimate genetic parameters for backfat fatty acid composition (FAC) and intramuscular fat content (IMF) of semimembranosus muscle in a sample of 949 Sib-tested Italian Large White pigs. Backfat tissue and semimembranosus muscle tissue samples were collected at slaughter and stored at -80°C. IMF content and backfat FAC were determined by gas chromatography and analysis of the fatty acid methyl esters. Heritability (h2) and genetic correlations between subcutaneous fatty acids composition and carcass and meat quality traits were estimated using the restricted maximum likelihood methodology applied to a multiple-trait Animal Model. The model was: Yijklm = μ + sexi + dj + b1ak + animall + eijklm where Y = analysed traits; μ = general mean; sex = fixed effect of sex; d = fixed effect of slaugher day; b1 = regression coefficient of the fixed effect of age at slaughtering (ak); animal = random additive effect of each animal; eijklm = error of the model. Results showed that the estimate of h2 for FAC were of moderate magnitude. This study suggest that backfat FAC is genetically correlated with carcass quality traits and the selection for carcass and meat quality traits produces a correlated response on FAC of subcutaneous fat. The reduction in fat covering tissue is associated with an increase of lipid insaturation and a decrease in subcutaneous fat firmness that determines technological problems in dry cured ham quality. Very low genetic correlations were found between IMF content and backfat fatty acids. The results found suggest the possibility of genetic improvement of FAC of subcutaneous fat by a partial substitution of saturated fatty acids with monounsaturated ones. This would not hinder the technological characteristics of hams for seasoning while improving its eating quality.

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C-073

Perilipins and the complex gene network of lipid metabolism enzymes in muscle are related to porcine intramuscular fat deposition

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The characteristics of swine seasoned products are strongly affected by porcine meat quality and intramuscular fat (IMF) content. Most of the genetic processes influencing pork meat quality and composition are still poorly known, particularly for the genes and networks that regulate IMF deposition. Thus, a better knowledge of genes affecting IMF content and meat quality is of primary interest for pig industry. To this aim, we chosen 11 genes involved in lipid metabolism, including Fatty Acid Synthase, Stearoil-CoA Desaturase, Adipose Triglyceride Lipase, Hormon-Sensitive Lipase, Monoglyceride Lipase, Lipoprotein Lipase (respectively FASN, SCD, ATGL, HSL, MGLL, LPL) and the Perilipin family genes (PLIN1, PLIN2, PLIN3, PLIN4, PLIN5). Since today, Perilipins are poorly investigated in pig and, though they have been associated to obesity in human, their specific function and role are unclear. The aim of this research is to test Perilipins effect on IMF deposition and their possible interactions and linking with other lipid metabolism genes. Among a population of 949 Sib tested Italian Large White (ILW) pigs, a set of 47 individuals (25 pigs with low IMF and 22 with high IMF) was selected for an expression study of the above-reported genes. PLIN1, PLIN4, PLIN5, ATGL, LPL, FASN, MGLL showed a statistically significant difference in transcription levels between the two groups, with a higher expression in pigs with a major IMF deposition. In particular, PLIN4 transcriptional level seems to be correlated with most of the expression levels of the considered genes. Moreover, PLIN2 might cover a different role in IMF deposition as, unlike the other differentially expressed genes, the lower is IMF and the higher is PLIN2 transcription. Furthermore, a SNP identified in the downstream region flanking the 3’UTR of PLIN5 gene was investigated on 500 samples belonging to the 949 ILW pigs. An association study between the PLIN5 SNP and some carcass and meat traits was performed, revealing significant effects with backfat fatty acid composition and meat quality characteristics. The obtained results suggest a role of Perilipin genes on IMF deposition and meat quality traits. Further studies are in progress to enlighten the genetic links between these genes and IMF content and disentangle the relations linking PLINs to complex networks of genes related to fat deposition in pigs.

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C-074

Survey on pre-slaughter handling and skin damages on pig carcasses

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Various stages during pre-slaughter handling, such transportation, lairage, and stress immediately before slaughter can negatively affect animals welfare and quality of carcasses. Rough practices at loading and unloading and inappropriate driving style of trucks and pigs may lead to serious skin damages. They represent a relevant economic losses, especially if are located on the most valuable cuts such as hams, loins and shoulders. In order to study the relationship between pre-slaughter handling and skin damages, a survey was carried out from January to June 2014 at one abattoir located in the Lombardia region. Data were collected from 1680 heavy pigs delivered from one farm to the slaughterhouse in 12 batches, each containing 140 subjects. Double trailer lorries with three hydraulic deck were used. At unloading of each deck, vocalizations, slips, falls, overlapping, balking and reversal were recorded. Resting duration and time requested to drive pigs to the stunning cage were measured. During this latter procedure, the same behavioural occurrences considered at the unloading were also recorded. Before evisceration, skin damages on regions of ham, loin, shoulder and head of each carcass were assessed by one trained inspector assigning for each region a score from 1 (absent) to 5 (serious). Score's averages were found higher on carcasses of pigs transported into the trailer with respect to those transported into the lorry. Scores were affected by deck of the lorry, the upper showing the higher score. Behavioural occurrences, resting time and driving showed weak relationships with the damage scores. Probably, the handling carried out by the staff of slaughterhouse could be responsible of this result. This research highlights that some stages before and during transport could be more involved in skin damages than handling at the slaughterhouse. However, additional research is necessary to understand the role of pre-slaughter handling on the presence of skin damages on pig carcasses.

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C-075

An investigation of the shelf-life of PDO *Vastedda della Valle del Belice* cheese subjected to different packaging systems alternative to vacuum

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The aim of this study was to evaluate the effect of different packaging systems alternatives to vacuum that is commonly applied for PDO *Vastedda della Valle del Belice* (VdB) cheese, a tipical pasta filata cheese, made with raw ewes' milk. After production following the traditional cheesemaking protocol, VdB cheeses were packaged as follows: vacuum, paraffin wax and two different Modified Atmosphere Packaging (MAP) consisting of a gas mixture of 70% CO2-30% N2 (MAP1) and 100% N2 (MAP2). Cheeses were stored at 4°C and analyzed (25 g) at time 0 and after 15, 30, 60, 90, 120 and 180 days. All the samples were subjected to microbiological analyses for the evaluation of total mesophilic aerobic bacteria, Enterobacteriaceae, total coliform, *Escherichia coli*, enterococci, lactic acid bacteria, positive coagulase staphylococci, sulphite reducing anaerobes, *Pseudomonas* spp., *Listeria monocytogenes* and *Salmonella* spp. Moreover, the physicochemical parameters such as pH, water activity, dry matter, fat, protein, salt, total solids, total and soluble nitrogen and ash content were also determined. Chemical, physical and microbiological data were analyzed by a two factors ANOVA model (GLM procedure of SAS) which included the effect of packaging and time of storage. The results did not show significant differences among chemical and physical parameters of the cheeses subjected to the different packaging during storage. No pathogens and spoilage microbial species/group were detected in any of the samples examined. Lactic acid bacteria reached the highest levels for all samples. Total microbial counts detected for paraffin packaged cheeses were higher than those of the other cheeses, regardless the storage time. This observation was probably due to the higher percentage of oxygen allowed by paraffin respect to the other packaging strategies. For this reason, paraffin was excluded as a possible tool for long term storage of VdB cheese. It was also observed an increase of the total microbial load in all packaging systems after 60 days of storage. Sensory analyses are still ongoing.

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C-076

Plant secondary metabolites and traceability of dairy products: a key role for livestock production and efficiency in ruminant dairy products

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Primary metabolites, such as amino acids, organic acids and carbohydrates are essential for life and exist in all plants. Secondary metabolites (PSM) are not directly involved in the normal life cycle and they play an important role in plant defence, adaptation to environment, colouring and other metabolic functions. The term ‚Äòsecondary’ is based on early research in which metabolism was thought to be partitioned into a primary component that directly supports growth, development, and reproduction, and a secondary component that has no direct role in these primary functions. Traditionally, PSM have been considered as ‚Äòanti-nutritive’ since they are often bitter, deter animals from eating the plant, or cause aversion through interfering with animal digestive and fermentation processes or directly affecting host metabolism. However, more recent trends and research for feed in the livestock production have stimulated interest in PSM, as they may benefit animal production when they are fed in the correct form and dose. It is possible to classify secondary metabolites as: phenols, sulphur-containing compounds, terpenes alkaloids, lipids (coronaric acid) and enzymes (polyphenol oxidase, PPO). To improve the nitrogen use efficiency during ensiling the use of tannin containing forages has been beneficial (2-4% DM in tannins contents) with an estimated profit in US dairy farms estimated at $300 million/y with reduced nitrogen losses of 25%. Tannins have shown antimethanogenic properties (-15%) with different tannin sources showing variable results on on palatability with low adverse effects on animal performance and organic matter digestibility. In Italy several PDO cheeses are produced from grazed pastures with strong seasonality, typical of the mountainous nature and Mediterranean climate. The cheeses sourced from grazed herbage are characterized by a higher content of volatile compounds compared to cheese made from animal fed at stall. The volatile compounds, besides giving a characteristic flavour to the cheese, can also be used as bio-markers as indicative compounds pass from the pasture to the cheese. Polyphenols oxidase, tannins, coronaric acid are capable when properly evaluated, to modulate the livestock system making it more efficient and more environmentally sustainable. Finally of particular interest is the role of grazing ruminants in land management and landscape re-evaluation for tourism, a key element to prevent the depopulation of rural areas.

C-077

Cytotoxicity, DNA integrity and methylation in mammary and kidney epithelial cell lines exposed to Ochratoxin A

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Ochratoxin A (OTA) is a secondary metabolite of moulds that may contaminate food and feedstuffs. OTA is recognized as a nephrotoxic, hepatotoxic and teratogenic substance in different animal species. The kidney is the target organ of OTA, whereas lower OTA concentrations could be detected in other tissues, such as adipose tissue, liver and muscles. In addition, OTA transfer to milk has been demonstrated in several species, such as humans, rabbits, rats and ruminants, identifying the mammary gland as one of the potential target of this mycotoxin. This study aimed to investigate the *in vitro* damage induced by OTA using two well established epithelial cell lines. MDCK cells have been used as a model of the kidney epithelium, while BME-UV1 have been employed as a model of the mammary gland epithelium. The effect of OTA on cultured cell lines, with subsequent evaluation of cell viability (MTT test) and membrane stability (LDH test), was assessed. In all experiments performed, control consisted of MDCK and BME-UV1 cells exposed to basal medium alone. After 24h of OTA treatment (concentration range 0.15 up to 10 μg/mL), MDCK and BME-UV1 cell viability was strongly reduced in a dose-dependent way and LC50 has been calculated. LC50 for MDCK cells was 1 μg/mL while, for BME-UV1 cells, LC50 was 0.8 μg/mL. In light of LC50, the range of concentrations for further experiments was determined (0.3 up to 1.25μg/mL). The percentage of LDH released by MDCK and BME-UV1 cells increased in a dose-dependent way. In particular, 1.25 μg/mL of OTA determined a 35% of LDH released in MDCK cells and a 46% of LDH released in BME-UV1 cell line. Subsequently, the effect of the addition of OTA to MDCK and BME-UV1 cells has been evaluated on DNA integrity, which was detected by gel electrophoresis, by the analysis of DNA oxidation biomarker 8-OHdG (8-OHdG adduct) and the global DNA methylation status (5-mC). The level of 8-OHdG adduct was significantly ($P<0.05$) increased in BME-UV1 cells treated with 1.25 μg/ml of OTA. The analysis of 5-mC revealed that in MDCK and BME-UV1 cells, OTA has not induced alterations in the global DNA methylation status. The results obtained herein could represent the basis for the development of future studies investigating the *in vitro* relationship between DNA damage and the global DNA methylation status, promoting new strategies to control OTA cytotoxicity at different tissue level.

C-078

Predicting survivors of neonatal calf diarrhea using logistic regression or gradient boosting

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Neonatal calf diarrhea is a serious welfare problem and a cause of economic loss due to mortality, treatment costs and poor growth. Moreover, it is a good example of a complex and multifactorial disease, resulting from the interaction between the calf, the environment, the nutrition and the different infectious agents. The objective of this retrospective study was to use clinical data and ancillary test examination findings from dairy calves diagnosed with NCD to predict the outcome of the disease. Data refer to medical records of 131 Holstein calves (males=16, females=115), affected by NCD and hospitalized from January 2006 until August 2014 at the Clinic For Ruminants, Swine and Management (CRSM) of the Large Animals Veterinary Teaching Hospital, University of Milan. Age, emogas analysis, total protein, dehydration and vitality scores and rectal temperature were available for each calf. Logistic Regression (LR) and Gradient Boosting Machine (GBM) were used to estimate the probability of the disease outcome (coded as 0 or 1 if the calf survived or died, respectively) based on the available medical records. The effect of sex and month of hospitalization were also fitted. To build the predictive model a cross validation procedure was adopted. First, the 131 records were randomly split in a training and a testing datasets including 75% (n=92) and 25% (n=39) of the observations, respectively. Then, the training dataset was used to build the predictive model with a 5-fold cross validation scheme. Finally, the obtained predictive model was used to predict the probability of the disease outcome (dead/survived) in the testing data. Before implementation of the cross-validation procedure data were checked for multicollinearity and standardized. The following statistics were obtained: 1) the Accuracy (AC), i.e. the proportion of the total number of predictions that were correct 2) the Sensitivity or true positive rate (TPR), and 3) the Specificity or True Negative Rate (TNR). The absolute value of the t-statistic for each parameter included in the predictive model was used for estimating its contribution to the model itself. The AC, TPR and TNR were 0.70, 0.74 and 0.58 and 0.54, 0.63 and 0.33 for LR and GBM, respectively. Total protein and Anion Gap were the major factors associated with mortality. These results represent a first attempt to assist the veterinarian in defining a more accurate prognosis and choosing the best therapeutic protocol.

C-079

The hidden costs of animal health in dairy cattle breeding: evidences from a case study

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Inaccurate management of dairy cattle health entails problems of animal health and represents a cost that need to be correctly evaluated in order to ensure the economic success of breeding activity. In term of organizational and accountancy aspects, the agricultural sector seems to be underdeveloped compared to technologically advanced food firms, and farmers likely underestimate these costs. The present research aims at quantifying the economic loss due to animal health and focusing on the "hidden costs" of sanitary treatments in dairy cattle breeding. According to accounting theory, the costs of animal health management are divided into two parts: explicit and implicit costs. In our analysis, the former include the amount of money required for the purchase of veterinary medicinal products, while the latter result from the revenues' losses due to milking suspension of treated cows. We refer to implicit costs as "hidden costs", because they do not imply a tangible expenditure, but they rather need to be accounted as income losses. In the present research, a sample of farms have been analyzed calculating the real (explicit) and estimated (implicit/hidden) accountable costs due to pathologies occurred over two years. The analysis proposes an economic efficiency index of veterinary cost per livestock unit (€/LU) in order to evaluate the total costs of sanitary treatments in dairy cattle breeding and compare different farms' performance. Results shows that farmers can spend from 89.43 to 211.98 €/LU per years due to cows' pathologies, and sometime hidden costs (i.e. implicit costs) overcome explicit expenditures. The study suggests the importance of managing and monitoring in order to provide targeted interventions. No process monitoring, i.e. lack of information, implies difficulties related to problem solving and decision-making issues. Considering that the case study focuses on a small sample of farms, results may not be generalizable to the dairy cows sector. Nonetheless, the calculated losses should encourage farmers in introducing an accurate plan for collection and processing of sanitary data in order to: (1) assess the health status of the herd; (2) identification of the priority animal care action area; and (3) optimization of resources and maximization of profits.

C-080

Nutritional and functional evaluation of cereals and legumes seeds

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Starch digestibility is generally high but is affected by several factors (i.e. amylose/amylopectin ratio, particle size, heat treatments, processing method, associative effects in mixed diet). Starch is primarily digested in small intestine by enzymatic degradation (α-amilase and glucoamylase), but some can escape in the large intestine where it is fermented by microflora. The glycemic index is used in human nutrition to classify carbohydrates sources in function of their potential to increase blood glucose. Recently, several authors have suggested a similar approach in pig nutrition. In this study, the feed factors influencing starch digestibility were evaluated investigating the carbohydrates profile of cereal and legume grains. In particular, 5 cereals (corn, oat, barley, rice, wheat) and 4 legumes (pea, lentil, faba bean, bean) were analysed. Structural carbohydrates composition and Prosky and De Vries and starch characterization were done. In order to predict the potential glycemic index, the digestibility of starch was evaluated *in vitro* by measuring glucose release at 0, 15, 30, 60, 90, 120, 180, 240, 300, 360, 420 and 480 min after the enzymatic treatments (Englyst et al., 1992 Eur J Clin Nutr, 46: 33-50; Sun et al. 2006 Anim Feed Sci Tech, 131: 66-85). Total dietary fiber (TDF) resulted highly variable (24±13 and 27±5% a.f. for cereals and legume, respectively). Excepted oat, all samples showed a proportion of insoluble fiber higher than 80%. The soluble and insoluble fibre in oat was 41 and 59% of TDF, respectively. Total starch level in cereals was about double (76±15% a.f) than in legume grains (43±11% a.f). The main differences were observed for starch fraction: legume seeds showed higher resistant starch level (56±7 *vs.* 20±7 % of starch). The rapidly digestible starch (RDS) and slowly digestible starch (SDS) were also different between cereals and legume grains. In particular, cereals RDS (33±10% of starch) was triple than in legumes (10±4% of starch), probably due to the higher proportion of amylose respect to amylopectin and of the relative encapsulation of starch in legumes. The potential glycemic index values varied among the feeds, but the relative starch digestion kinetics only partially reflected starch classification.

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C-081

Nutritional value of two different insects meal (*Tenebrio molitor* and *Hermetia illucens*) for broiler chickens: apparent ileal amino acid digestibility and apparent metabolizable energy

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The aim of this study was to determine the apparent metabolizable energy (AME) and the apparent ileal amino acid digestibility coefficients (AIDC) of two insect meals (*Tenebrio molitor* and *Hermetia illucens*) in broiler chickens. Day-old male broiler chickens (Ross 708) were raised in floor pen till the age of 19 d. On day 19, ninety birds (3 each cage/10 replicates) of uniform body weight were selected and kept in thirty 2-floor cages. On d 26, birds were randomly allotted to the three experimental diets. A basal diet (BD) was administered for the adaptation period lasting 5 d. Two assay diets were developed by substituting 25% of the basal diet with *Tenebrio molitor* meal (TM) or *Hermetia illucens* meal (HI). For each diet, in order to calculate digestibility of amino acids (AA), celite was added. Digestibility was evaluated by the total collection of feces method from 31 to 34 d. AME was calculated using the following formula:
AME insect meal (%; MJ/kg) = [AME of insect meal diet – (AME basal diet × 0.75)] / 0.25
Correction for zero nitrogen (N) retention was made using a factor of 36.54 kJ per gram N retained in the body in order to estimate the N-corrected apparent metabolizable energy (AMEn) (Hill and Anderson, 1958). N retention was calculated using the following formula:
N retention = [(feed intake × N diet) – (excreta output × N excreta)]/feed intake (kg)
On day 35, all birds were euthanized to sample the content of the lower half of the ileum to perform the apparent ileal amino acid digestibility. Data were analyzed using Student's t-test for independent samples, with cage as the experimental unit. Differences were considered to be significant at $P<0.05$. No statistical differences between TM and HI were observed for AME and AMEn. The AME and AMEn values of TM and HI meal resulted 16.86 and 17.38 MJ/kg DM and 16.02 and 16.60 MJ/kg DM, respectively. The average AIDC of the 17 AA studied was higher ($P<0.001$) in TM than in HI, because the AIDC of isoleucine

(0.822 *vs.* 0.446), lysine (0.852 *vs.* 0.463), methionine (0.804 *vs.* 0.424), phenylalanine (0.912 *vs.* 0.630), valine (0.819 v 0.622), alanine (0.928 *vs.* 0.861), aspartic acid (0.892 *vs.* 0.615), glycine (0.888 *vs.* 0.666), glutamic acid (0.855 *vs.* 0.741) and tyrosine (0.832 *vs.* 0.427) was higher (P<0.05) in TM than in HI. Overall, the present results showed that TM and HI meals are an excellent source of AME. TM is a good source of digestible AA, while HI is a moderate one.

C-082

Effect of alfalfa hay digestibility and inclusion rate in lactating dairy cows diets on feed intake, digestibility, ruminal pH and productive performance

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The objective of this study was to investigate the effects of two alfalfa hay differing in digestibility in high producing cows diet with two levels of forage neutral detergent fiber (NDF) inclusion (HF and LF respectively), on feed intake, digestibility, ruminal pH and productive performance. Alfalfa hay NDF *in vitro* digestibility at 24 and 240 hours were 40.2% and 31.2%, and 53.6 and 45.7% for high (HD) and low digestibility (LD) respectively. The four experimental diets (HF-HD; LF-HD; HF-LD and LF-LD) contained, on a dry matter basis, 46.8, 36.8, 38.8 and 30.1% of alfalfa hay respectively, 8.6% straw and 34.4% corn (50% flake and 50% meal), while soyhulls and soybean meal were used to replace part of the hay in order to balance protein and energy among diets. Eight multiparous Holstein cows (average milk production 46.0±5.2 kg/d, 101±38 days in milk and 662±42 kg average BW), were assigned to a 4 x 4 Latin square design, with 3 weeks adaptation and 1 week of collection. Dry matter intake, milk production and quality, and rumination time were measured daily. Ruminal pH was measured continuously at 10 min intervals. Fecal samples were collected at multiple time points and averaged daily. Diets and feces were analysed for aNDFom, ADF, ADL, undigestible NDF (uNDF) and potentially digestible NDF (pdNDF), in order to calculate total tract fiber digestibility. Starch and protein analysis were performed on each diet. All data were statistically analyzed with a factorial arrangement of treatments using the PROC MIXED model in SAS. Milk production was not different, while dry matter intake was higher in HD diets (P≤0.01). Rumination time was significantly higher for HD diets when compared to LD diets (P≤0.01), regardless of forage amount. Rumination time was constant per unit of dry matter and differed when related to uNDF (P≤0.05), aNDFom (P≤0.01) or peNDF intake (P≤0.01). No differences were found among treatments on average ruminal pH, but the amount of time with pH <5.8 tend to be lower in HF-HD diets (P≤0.10). Total tract NDF and pdNDF digestibility showed good results for all the experimental diets. Higher digestion was observed in the LD diets (88.3 *vs.* 85.8 in HDs) (P≤0.01), in which a lower feed intake was also observed. This fact could suggest that retention time is the first limiting factor for ruminal fiber digestibility. HF-HD diet allowed the higher dry matter intake, rumination time and the best ruminal pH values.

C-083

Relationship between horse faecal particle size and forage type/dry matter digestibility

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The aim was to compare the relationship between horse faecal particle size (FPS) and dry matter digestibility (DMdig) of several forages. Six horses (live-weight 110-840kg) housed in individual boxes with rubber bedding, access to paddock 3 hours/d except 24 h before sampling were considered. Horses were fed *ad libitum* four forages successively: lucerne; fescue; mix perennial rye-grass/clover; straw. The trial lasted from October to January. Adaptation period between the different forages: minimum two weeks. Faecal samples, representative of 24h of feeding, were collected in the morning and preserved at -20°C for the analysis. Forage samples were dried at 65°C for 18h, ground in a mill to pass a 1.0 mm screen. Forages composition was determined by NIR (ash, protein, fat, NDF, ADF, ADL, NDFD 24h). FPS determined by wet sieving on a rack of six sieves (mesh size: S0: 6.50; S1: 4.60; S2: 2.36; S3: 1.18; S4: 0.6; S5: 0.15 mm). Dry matter digestibility (DMdig) was determined by acid insoluble ash (AIA) method. Average DMdig of forages: lucerne 93.92%; fescue 53.19%; mix perennial rye-grass/clover 73.13%; straw 44.58%. Statistical analysis was carried out using SPSS (vers. 21). GLM multivariate procedure was applied for the general analysis, considering as independent variable forage type-average DMdig or horse; as dependent variable sieve residue percentage (RP). Lucerne (highest digestibility) showed the highest RP on S0 (9.37%) and S1 (11.82%), the lowest (18.16%) on S5 (P<0.001). Similar results were obtained for the mix (medium-high digestibility) which showed a high RP on S1 (6.05%), a low RP on S5 (18.75%) in comparison to fescue and straw. Straw (lowest digestibility) showed a low RP on S0 (0.13%) a high RP on S5 (27.93%) and the highest RP on S4 (38.26% P<0.001). Fescue showed the highest RP on S5 (33.50 P<0.001). An explanatory model of the relationship between forage digestibility and FPS was obtained calculating the correlation and the regression between forage DMdig and dry matter residue on the different sieves. The higher correlation and the more robust regressions were found for sieve S1 (r=0.942 P<0.001;

$y=3.889x+46.495$; $R^2=0.888$) and S4 ($r=-0.707$ $P<0.001$; $y=-1.416x+100.389$; $R^2=0.499$). It appears that in general FPS distribution is different among forage types. Digestibility seems to deeply affect FPS, reduced with the decrease in digestibility. The measure of residues of particles <6.50 and >4.60 (S1) is the most correlate with DMdig in horse.

C-084

In vitro rumen fermentation of barley grain contaminated with *Fusarium* mycotoxins

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Grains from nine barley cultivars (Aldebaran, Concerto, Dasio, Grace, Kangoo, Odyssey, Otis, Scarlett, Tunika) either naturally contaminated with *Fusarium* toxin (T2/HT2: 312.3±154.2 ppb) or not-contaminated were incubated *in vitro* at 39°C under anaerobic condition with buffered bovine rumen fluid. In order to estimate the fermentation process, the gas production was recorded for 72 h using a manual system. At the end of incubation, pH and organic matter degradability (dOM, %) were determined. Data were statistically processed to analyse the contamination effect. According to our previous results all barley grains showed a high gas production and dOM and a fast fermentation process, due to their high fermentable carbohydrates content. The T2/HT2 toxin significantly ($P<0.05$) lowered dOM (82.75 *vs.* 85.28 %) and gas production (OMCV: 330 *vs.* 335 ml/g) whereas the contaminated sample of cv. Scarlett showed a significantly higher OMCV (356 *vs.* 336 ml/g, $P<0.01$). Concerning fermentation kinetics, in most of cultivars the toxin contamination significantly ($P<0.001$) lowered the maximum fermentation rate (Rmax: 16.37 *vs.* 18.17 ml/h) and the time at which it occurs (Tmax: 2.58 *vs.* 2.71 h). By contrary, cv. Otis and Kangoo showed a higher ($P<0.05$) fermentation rate in contaminated samples. According to other authors mycotoxins affected the activity of rumen microbes but, in the present trial, the level of contamination didn't seem to be related to the *in vitro* fermentation and the different chemical composition of cultivars probably affected the results. Further information will arise from the in progress determination of volatile fatty acids at the end of fermentation.

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C-085

Evaluation of inoculants addition on corn silage nutritional value

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Utilization of chemical or biological additives to optimize ensiling process is focused into an improvement of the nutritional fractions of forages. In this fashion, Silo-King® would act in stabilizing pH, reducing silage temperature and dry matter loss, improving organic matter digestibility, and, overall, an increased nutrient availability. Harvested corn was treated according to the experimental protocol: the first portion was treated with 10 mg/kg solution of inoculant (T2), the second one with 6 mg/kg solution (T1), while the third one with de-ionized water at 10 mg/kg (C). From every treatment, 42 samples (0.9 to 1.0 kg weight) were randomly collected, pressed in mini-silos and sealed. At 0, 15, 30, 60, 90, 120, 180 and 240 days of ensiling, replicates per each treatment were opened and analyzed for dry matter, ashes, aNDFom, ADF, ADL, NDIP, ADIP, SolP, ammonia, starch digestibility at 7h, sugars and *in vitro* aNDFom digestibility at 24, 48 and 240 hours. All data were analyzed with statistical software via factorial ANOVA. Dry matter and ash content were not different among treatments. Averaged values of CP were statistically different for T2 *vs.* T1 and C, (9.27, 8.89, 8.92% respectively $P\leq0.05$), while no differences were found for ADIP and NDIP. Ammonia was higher in control samples, with significance at 120, 180 and 240 days (0.68, 0.9 and 1.58% for T2, T1 and C respectively at 240d. $P\leq0.05$). Results about fibrous fractions suggest no impact of the treatment. Values of aNDFom were similar among different thesis (~37-40, % on DM basis), and in line with the expected. No differences were observed for ADF or ADL. *in vitro* NDF digestibility at 24 and 48 hours, showed higher values for T2 and T1 compared to C ($P\leq0.05$). After 180 days of ensiling, observed values were 36.9, 38.9 and 31.9 % at 24h, 53.9, 50.6 and 42.9% at 48h, for T2, T1 and C, respectively. Sugar content decreased as the ensiling process, while starch digestibility resulted differently among treatment, being higher for treated samples compared to control after different ensiling points (82.7, 65.2 and 57.1% at 180d for T2, T1 and C. $P\leq0.05$). Present study demonstrates that inoculants led to higher amount of soluble protein preserved, resulting in less ammonia formation, and to an increased fiber and starch digestibility. From a nutritional point of view, Silo-King® improved energetic yield of the treated substrate, for both nitrogen source and carbohydrates.

C-086

Pasta by-product as alternative source of starch in diets for finishing pigs

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Corn is the most cereal grain used in heavy pig diets. However, high level of corn in diets might yield yellow-coloured ham with soft subcutaneous fats prone to oxidation. The study evaluated the effect of pasta by-product (Pbp) as alternative starch source to corn in diets for finishing pig on growth performance and carcass characteristics. 144 Italian Duroc×PIC pigs (72 females (F) and 72 castrated males (CM); BW: 103±3kg) were randomly allotted to 1 of 4 dietary treatments (0, 30, 60, 80% Pbp inclusion) for 91 days in a completely randomized design with a 2x4 factorial arrangement. There were 6 pens per treatment (3 F and 3 CM). Diets were: corn-based diet (CTR); T1: 30% of Pbp; T2: 60% of Pbp; T3: 80% of Pbp. The diets were formulated according to requirements, iso-nutritive and pelleted. The feed intake was recorded daily and average daily gain (ADG), average daily feed intake (ADFI,) and gain to feed ratio (G:F) were obtained. The carcasses and trimmed thighs were weighed at slaughter and yields calculated. The thicknesses of the back fat and Longissimus thoracis et lumborum muscle (LTL) were recorded and the carcass lean percentage estimated. The pH was measured at 45 min and 24 h post mortem on Biceps femoris on cooled thighs. Pen was the experimental unit and gender and Pbp were considered fixed effects. There were no Pbp × sex interactions. Pigs fed Pbp had greater (linear, $P<0.05$) ADFI than pigs fed the CTR diet. Nevertheless, no differences were detected on final BW, ADG and G:F ratio. The carcass weight and dressing percentage were higher ($P<0.05$) in CM compared with F (131 *vs.* 128kg and 78.7 *vs.* 76.8%, respectively) and there was a quadratic effect of Pbp (126, 132, 130, 130kg for carcass weight and 76.1, 80, 78, 76.8% for dressing percentage, respectively for CTR, T1, T2 and T3). Females had lower ($P<0.05$) pH measured on Biceps femoris at 45 min post mortem (6.07 *vs.* 6.1) than CM and there was an effect (quadratic, $P<0.05$) of Pbp (6.17, 6.06, 6.02 and 6.04, respectively for CTR, T1, T2 and T3). The trimmed thigh weight were similar across gender and Pbp, however the yield was lower ($P<0.05$) in CM compared with F (22.0 *vs.* 22.7%) with a quadratic effect ($P<0.05$) of Pbp (22.7, 21.7, 22.4 and 22.7%, respectively for CTR, T1, T2 and T3). Increasing Pbp linearly reduced ($P<0.05$) the LTL thickness and carcass lean percentage. The Pbp did not affect animal performance and could partially replace corn in diets for finishing pigs.

C-087

Digestibility and energy balance of heavy pig fed high cut corn silage

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In the last years the interest in silage corn products as alternative to dry corn grain has strongly increased in pigs feeding, in order to reduce diet costs although whole-plant corn silage is considered too high in fiber for heavy pigs. However, a high cut (75 cm from soil) of corn plant can reduce its fiber content and makes it promising for pigs feeding. Aim of this work was to evaluate the effects of the inclusion of high cut corn silage (HCCS) in diets for heavy pigs in substitution of 20% DM of dry corn grain and wheat bran, on the digestibility of main nutrients and the energy balance. Two experimental diets were fed to 8 barrows (143 kg BW) divided into 2 groups, 4 fed the control diet (C) and 4 fed the HCCS diet in a 2x2 cross-over design. The diets were based on cereal and soya bean meals, had similar CP content (13.7 and 12.8% on DM for C and HCCS diet, respectively) and different NDF content (13.8 and 18.8% on DM). The animals were housed in metabolic cages to collect feces and urine separately, in 2 collection periods of 7 days. Each animal was housed in open circuit respiration chamber to determine the energy metabolism. The pigs were fed restricted ratio, daily DMI was 6.3% BW0.75. The digestibility of DM, OM and CP of the pigs fed the HCCS diet were 83.5, 85.5 and 81.1%, respectively. These data were significantly ($P<0.05$) lower than those of pigs fed the C diet: 86.3, 88.5 and 85.1% for DM, OM and CP, respectively. No differences were observed between treatments for EE, NDF and ADF digestibility. The energy balance (in percentage of the energy intake) showed, for the HCCS diet, a lower digestibility (86.0 and 83.3% for C and HCCS diet, respectively, $P=0.04$), a higher energy lost as feces (14.0 *vs.* 16.7%, $P=0.01$) and lower urinary energy (2.2 *vs.* 1.8%, $P=0.01$). As a result metabolizable energy was numerically lower for HCCS (81.1 *vs.* 83.1%, $P=0.09$). However, the retained energy was similar for both diets: 36.9 and 37.0% for C and HCCS, respectively. The overall experimental data obtained indicate that the substitution of corn meal and wheat bran for HCCS (20% of DM) slightly reduces the digestibility but not the energy retention. Considering the lower diet cost, the inclusion of HCCS in finishing heavy pig feeding deserves attention. However, growing performance should be checked as well as the feasibility of the automatic distribution of liquid diets containing HCCS.

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C-088

Effect of genetic group and dietary crude protein content on technological properties of thighs intended for dry-cured ham production

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This study (supported by AGER, grant n. 2011-0280) investigated the effects of commercial genetic group (GG: Anas, Danbred, Goland and Topigs) and dietary crude protein (CP) content on several technological properties of thighs aimed to dry-cured ham production. Thighs data from 282 pigs from a feeding study that involved 3 batches of 96 pigs each were used. In each batch, pigs were housed in 8 pens balanced for GG and assigned either to conventional (CONV) or low (LP) CP diets providing 147 or 119 g/kg CP and 6.0 or 4.8 g/kg standardized ileal digestible (SID) lysine in early (90 to 120 kg BW) and 132 or 103 g/kg CP and 4.4 or 3.5 g/kg SID lysine, respectively (12 pigs per pen, gilts and barrows, and 4 pens per treatment in each batch). Pigs were slaughtered when BW averaged 166 kg and carcasses were dissected. After a 24 h chilling period, thighs were dressed, subcutaneous fat thickness was measured and a trained operator scored the dressed thighs. A sample of thighs subcutaneous fat was individually collected to determine fatty acid (FA) composition and iodine number. Thighs were seasoned according to S. Daniele procedures and the weight of raw and dry-cured hams was recorded. Data were analysed according to a linear mixed model including the effects of batch, pen, diet, GG and sex and some interactions. The GG influenced the thigh dressed and seasoned weight and seasoning loss % ($P<0.001$), and affected marbling ($P<0.001$), fat cover ($P<0.001$) and lean colour ($P=0.001$). In particular ANAS, compared to Danbred, produced lighter thighs with a thicker fat cover and reduced seasoning loss. Thighs from Danbred were also more marbled and paler than those from the other GG. Subcutaneous fat of thighs from ANAS was richer in saturated FA than that of Topigs and contained a lower amount of polyunsaturated FA than that of Danbred, resulting in the lowest iodine number. Thighs from pigs fed LP showed higher fat covering thickness and score ($P<0.05$), a more saturated FA proportion and lower iodine number ($P<0.05$), and lower seasoning losses than those from CONV fed pigs, but also an increased marbling score. In conclusion, the provision of low CP and SID lysine diets seems not only economically and environmentally convenient, but it may also improve several important technological properties of raw hams. Nevertheless, the increased marbling score should be taken into account especially when using GG with high marbling tendency such as Dandred and Topigs.

C-089

Multivariate analysis of milk fatty acid composition is able to discriminate dairy farm according to feeding regimen and predicted methane emission

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Diet composition may change milk fatty acid (FA) profile by modulating the amount of acetate and butyrate for mammary neo-synthesis and influencing the rumen biohydrogenation (BH) of dietary FA. Therefore, milk FA may be considered as a marker of the feeding regimen. The aim of this study was to evaluate the ability of linear discriminant analysis (LDA) applied on the milk FA composition to discriminate the feeding regimen adopted in 29 commercial dairy farms, considering the type of roughage in the diet. LDA was also applied to discriminate the farms on the basis of predicted methane emission (PME) rate (kg CH4/head and day) calculated as follow: (0.37+0.0392 GEI+0.0189 NDF–0.156 EE+0.0014 BW)/55.65, where GEI= Gross Energy Intake (Mcal/d), NDF=%NDF in the diet, EE=% ether extract in the diet, and BW= Body Weight (Kg). A stepwise LDA was applied to select the FA that best discriminated the farms on the basis of roughage used (hay, corn silage, other type of silage) or of the PME (<0.41, 0.41-0.44, 0.44-0.48, >0.48). Only variables with a $R^2>0.1$ were retained at the end of the stepwise procedure. From the 56 initial FA detected in milk, 22 or 23 FA were retained in order to discriminate the farms according to roughage type or PME, respectively ($P<0.001$). The first canonical variable of both LDA explained more than 98% of variance. Results showed that farms adopting feeding regimens based on hay (no silage) were clearly discriminated from farms adopting silage as roughage basis of the feeding regimen, moreover farms with feeding regimen containing corn silage were discriminated from farms adopting other type of silage. The absence of silage in the diet was positive related with the content of α-linolenic acid and CLA and negative related with linoleic acid content in milk. Finally, LDA clearly discriminated also the four predetermined group of farms with different PME. High levels of PME were positively related with milk FA originated by rumen metabolism (branched FA and intermediates of rumen BH). On the contrary low levels of PME were related with milk FA originated by mammary metabolism (mainly short chain FA). LDA of milk FA was an effective method to discriminate between farms adopting feeding regimens with different roughage basis. Since this aspect may interfere also with methane emission, LDA may also effectively discriminate farms with different PME.

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C-090

Effects of rosemary extracts on sheep rumen microbiome

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Rumen microbiome has a great influence on ruminant health and productivity (Welkie et al. 2010). Over the years, different plant extracts have been tested for their ability to modulate the rumen microbiome and improve feed digestion and fermentation. Among the evaluated plant extracts, essential oils, tannins, and saponins appeared to have positive effects on rumen protein metabolism, VFA production, methane and ammonia production. The objective of this study was to evaluate the effect of rosemary (*Rosmarinus officinalis* L.) on the populations of major well characterized rumen bacteria. Four Bergamasca x Appenninica ruminally cannulated sheep were used in a replicated 4x4 Latin square design. Sheep received a basal diet composed of 1.5 kg/d of alfalfa hay (13.5% crude protein, CP; 50.6% neutral detergent fiber, NDF; 36.7% acid detergent fiber, ADF; and 5.3% sulphuric acid lignin) and one of the following concentrates (400 g/d): 1) control (CTR); 2) CTR with the addition of 1.75% rosemary essential oil adsorbed on inert support (EO); 3) CTR with the addition of 2.50% dried and ground rosemary leaves (RL); and 4) same as (3) but the rosemary leaves were included in the concentrate before pelleting (RL pellet). Feed was offered twice daily in equal meals (8:00 and 16:00 h) for periods of 21 day. At the end of each period rumen content samples were collected for microbial DNA extraction. Absolute quantification of total bacteria, archaea, protozoa and some select bacterial species were performed using specific quantitative real-time PCR. The results showed no differences in the abundances of total bacteria, protozoa, or Ruminococcus albus among the four treatments. Both the RL and the RL pellet diets decreased the abundance of archaea and Prevotella genus ($P<0.001$). Abundance of Ruminococcus flavefaciens ($P<0.001$), Clostridium aminophilum ($P<0.01$), and Fibrobacter succinogenes ($P<0.05$) were increased by the RL, RL pellet, and EO diets, respectively. Collectively, rosemary extracts can affect rumen microbial populations and potentially improve feed digestion and fermentation. The possibility of decreasing methane emission and protein degradation appears of special interest.

C-091

Integration of data to study goat diversity and adaptation: the AdaptMap project

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Goat breeds are bred successfully in a wide variety of environments making the goat species an optimal candidate to disentangle the genetics of adaptation. The ADAPTmap is an International effort to improve coordination among projects for genotyping and re-sequencing of goat breeds. The objective is to explore diversity and adaptation of goat populations around the world by using traditional and novel approaches. Several international projects aim to disentangle diversity of goat breeds/populations by recording and relating genomic diversity, morphological traits and several geo-climatic parameters characterizing the breeding environment. In addition, large projects have been carried out for genomic analyses of complex traits, providing extensive datasets. Integrating of data from the various sources is an essential step to allow a comprehensive application of population genomics. Heterogeneity of sampling designs, studies objectives, resources and methods of sample collection and recording poses a great challenge in data integration for storage, querying and analysis. Bioinformatic solutions have been developed for efficient merging of genomic data from independent sources. Information has been collected from 14 projects. The number of sampled breeds ranges from 3 to 15 (total 64) and covered in total 21 countries and a wide variability of environments and breeding systems. Most breeds are autochthonous (80%) and mostly bred for meat production (63%). Collection of FAO production environment descriptors (PED) is foreseen for about 40% of the populations. Geographical Information Systems (GIS) information is collected in 3 large projects Methods for identification of hidden relationships among morphological, phenotypic, environmental and geo-climatic data will be applied for the calculation of synthetic indicators. Ten working groups coordinated by leading scientists have been identified covering all aspects of the goat genome: i) the improvement of genome assembly; ii) genome annotation; iii) the study of the possibility to improve the current SNP panel; iv) the identification of parentage identification SNP panel; v) comparative genomics; vi) integration and standardization of phenotypic data; vii) population genetics analyses and population history (domestication reconstruction); viii) Landscape genomics; and, ix) Breeding and genetic improvement. The progress and first results of these working groups will be presented

C-092

Parentage assessment with 200 single nucleotide polymorphisms on 15 Italian goat breeds

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In this study we describe a panel of 200 SNPs for parentage testing in goat, optimized on 15 Italian breeds. Data on 350 goats genotyped with the Illumina 50K SNP array were provided by the Italian Goat Consortium (IGC). Animals belong to 15 breeds/populations farmed in North (Saanen, Alpine, Valdostana, Orobica, Bionda dell'Adamello and Valpassiria), Center (Teramana and Grigia Ciociara), South Italy and Islands (Aspromontana, Nicastrese, Girgentana, Argentata dell'Etna, Maltese, Maltese Sarda e Sarda). Quality editing excluded 2,211 SNPs with minor allele frequency <1%, genotype call rate <95% and individual call rate <90%. Genomic Parentage (GP) and Mendelian Errors (ME) were assessed on the 350 goats using the remaining 51,136 markers. Pairs of individuals were classified as Parent-Offspring (PO) when ME<1000 and GP≥0.4. A total of 34 PO were identified out of 61,075 pairwise comparisons. We developed a novel method based on multivariate discriminant analysis and stepwise regression for choosing the best SNPs for parentage testing. Following ISAG standards for parentage testing in cattle, we identified a 200 SNP subset suitable to parentage testing in goat based on pairwise ME calculation. We considered PO all pairs of animals sharing ≤1 ME, doubtful all pairs sharing 2-3 ME and unrelated all pairs sharing >3 ME. The sensibility, specificity and accuracy (false negative, false positive and true assignment ratio, respectively) of the panel were assessed. In addition, we estimated the probability of single parental exclusion (Pe) and the probability of a random coincidental match inclusion (Pi) for each breed. The parentage panel showed good assessment power, with high specificity (0.9705882), sensibility (1.0) and accuracy (0.9999836). Pe values ranged from a minimum of 0.9999995 for Teramana to a maximum of 1.0 for Alpine. Pi values ranged from 8.49×10^{-78} for Alpine to 1.21×10^{-61} for Teramana. Pe for single SNP ranged from 0.0677±0.0592 to 0.1085±0.0506 (mean±SD) for Teramana and Alpine, respectively. This is the

first SNP panel available for parentage testing in goat. Our results suggest that genomic research can help solve practical problems in breeding, such as pedigree registration errors. In this context, cost-effective parentage testing would help goat breeders in the management of consanguinity.

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C-093

The analysis of the methylome of *Capra hircus*

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Methylation at the carbon 5 position of cytosine residues is among the best studied epigenetic modification, and a fundamental layer of cellular differentiation through the control of transcriptional potential. DNA methylation patterns can be inherited and influenced by environment, diet and aging, and disrupted in diseases. Complete DNA methylomes for several organisms are now available, helping to clarify the evolutionary story of this epigenetic mark and its distribution in key genomic elements. At the present, methylome of the domestic goat, Capra hircus is unexplored, and hence to contribute information on epigenetic modification in the species, we analyzed the methylome of two tissues, ovary and hypothalamus, from 3 adult Saanen goats. DNA methylation patterns are increasingly studied using high throughput sequencing methods. In order to evaluate differentially methylated region, we used Methylated DNA binding domain sequencing (MBD-seq), with enrichment of methylated DNA fragments and next generation sequencing (NGS - Hiseq 2000 Illumina). We produced least 23 million reads per sample, which were aligned to the goat reference genome with a minimum success rate of 97.5%. Further analyses were performed to identify peaks corresponding to hyper-methylated regions. Matching the methylation pattern of hypothalamus versus ovaries of the three goats under study we found 4687 methylated fragments showing significant differences ($P<0.001$) between tissues. Chromosomes 19 and 23 showed the highest density of methylated fragment, respectively every 0.56 Mb and 0.52 Mb in ovaries. Chromosomes 19 and 25 had the highest density of methylated fragment in the hypothalamus, every 0.58 Mb for both the chromosomes. The chromosome with least methylated fragments was the X, with a statistically significant ($P<0.001$) methylated fragment every 9.38 Mb in the ovaries and 6.42 Mb in the hypothalamus. This is the first work dealing with methylome in Capra hircus: the outcoming pioneering preliminary results could be helpful for a deeper comprehension of the complex epigenetic machinery.

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C-094

MicroRNAs expression in hypothalamus and pituitary of Saanen goat

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MicroRNAs (miRNA) are small, noncoding RNA molecules that regulate gene expression by targeting messenger RNAs for post-transcriptional cleavage or translational inhibition. There is still limited knowledge about miRNAs expression profiles in farm animals, especially in small ruminant species. The identification of miRNAs expressed in different brain regions of the domestic goat, Capra hircus, will contribute to a better understanding of miRNA function and the control of the physiologic pathways in this species. We sequenced miRNAs expressed in hypothalamus and pituitary gland, from 3 adult Saanen goats using Illumina high-throughput sequencing (Miseq). Reads were mapped on the Capra hircus reference genome and both known and novel pre-miRNA, mature miRNAs, and miRNA target sites were identified using existing information collected in miRBase and using specific bioinformatic tools. This analysis found 313 and 304 miRNAs in the hypothalamus and pituitary respectively, 131 of which were differentially expressed between the two tissues. Mapping these small RNAs on Capra hircus genome revealed that 61 miRNA genes from hypothalamus and 54 from hypophysis were located on goat chromosome 21, and belonged to the largest conserved mammalian miRNA gene cluster. A large miRNA gene population was also identified on chromosome X, encompassing 38 miRNAs in the hypothalamus and 36 in the pituitary. We also discovered 46 and 48 putative novel miRNAs in hypothalamus and pituitary respectively, based on structural prediction and homology with known miRNA genes from goat and other ruminants. This study produced a comprehensive miRNA profile related to the biology of two brain areas in goat. The characterization of these miRNAs contributes to a better understanding of the molecular and epigenetic mechanisms of physiology and development in this species.

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C-095

Economic evaluation of genetic improvement with inbreeding control in the Verzaschese goat

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To analyse expected costs and benefits of closed nucleus selection in the Verzaschese goat. A semistocahstic model is used for simulating nucleus selection of a dairy trait, with inbreeding control constrained at 0.3%. Milk and pedigree recording, and artificial insemination, are carried out in the nucleus; the only management constrain in the commercial population is the use of males coming from the nucleus, alternatively for one or two years. Nuclei of 100, 200 and 400 goats, supporting commercial populations of 175, 350, and 650 goats are considered, for a total of 14 breeding schemes simulated. Returns from the breeding program are based on income from milk per unit of genetic gain, considering a net monetary value per kg of milk from .96 to 1.56 Euro. Breeding costs include housing, maintenance and transport of males, semen production (assuming working at station or in the field), milk and pedigree recording (with and without subsidies), and artificial insemination. Discounted profits are computed per breeding scheme as returns minus costs, considering a discount rate of 3.5%, across 8 economic scenarios. By enlarging the dimension of the nucleus, genetic gain increases from 4.1 to 4.9 kg of milk (nucleus of 100), from 5.3 to 6.0 Kg. (nucleus of 200), and from 5.9 to 6.6 Kg. (nucleus of 400). Profit increases by prolonging the selection programme time horizon, ranging across the simulated cases from -95,549 to 22,746 Euros (10 years period), from -42,479 to 124,047 Euros (15 years period), from 16,317 to 323,155 Euros (20 years period). Discounted profit per kg of milk of genetic gain ranges from -211,142 to 65,978 Euros. Discounted profit decreases substantially when we consider returns only from the genetic gain in the nucleus. In general discounted profit is largely affected by price of milk and costs for milk recording, and marginally by costs of semen collection. Costs used in this study refers to the specific breed-context of the Italian Verzaschese, however the simulated scenarios can be reasonable considered common to many dairy goat breeds in the Mediterranean area.

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C-096

Use of protease enzymes in poultry feed offers promising economic and environmental benefits

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Enzymes are widely used in feed to improve utilization of nutrients and it is generally acknowledged that phytases, by improving phytate phosphorus utilization are an important tool to reduce phosphorus pollution and the environmental problems derived from this such as eutrophication. Lately, the use of proteases as feed enzymes has gained interest. Proteases are added to feed with the purpose of increasing dietary protein hydrolysis and thus enabling improved nitrogen utilization. When animals utilize nitrogen better, there is a possibility to decrease the diet protein content which can result in economic benefits and also reduce the content of nitrogen in manure. The environmental consequences of decreased dietary protein content and reduced nitrogen excretion when using a protease in feed have been investigated using Life Cycle Assessment (LCA), which considers all steps in the broiler and broiler feed production chains from production of feed ingredients, to the broilers leaving the chicken house, including the use of manure. Data from trials using both standard soya-based broiler diets and reduced protein diets with added protease (RONOZYME ProAct, DSM Nutritional Products, Basel, Switzerland) were used for modelling. Different environmental impacts were analysed, covering emissions of nitrous compounds to both air and the aquatic environment. Results for the feed production chain showed that there was a reduction in all environmental impact categories, when protease was used in the diets. The biggest reduction occurred in the category of Global Warming Potential, mainly as a result of decreased CO_2 emissions from land use changes related to soya production. In the results for the broiler production chain, there were relatively bigger reductions in Eutrophication Potential and especially in Acidification Potential, mainly as a result of reduced feed protein content and subsequent nitrogen emissions from housing and manure management. It is concluded that the use of protease can contribute significantly to current efforts to reduce nitrogen emissions whilst increasing the economic viability of broiler production.

C-097

Effects of a blend of essential oils and an enzyme combination on growth performance, microbial counts, and ileum morphology in *Escherichia coli* K88 challenged piglets

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This study was conducted to evaluate the effects of nutritional supplement of a blend of essential oils (EO; thymol and cinnamaldehyde) and an enzyme combination (XB; xylanase and β-glucanase) either alone or in combination on the growth performance, microbial counts, and morphology in the ileum of weaned piglets challenged with *Escherichia coli* K88. One hundred and ninety-two piglets were weaned at 24 days and allocated to 8 treatments for a 35-d experiment with a 2×4 factorial design comparing oral E. coli challenges [sham (-) or infected (+)] and different dietary treatments [fed the basal diet (CTR) either without or with EO, XB, or their combination (EO + XB)]. On day 8, half of the piglets were orally inoculated with 4 mL E. coli 0149: F4 (K88). On day 35, forty-eight piglets (6 piglets/treatment) were slaughtered. E. coli challenge significantly ($P<0.05$) impaired the growth performance, induced severe diarrhea, and increased the populations of *Clostridia, E. coli* and Coliforms. In the unchallenged group, dietary EO reduced the *Clostridia* population in the feces on day 14 compared to the CTR group ($P<0.05$), and dietary XB or EO + XB decreased the E. coli count in the cecum digesta compared to the CTR group ($P<0.05$). In addition, dietary EO + XB decreased the crypt depth and increased the villus to crypt ratio of piglets compared to the CTR group ($P<0.05$). In the challenged group, XB and EO + XB supplementation improved the feed efficiency when compared to the CTR group during the last week of the trial ($P<0.05$), and dietary EO + XB reduced fecal consistency compared to the EO and XB groups at 1 day post-infection ($P<0.05$). Challenged piglets fed EO + XB had less Coliforms populations than the CTR animals in the feces on day 14 and in the digesta of cecum ($P<0.05$). Moreover, EO + XB piglets had lower crypt depth and higher V:C ratio than the CTR animals ($P<0.05$). The results suggest that the combination of EO and XB supplementation may improve the gut health status of post-weaning piglets by modulating fecal consistency, microbial count and ileum morphology when piglets are challenged with *E. coli.*

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C-098

Effects of a novel emulsifier supplementation on growth performance and carcass yield of female and male broiler chicks

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The aim of present study was to evaluate the effects of a novel emulsifier supplementation on growth performance and carcass yield of female and male broiler chicks. A total of 1200 ROSS308 broiler chicks with 0-d-old were allocated into 4 treatments with a 2 × 2 factorial design comparing sex (female or male) and different dietary treatments (basal diet supplemented without (CTR) or with supplemental emulsifier (AVI-MUL TOP/GP10 (AMT), 1 g/kg from d 0 to 12, 0.75 g/kg from d 12 to 22 and 0.5 g/kg from d 22 to 44, respectively)). Each group consisted of 15 pens, 20 birds per pen. Growth performance was determined at days 0, 12, 22 and 44 (37 for females). At day 44 (37 for females), all birds were slaughtered. Dressing and breast muscle percentages were determined from one bird of each pen. Males had higher BW than female chicks on days 0 ($P=0.014$), 12 ($P<0.01$) and 22 ($P<0.01$). Compared to females, male chicks had higher ADG and ADFI from day 0 to 12 and from day 12 to 22 ($P<0.01$), and lower FCR from day 12 to 22 ($P<0.01$). AMT supplementation increased the BW compared to the CTR group on days 12 ($P=0.02$), and AMT chicks had higher ADG compared to the CTR birds from day 0 to 12 ($P=0.02$). Among the males, AMT increased BW on days 12 and 22 ($P=0.02$ and 0.02, respectively), ADG from day 0 to 12 ($P=0.02$), and ADFI from day 12 to 22 ($P=0.03$). AMT male chicks had lower FCR compared to the CTR males from day 22 to 44 and day 0 to 44 ($P=0.047$ and 0.02). In addition, dietary AMT tended to increase ADG of male chicks compared to the CTR group from day 12 to 22 ($P=0.08$). However, there was no diet effect on growth performance of female chicks during the trial. AMT supplementation significantly increased dressing percentage compared to the CTR group ($P=0.02$), and mainly showed in males ($P=0.014$). In conclusion, supplementation of AMT can improve growth performance and carcass yield of male broiler chicks.

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C-099

In vitro studies to assess the efficacy and potential toxicity of multi-mycotoxins adsorbing agents used as feed additives

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Mycotoxins have become one of the most recognised feed chain contaminants, with hundreds of toxins identified to date. Management of mycotoxins includes prevention, regulation, monitoring, decontamination, and animal treatments. Even with good management, unavoidably low levels of several mycotoxins can cause loss of feedstuffs, increased animal disease, reduced animal performance, and food residues. A promising approach to protect animals against the harmful effects of contaminated feed is based on the use of feed additives. These additives are defined as substances that, when included into contaminated feed, can adsorb or denature mycotoxins in the digestive tract of animals. Since 2009, they are officially allowed in the UE as technological feed additives. Mycotoxin adsorbents are the most studied additives and a variety of products are on the market claiming multi-toxin adsorption capacity. The efficacy of adsorbents in sequestering different mycotoxins has been poorly addressed. The aim of this study was the screening of commercial products for preparing a nutritional composition intended to reduce bioavailability of a large range of mycotoxins. 52 commercial products from 26 industrial partners, including minerals, yeast-based products and blend of components, were tested. Preliminary adsorption tests allowed the selection of 4 commercial products as effective in sequestering simultaneously aflatoxin B1, zearalenone, ochratoxin A and fumonisin B1. All products failed in adsorbing deoxynivalenol, but activated carbon. Adsorption experiments were performed with selected binders, at physiologically relevant pH values commonly found in the stomach and intestine, to determine adsorption parameters (capacity, affinity, chemisorption index). Mineralogical analyses showed that 3 out of the 4 commercial products selected as best multi-toxin adsorbents (designated by the supplying companies as minerals) were organoclays. Organoclays are not suitable for feed ingredients due to toxicity of the interlayer quaternary alkylammonium ions. Two organoclays and one yeast cell wall product, out of 52 commercial products, were found toxic in 2 bioassays. In conclusion, multi-toxin adsorbents covering major mycotoxins are not commercially available. Most of them lack effectiveness towards trichotechenes. The identity/composition of commercial products could be counterfeit and misleading. Some commercial products can be even highly toxic in toxicity bioassays.

C-100

Organic laminaria and selenized yeast as dietary integrators of iodine and selenium in organic dairy goat farming

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Dairy animals reared according to the organic farming rules may experience under-nutritionally intake of some trace elements. This work aimed at evaluating the effectiveness of organic Laminaria digitata meal (LAM) and selenized yeast (SY) as integrators of iodine (I) and selenium (Se) for organic dairy goats. Five groups of healthy 8-months old Saanen goats (four animals per group) were housed in separate boxes and fed on a basal diet (CON: 1 mg I/day and 0.24 mg Se/day) composed of Lucerne hay, concentrate mix (corn, barley, soybean) and Ryegrass hay. During the first four weeks of the experimental period (Trial 1), two groups were supplemented with 4 (LAM-1) and 8 mg I/day (LAM-2) through LAM (0.57% as I). In the Trial 2 (further four weeks), two groups of goats were supplemented with SY (0.23% as Se) providing 0.26 (SY-1) and 0.52 mg (SY-2) Se/day and the two groups treated with LAM in Trial 1 were co-supplemented in iodine and selenium using LAM and SY (LAMSY-1: 4 mg I/day and 0.26 mg Se/day; LAMSY-2: double dosage of I and Se). Periodically, animals were weighed, and blood samples were taken every two weeks. Plasmatic iodine and selenium, hematic albumin, glucose, β-glutamyl transferase, urea, β-hydroxybutyrate and NEFA were monitored. As a marker of oxidative stress, the plasma TBARS content also was assessed. Compared to the control diet, LAM and SY supplementation did not affect the average daily gain of goats. The iodine and selenium blood content increased significantly already within the first two weeks of treatment. Overall the iodine blood content exhibited a dose-dependent pattern according to the level of LAM inclusion in the diet. Regardless the supplementation level, in LAMSY treated groups, a sort of saturation effect was observed for the plasmatic I but not for Se that increased almost linearly up to the end of the trial. Overall, the LAM or/and SY inclusion in the goats' diet did not affect the hematic parameters monitored with the exception of NEFA that were higher than the control after four weeks of LAM supplementation, urea that increased after two weeks of SY administration and β-hydroxybutyrate that exhibited a time-dependent effect of the LAMSY administration. Taken together, these findings suggest that organic LAM and SY may be viable options as I and Se integrators for organic diets low in trace minerals without interfering with the hepatic functionality.

C-101

Effect of dried extracts of Aspergillus oryzae and their association with Saccharomyces cerevisiae on mid lactation dairy cattle productivity under moderate heat stress conditions.

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A trial was conducted in Parmigiano Reggiano area with the aim to study the effects of Aspergillus Oryzae fermentation extract pure or in association with Saccharomyces cerevisiae yeast as direct feed microbials on the productivity and milk quality of dairy cattle diet during moderate heat stress. Twelve Holstein multiparous cows with average parity of 2.7 and average DIM of 193, were involved in sequential study design of three periods of 21 days including 14 days of adaptation and 7 days of measurements and sampling. During the first period animals were treated with 7 g/d of a commercial product containing 50% of Saccharomyces cerevisiae yeast as direct feed microbials and 50% of fermentation extracts of Aspergillus oryzae –treatment "SC+AO"-. During the second period animals were fed the base diet –"CON"- while during the third period the diet included 7 g/cow/d of fermentation extracts of Aspergillus oryzae –treatment "AO"-. Diet was composed of a mix of hays, straw, soybean meal and a commercial protein mix including mineral and vitamin supplement and was administered as Total Mixed Ration (36.18% NDF, 14.18% CP, 24.07% Starch). Measurements included environmental temperature and humidity (to calculate Thermal Humidity Index –THI-), group feed intake and orts, individual milk production (daily), BCS, fecal score (FS) and rectal temperature (RT) (twice weekly). Sampling included diet collection, individual faeces and individual milk (twice a week). Milk composition, diet composition and indigestible NDF (uNDF) and faeces uNDFwere determined to calculate *in vivo* NDF digestibility (NDFD) and dry matter digestibility (DMD). Statistical analysis of data was performed using the SPSS for windows (vers.21); the repeated measures procedure of the GLM model was adopted using THI as covariate. The treatment AO induced an increase of dry matter intake (25.55 *vs.* 23.93 and 22.12 kg of CON and SC+AO thesis; $P<0.001$), of *in vivo* NDFD (53.18 *vs.* 48.49 and 49.23 %NDF; $P<0.05$) compared to the other treatments and of *in vivo* DMD with respect to the CON group (70.50 *vs.* 69.06; $P<0.05$). AO has also been observed to decrease rectal temperature (38.31 *vs.* 38.77 and 38.80°C; $P<0.01$). Energy Corrected Milk production such as the percentage of protein and LDG parameters (r and A30) appeared improved. Dried extracts of Aspergillus orizae had some beneficial effects on DMI, showing a higher activity if compared with SC+AO, but did not heavily affected milk production and quality.

C-102

Factors contributing to meat tenderness: interaction between myofibril structure and proteolytic changes in beef

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Large muscle to muscle variations in tenderness exist, giving rise to economical differences among different muscles within the same animal. Understanding the mechanisms of meat tenderness among different muscles may be useful in improving overall tenderness and reducing inherent variability.
Therefore, in order to study the relationship between the stability of the myofibril structure and the changes in the protein profile, in the present study, three bovine muscles (Psoas major, PM; *Longissimus Dorsi*, LD; and semitendinosus, ST) aged 1, 7, 14 and 21 days, were analyzed. Meat chemical composition, Warner Bratzler shear force (WBSF), myofibril fragmentation index (MFI), total collagen content and changes in myofibrillar proteins were estimated. ST muscle showed the lowest intramuscular fat percentage ($P<0.01$) and the highest total collagen content ($P<0.01$) with respect to LD and PM. WBSF decreased during aging in all muscles ($P<0.001$), ST was the toughest muscle whereas PM was the most tender ($P<0.001$). MFI significantly increased ($P<0.001$) in LD and PM meat throughout aging time whereas in semitendinosus it increased from 14 days of aging. Proteolysis was investigated by SDS-PAGE, western blotting and two-dimensional electrophoresis coupled to mass-spectrometry. Throughout postmortem aging some structural proteins changed in intensity in all muscles analyzed. Blotting profile highlighted that desmin and Troponin T bands were affected by both muscle and aging effects. Desmin degradation was more intense and faster in LD muscle than in ST and PM muscles. A progressive increase of the degraded isoforms of TnT (33 kDa and 30 kDa polypeptides) was found during aging in *Longissimus Dorsi*, while, in psoas major these bands appeared earlier showing a greater intensity from 1 day. During aging, 2DE image analyses results showed a significant increase of the total number of spots reaching the highest value in the *Longissimus Dorsi* muscle at 21 days of aging ($P<0.01$). Proteins separation also revealed differences in the spot number and expression patterns of MLCs isoforms among muscles. Data highlight that aging affect the meat tenderness and proteolysis with different intensities in each muscle. These results provides knowledge on the tenderness mechanism in different muscles and it could be useful for the development of muscle specific strategies for improving the quality and value in different commercial cuts.

C-103

Quality characterization of products from Romagnola chicken breed: 2. Eggs

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In poultry production the interest toward local breeds has been increasing in recent years either for the recovery and preservation of chicken biodiversity or for their potential use in free-range and organic rearing systems considering their better adaptability to outdoor conditions in respect to highly productive genotypes. The objective of this study was to characterize quality traits of eggs from Romagnola hens in comparison with a hybrid strain used for intensive egg production. Ten Romagnola (RMG) and 10 Hy-Line brown (HLB) laying hens of similar age were reared from June to September in free-range conditions with 20 m2/hen of pasture availability. Hens received the same commercial feed (2,900 kcal/kg ME and 16.5% CP) on *ad libitum* basis. After 5 and 10 wks of rearing, eggs were collected for 3 consecutive days and used for assessing physical and technological properties (weight of whole egg, yolk, albumen and eggshell, shape index, eggshell surface, Haugh and yolk indexes and eggshell breaking strength), yolk chemical composition (moisture, protein, lipid, ash, fatty acids and total cholesterol) and colour (β-carotene equivalents and CIE L*a*b* system). The weight of eggs and of their components resulted significantly lower in RMG than HLB with the exception of egg yolk which was similar (16.5 *vs.* 16.0 g respectively). Yolk:egg ratio resulted thus considerably higher in RMG (0.31 *vs.* 0.25; $P<0.01$) while the albumen:egg ratio was lower (0.60 *vs.* 0.65; $P<0.01$). Moreover, RMG showed lower shape index (0.74 *vs.* 0.79; $P<0.01$) and similar Haugh and yolk indexes to HLB. Eggshell:whole egg ratio resulted lower in RMG (0.088 *vs.* 0.097; $P<0.01$) as well as eggshell breaking strength (3.26 *vs.* 4.56 kg; $P<0.01$). Regarding yolk chemical composition, RMG showed lower ash (1.62 *vs.* 1.79%; $P<0.01$), higher cholesterol (12.8 *vs.* 11.7 mg/g yolk; $P<0.01$) and similar moisture, protein and lipid contents. As for fatty acid profile, RMG exhibited higher SFA (34.5 *vs.* 31.6%; $P<0.01$) and MUFA (40.6 *vs.* 37.3%; $P<0.01$) and lower PUFA content (22.6 *vs.* 28.4%; $P<0.01$). Furthermore, n-6/n-3 ratio resulted lower (11.3 *vs.* 13.5; $P<0.01$). Yolk from RMG showed higher redness (a*, -4.32 *vs.* -6.53; $P<0.01$), yellowness (b*, 48.9 *vs.* 42.2; $P<0.01$) and β-carotene equivalents (36.8 *vs.* 20.2 mg/kg yolk; $P<0.01$). In conclusion, egg quality traits were substantially different in RMG breed in comparison to the commercial hybrid, and their knowledge maybe useful for an aware food preparation and valorisation.

C-104

Broiler *Pectoralis major* muscle affected by emerging abnormalities: composition and technological traits

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In the last few years, modern chicken hybrids exhibited new breast muscle abnormalities, termed as "wooden breast" and "white-striping", which impaired both product appearance and quality properties. This study aimed to assess the implications of wooden breast and white striping abnormalities on quality traits of the meat. For this purpose, 10 normal (NORM) and 10 wooden and white-striped (WB/WS) P. major muscles were obtained from the same flock of heavy broilers (males Ross 708 weighing 3.8 kg) towards a processing plant and used to assess proximate (moisture, protein, fat, ash and collagen) and mineral composition (Mg, K, P, Na and Ca), sarcoplasmic and myofibrillar protein profile (SDS-PAGE) as well as technological traits of raw (pH, colour, drip loss, cooking losses and Allo-Kramer shear force) and marinated (marinade uptake, cooking losses and Allo-Kramer shear force) meat. If compared with NORM group, WB/WS fillets exhibited significantly higher fat (1.79 *vs.* 0.79%; P<0.001), moisture (76.8 *vs.* 74.6%; P<0.001) and collagen contents (1.35 *vs.* 1.16%; P<0.05) coupled with lower amount of protein (18.5 *vs.* 23.4%; P<0.001) and ash (1.19 *vs.* 1.46%; P<0.05). Besides, WB/WS breast meat samples showed increased calcium (11.3 *vs.* 7.8 mg/100g; P<0.05) and sodium (75.1 *vs.* 37.8 mg/100g; P<0.001) levels. With regard to protein pattern, a couple of significant (P<0.05) differences were found in myofibrillar proteins such as LC1 and troponin, while almost all sarcoplasmic proteins involved in glucose metabolism differed. In addition, calcium-ATPase was only detected in WB/WS fillets. As for raw meat quality, WB/WS fillets were paler (L*, 52.5 *vs.* 54.5; P<0.05) and exhibited higher ultimate pH values (6.06 *vs.* 5.87; P<0.05) and extremely greater cooking losses (34.0 *vs.* 21.5%; P<0.001) and AK-shear force (7.54 *vs.* 4.26 kg/g; P<0.05) in respect to NORM group. In addition, marinated WB/WS fillets had a dramatically reduced marinade uptake (7.4 *vs.* 18.3%; P<0.001), but also higher cooking losses (21.9 *vs.* 14.5%; P<0.001) and shear force values (4.08 *vs.* 2.13 kg/g; P<0.001). In conclusion, this study revealed that emerging breast abnormalities severely compromise nutritional value and technological properties of the meat. Furthermore, the increased concentrations of sodium and calcium as well as modification of sarcoplasmic protein pattern suggest that these muscle abnormalities may be associated with a perturbation of ion homeostasis and glycolytic metabolism.

C-105

Meat and carcass quality in Martina Franca donkey foals

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The effects of slaughter age (8 *vs.* 12 months) were investigated on meat and carcass quality obtained from Martina Franca donkey foals. Sixteen male entire foals had been used, half of them slaughtered at 8 months of age with a mean final body weight of 101±18 kg and the remaining 8 foals slaughtered at 12 months of age with a mean final body weight of 122±13 kg. Carcass weight and dressing percentage were significantly higher (P<0.05) in older foals; 8 months old foals had 49.2 % warm dressing percentage compared to 53.9 % obtained in older animals, while cold dressing percentage was 47.1 % in younger foals and 52.5% in the 12 months old animals. Donkey foals meat analysed in this study was characterised by a low fat (1.76±0.23 g/100 g in 8 months old animals and 1.87±0.18 g/100g in 12 months old foals) and a low cholesterol content (62.4±2.33 mg/100 g in 8 months old foals and 63.9±3.08 mg/100 g in 12 months old foals), while protein content was very interesting and similar to other red meats (19.8±0.24 g/100 g in younger foals, 21.0±2.32 g/100 g in older foals). Glycogen content was significantly higher (P<0.05) in foals slaughtered at 12 months of age (0.38±0.02 %) compared to foals slaughtered at 8 months of age (0.22±0.05). Shear force value was significantly lower (P<0.05) in donkeys slaughtered at 8 months of age (5.51 kg/cm^2) compared to older animals (6.39 kg/cm^2). The percentages of essential amino acids were higher compared with the total amino acid content, respectively 52.80% in foals slaughtered at 8 months, while in older animals the value obtained was 51.28%.

C-106

Chemical characterization of European eels (*Anguilla anguilla*) of different origins collected from Italian market

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European eels farming is based on different production systems: an extensive culture in brackish water of lagoon known as valliculture; an intensive farming in earth tanks with the use of moist feed, common in Italy, and an iper-intensive culture in recirculation systems with the use of extruded feed, typical of Northern European countries. Generally, eels are marketed into two different sizes: large eels named Capitoni (C) (>400 g live weight) and small eels, Buratelli (B) (100-200 g live weight).

In order to investigate the influence of production system on the proximate composition of eels a total of 37 eels were collected: 14 were from Italian intensive farming (C+B), 12 (C+B) from iper-intensive farming (6 raised in Netherland and 6 in Denmark), 6 were captured in Adriatic Sea and 5 were purchased in an Italian valliculture. The fillet proximate composition (AOAC standard methods) and fatty acid profile were determined and data were analysed by SPSS 22 statistical package. Intensively and iper-intensively farmed eels presented a higher lipid content (30.2%) than lagoon raised (16.7%) and wild eels (15.9%). In addition, the protein content showed differences among groups, resulting 14.1%, 16.7%, 18.5% in intensive farmed, lagoon and wild eels, respectively. Fatty acid profile revealed some interesting differences among origins. Eels fed with wet feed did not show differences between sizes C and B; they presented the highest amount of oleic acid (41.0%) and linoleic acid (7.7%) and the lowest amount of n-3 polyunsaturated fatty acid (n-3 PUFAs, 9.8%) when compared to others eels. No differences were found between Danish and Dutch eels where gondoic and cetoleic acids were found to be more abundant in B than C. As concerns valliculture and wild eels, they presented different content of linoleic acid, which was 3.6% in lagoon eels and 1.3% wild eels. Even if the total n-3 PUFAs did not vary between these two groups, a different ratio between EPA, dominant in wild fish, and DHA, more abundant in lagoon eels, was observed. Our results confirm the correlation between fatty acid profile of muscle and the production system, with emphasis on the impact of fish diet. It appeared that wet and extruded feed were formulated using different lipid sources and that B and C farmed in Northern Europe received different diets. Differences were also found between wild and lagoon eels, suggesting that apparently similar habitats could present different food web structure.

C-107

The β-hexachlorocyclohexane (β-HCH) affects the oxidative status of bovine peripheral blood mononuclear cell *in vitro*

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The β-hexachlorocyclohexane (β-HCH) is an environmental contaminant able to migrate along the food chain by which it can cause adverse effects on the health of humans and farm animals. In our previous work, bovine Peripheral Blood Mononuclear Cells (PBMC) exposed for 7 days to β-HCH (10-100-1000 µM) showed an altered cell functionality (assayed by XTT). This condition was hypothesized as linked to an oxidative impairment of the cells. Based on those preliminary results, the aim of this work was to monitor the free radicals production in bovine PBMCs exposed to β-HCH. The expression of mRNA for superoxide dismutase (SOD) and Catalase (CAT) were also monitored as first enzymatic mechanisms of cell defense against oxidative stress conditions. Four healthy Friesian heifers were used as blood donors. After isolation, PBMCs were incubated at 39°C for 2 or 7 days in an atmosphere of 5% CO2 and in the presence of β-HCH at 0 (control), 0.1, 1, 10, 100 and 1000 µM. Dimethylsulfoxide was used as solubilizing agent. The intracellular reactive oxygen species (ROS) concentration was measured by 2',7'-dicholrodihydrofluorescein diacetate (DCHF-DA). The mRNA expression of SOD and CAT was monitored by real-time PCR (rt-PCR). The data were analyzed by ANOVA and differences were declared significant at $P<0.05$. Compared to the control, a significant increase of intracellular ROS in PBMCs exposed to β-HCH was observed, particularly after seven days of exposure (Tab. 1). The rt-PCR analysis showed that both SOD and CAT mRNA expression did not change after two days of exposure to β-HCH. However, after seven days of incubation a significant increase in CAT mRNA expression was observed in PBMCs treated with β-HCH 100-1000 µM, but SOD mRNA expression increased only at β-HCH 1000 µM (Tab. 1), if compared with the control. These results suggest that exposure to β-HCH at low concentrations (i.e. 1-10 µM), even at the shortest exposure time tested (2 days), may increase the ROS content of bovine PBMC without any appreciable up-regulation of both SOD and CAT expression. These results may be regarded as a direct effect of the β-HCH exposure on the cell's antioxidant system possibly leading to the onset of oxidative stress conditions.

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C-108

Milking procedure can affect anaerobic spore-forming bacteria in milk

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Anerobic spore-forming bacteria (ASFB) in milk can cause problems during the ripening of hard cheeses. The relationship between ASFB content in dairy cows feed and milk is well documented, while there are only few evidences on the effects of different milking hygienic procedures on milk contamination. Three milking schemes were applied in an intensive dairy farm (70 lactating cows; 24.7 kg milk/d per cow on average) for three consecutive weeks during autumn over 2 years: forestripping (F), forestripping + postdipping (FP) or predipping + forestripping + postdipping (PFP). The pre and post dipping were carried out with commercial detergent solutions. Milk, feed and faeces samples and swabs of teat skin were collected in the last three days of each experimental week. The ASFB contents of hays and silages were not different between the years: maize silage had a slightly higher contamination compared to lucerne and Italian ryegrass hays (4.17±0.42 *vs.* 2.22±0.22 $\log_{10}$ MPN/g, average of the two hays), total mixed ration showed a different level of ASFB in the two years: 5.66 and 4.18 lo$_{g10}$ MPN/g. The different milking procedure determined a decrease in the standard plate count and coliform count of milk: from 4.51 to 3.94 $\log_{10}$ CFU/mL and from 4.68 to 1.48 $\log_{10}$ CFU/mL respectively for F and PFP treatments. Milk ASFB was positively affected by the introduction of predipping: ASFB counts in milk were 2.48, 2.63 and 1.95 $\log_{10}$ MPN/l in the first year and 2.45, 2.94 and 1.82 $\log_{10}$ MPN/l in the second year for treatment F, FP and PFP, respectively. The higher FP value suggests that the postdipping procedure does not have any effect on the reduction of ASFB contamination. Faeces showed a higher contamination of ASFB in comparison with milk: 3.52, 4.16 and 3.47 $\log_{10}$ MPN/g in the three treatments (average of the two years). The intensification of milking procedures had a positive effect also on the ASFB count of swabs applied on teat skin before clusters attachment: 2.18, 1.94 and 1.30 $\log_{10}$ MPN/swab for F, FP and PFP treatments, respectively, probably because the predipping reduced the faeces load on teat. The results obtained from this study underline the importance of the application of a proper milking procedure, in particular predipping, to reduce the ASFB contamination of milk.

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C-109

Global milk quality: evaluating tool for dairy farm

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The concept of food quality is gradually expanding to include, in addition to intrinsic aspects of the quality, even the manner in which the food has been produced. Consumers are looking for nutrient, healthy and safe food, produced from environmentally sustainable and ethical processes. To evaluate the quality of both production processes and product, a multidisciplinary study was conducted in 29 Italian dairy farms. Farms were located in northern Italy and their total production was destined to produce Grana Padano PDO cheese. Average milk production, expressed as Fat and Protein Corrected Milk, was 27.0 kg/d per cow (CV=16.5%) and the farms had on average 78.6 lactating cows (CV=61.1%). Feed self-sufficiency was 65.9% (CV=25.7%); 82.3% of the concentrate feed and 15.9% of the forages was purchased. Each farm was visited to obtain milk samples for analyses, to gather information on farm management for the evaluation of profitability and environmental sustainability through Life Cycle Assessment method and to carry out animal welfare assessment. A pool of variables was used to define 6 aspects of global milk quality: animal welfare, environmental and economic sustainability, microbiological, nutraceutical and nutritional quality. For each quality variable, minimum and maximum benchmark values were identified to rescale indicator values into scores between 1 and 3. The 25% best performing farms for each quality variable received the score of 3, while the 25% worst performing farms were set to a score of 1; all other farms were scored 2. By matching the indices of the variables of the same quality aspect, a general index for each quality aspect was calculated. Figure 1 shows the scheme used to evaluate the scores of each quality aspect with the reference thresholds that identify the 25% best farms. No farm achieved the maximum score in the 6 aspects together; farms with high efficiency (>1.4 kg FPCM/kg dry matter intake) had the best scores for environmental, economic and microbiological index. This multidisciplinary evaluating system could find useful applications such as the development of support tools for managerial decisions, the provision to consumers of additional information on the quality of the production process, the development of a milk payment system based on a concept of global quality.

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C-110

Animal husbandry as a tool for post-war recovery and reconciliation. An update from The Transhumance of the Peace project

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This follow-up study analyzed the performance of 3 batches of Rendena cattle after their transfer to Bosnia within the solidarity project The Transhumance of the Peace. The project, supported by the Province of Trento (Italy), aimed at promoting the reconciliation between Bosnians and Serbs through the recovery of cattle husbandry in the area of Srebrenica after the mass slaughter of July 1995. Ninety-nine heifers (average age at the transfer = 19.3±6.6 months) and 8 multiparous cows (average age at the transfer = 77.7±14.6 months) were transferred to Bosnia between December 2010 and January 2013. The animals were assigned through a free loan to over 80 micro family farms scattered over 30 districts of Srebrenica's territory. Data and information were collected during two visits to Bosnia in the year 2014 and they considered health, productive and reproductive parameters useful to identify the main strength and weakness of the project after 4 years of activity. Twenty animals were lost during this period: 8 animals died from causes mainly attributable to the intake of *Pteridium aquilinum* (L.), a poisoning weed of the local meadows and pastures. Six subjects were culled due to fertility problems, and 6 animals were illegally sold to third parties. Despite these losses, the consistency of the Bosnian Rendena population showed an increasing trend with 99 calves born during the 4 years period. An encouraging average calving to conception interval of 119±77 d was observed for primiparous cows with 1.7±1.0 services for conception. However, low fertility was the main management problem of the animals. Only 74% of the heifers and cows calved at least once in Bosnia and the number of repeated breeders increased according to parity. The peak in milk yield reported by the farmers was on average 18.6±2.8 kg/d and 19.9±3.8 kg/d for primiparous (n. 37) and secondiparous cows (n. 13), respectively. Milk is mostly used within farm gate, but there is an increasing number of producers who are starting to sell it to a milk factory. The cooperation among farmers of the two ethnic groups represents the main strength point of the project which has also promoted a relevant recovery of meadows and pastures. Rendena breed has shown a good adaptation to the harsh environment of Srebrenica mountain while uncomfortable livestock facilities along with a poor feeding and health management are the most frequent weaknesses that penalize cattle reproductive performance.

C-111

Integrated approach to evaluate primiparous dairy cows performance

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Behavioral patterns of dairy cows are useful to identify health problems and reproductive status, it is possible to have a lot of alternative information, particularly in continuous monitoring system. Those automatic systems can help dairy farm management, reporting individual problems that need care and attention. In primiparous dairy cows, typical changes in the transition period lead to accentuate every critical status, particularly in metabolic and productive point of view. Many studies focused on those aspects, but an integrated approach is needful to study the multifactor response at production or reproduction level. In a commercial farm, a trial monitoring 20 primiparous dairy cows (in a larger group of 35 primiparous) during all lactation period was performed. Parturition period ranged from 15 september to 31 march and in the first 7 lactation days all cows were equipped with individual HOBO Pendant G loggers (Onset Computer Corporation, Pocasset, MA) for activity measuring. Those data loggers use an internal 3-axis accelerometer, and were attached to the lateral side of the right hind leg of the cows. Individual Body Condition Scores (BCS) were estimated weekly, while milk production (in 2 daily milking), time of milking and humidity and temperature air data were collected daily. Average individual milk yield was 27.3±6.24 kg/d (50.5 % in the morning milking) and milking duration was 4.95±1.94 min. Milk fat was 3.96±0.53 % and milk protein was 3.34±0.32 %. Individual BCS was on average 3.46±0.30 and individual standing daily time was 13.5±2.26 h. On the basis of individual BCS at parturition time, cows were divided in 2 homogeneous groups with the threshold of 3.25, and on individual daily time standing in the first 21 days of lactation cows were divided in 2 groups with the threshold of 14 h/d. Primiparous cows with BCS at parturition <3.25 quite regained BCS after 20 weeks of lactation, but produced 2.3 kg/d ($P<0.001$) on average less than the other group. On the contrary, primiparous cows with standing time <14 h/d showed higher BCS than the others cows (+0.11 on average, $P<0.001$), but did not produce more milk (+0.1 kg/d, $P=0.25$) on average (Table 1) and showed smaller Linear Score than other cows. Moreover primiparous cows with standing time <14 h/d had higher number of lying bouts and less minutes between two lying bouts, probably due to a higher cow activity to feeding.

C-112

Light conditions affect development and cortisol response in common sole (*Solea solea* l.) eggs and larvae: preliminary findings

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Recently, some improvements in the study of common sole larvae (*Solea solea*) have been achieved by focusing on larval feeding, nutrition and physiology. However, abiotic factors such as light intensity and photoperiod during egg development and yolk sac larvae are poorly investigated in this species. Two experiments were conducted to study the effects of light intensity and photoperiod on hatching, survival, egg development and cortisol response in common sole from fertilized eggs to 5 days post hatching larvae (DPH), to provide useful information for hatcheries. Experiment 1: common sole eggs were randomly allocated at 30±2 hours post fertilization (HPF) in 5 L egg incubators (360 eggs/L-1) and were exposed to six light intensities: 50 (LI-50), 250 (LI-250), 500 (LI-500), 1000 (LI-1000), 2000 (LI-2000) and 3000 (LI-3000) lux under a photoperiod of 16h light:8h dark. The trial was conducted in triplicate and lasted until 5 DPH. During the trial the hatching rate (HR), hatching time (HT) and survival rate (SR) of larvae were estimated at 36, 44, 52, 60, 68, 76, 84, 92, and 100 HPF, while egg development was assessed according to a six-stage classification. The percentage of larvae with malformation was estimated at the end of the trial. Cortisol level of egg was determined at 36 and 100 (pre-hatching) HPF while cortisol level of larvae was determined at 1 and 5 DPH. Cortisol was measured in 3 pools of egg and larvae (50-60) at 30-100 HPF and at 1-5 DPH by a specific microtitre radioimmunoassay (RIA) as described by Bertotto et al. (2011). Experiment 2: eggs were exposed to five photoperiod: P0 (0h light:24 h dark), P6 (6h light:18h dark), P12 (12h light:12h dark), P18 (18h light:6h dark), P24 (24h light:0h dark). The light intensity was maintained at 250 lux for all the treatments. The rearing techniques, sampling, and parameters analyzed were the same described in the Experiment 1. High light intensity seems to accelerate the developmental rhythms of embryos and increased larval malformation. Cortisol level in the eggs displayed similar trend among the treatments. However, an increasing pattern was observed in the eggs at 100 HPF under 2000 and 3000 lux. This could suggest an activation of hypothalamus-pituitary interrenal (HPI) axis for cortisol synthesis in common sole embryos prior hatching. Different photoperiods did not affected HR, SR and malformation, however short photoperiods led to a delay in the HT. Cortisol level in the eggs displayed similar trend among the treatments possibly indicating that eggs and larvae were not so much sensitive to changes of this parameter. To our knowledge, this is the first study describing the effects of light intensity and photoperiod on cortisol level in common sole eggs and yolk sac larvae. On the basis of the results of the present trial, the light intensity regime could be kept between 50 and 1000 lux without any detrimental effect on embryo development and cortisol response, while a photoperiod of 18h light:6h dark seems to guarantee an appropriate hatching time and a low cortisol response. Further studies are necessary to explore the effects of light conditions during embryo development on subsequent larval stages in particular on larval pigmentation.

C-113

Compensatory growth following long term multi-phase cyclic feeding in rainbow trout (*Oncorhynchus mykiss*)

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Compensatory growth (CG) during recovery from feed deprivation is a well-known phenomenon in fish, making the practice of cyclic feed restriction-refeeding a possible tool for aquaculturists to optimize growth performance. While earlier studies in this direction focused on relatively short single feed restriction-refeeding protocols, the present trial was designed to evaluate the impact of different repeated cyclic feeding schemes on the zootechnical response of rainbow trout (O. mykiss) over a complete growing phase up to the commercial size. Three hundred trout (body weight 72±6 g) were randomly distributed among 12 tanks, each of 0.5 m^3 capacity and supplied with 8 L min-1 of well water at a temperature of 12.7±0.8°C. Triplicated groups of fish were subjected over 27 weeks to one of the following treatments: C, control, continuous feeding to visual satiety 6 days a week; T1, cyclic feeding regularly alternating 1 week starvation (S) and 3 weeks refeeding (F) (1S+3F); T2, cyclic feeding consisting in 3 consecutive phases: 1S+3F, 2S+6F, and 3S+12F; T3, where a feed restriction (70% of the satiety level observed in the previous week) was applied instead of starvation with the same schedule as T2. The same trout feed (45% crude protein, 28% crude lipid) was used throughout the trial. At the end of the trial the different cyclic feeding protocols resulted in the same zootechnical outcome (P>0.05). A nearly complete convergence of body mass was evident as no significant differences were found among treatments in individual weight (543±28 g), specific growth rate (1.06±0.03%), feed conversion ratio (0.84±0.03) and protein efficiency ratio (2.64±0.12) despite a lower feed consumption in treatments T1, T2 and T3 relative to controls (357 *vs.* 390 g fish-1, P<0.05). All protocols imposing fasting or feed restriction resulted in CG at the end of each re-feeding phase. Hyperphagia was a major cause of CG. Both phenomena were emphasized with treatment T3 after recovering from the last 3-week fasting period, when they were associated to a marked improvement of feed conversion ratio relative to controls (0.75 *vs.* 0.85, P<0.05). The results obtained so far suggest repeated cyclic feeding as a reliable practice in trout farming, provided fasting or feed restriction periods are followed by refeeding phases of suitable length to allow recovery of body mass. This could result in improved profitability and environmental sustainability.

C-114

Not published

C-115

Genetic correlations among selected traits and inbreeding depression in dual purpose Rendena breed

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Selection in native cattle breeds requires great attention because of the smaller population size and the greater risk of inbreeding (F) as compare to bigger populations. Moreover, native breeds are usually dual purpose, and selection involves antagonistic traits, i.e., milk and meat attitudes. Focusing on Rendena cattle (~4,000 breeding cows), the present work aimed to study F depression and genetic correlations (r) in the following traits: milk, fat, protein (milk yield traits, MY), and udder correctness (Ud), for milk attitude; muscularity (Mu), average daily gain (ADG) and *in vivo* estimated carcass traits (Ca) for meat production. An amount of 28^{1},497 MY test-day data collected on ^{1}6,974 cows during routinely milk recording were considered, whereas Ud and Mu were obtained from linear type traits scored on ^{11},992 primiparous cows. ADG and Ca were measured on ^{1},428 young bulls at performance test (i.e., up to ^{11} months of age). Individual F was estimated including incomplete pedigree information. A series of bi-trait REML animal models were used to assess genetic parameters, and genetic trends were drawn as linear regression (b) on estimated breeding values (EBV) standardized on mean ^{1}00 and standard deviation of ^{1}0 points. The effect of F was evaluated by introducing individual F in the models and considering the change in EBV for each percentage point of F increase (b'). Heritabilities varied from 0.^{1}7 (MY) to 0.38 (Ca). Low but negative r were found within milk traits MY-Ud (r=-0.^{1}4), suggesting negative impact of milk yield on udder form. About meat traits, positive r were found in Mu-Ca (r=0.57) and ADG-Ca (r =0.38). ADG showed low r with all other traits. The antagonism of dual purpose selection was evident in MY-Mu (r=-0.33), whereas close to zero r were found in MY-Ca (r=-0.06). Genetic trends reflect the stronger selection for milk attitude realized: MY EBVs are increasing (b=^{1}.4 since 2000), but Mu EBVs are decreasing (b=-0.5), while ADG and Ca are slightly increasing (b=0.8). An F depression was found in all traits (average b' =-0.33), except for MY (b'=0.35), meaning that the greater MY EBVs are in more inbred animals. Results suggest a slight but progressive change toward a milk conformation in the breed, and a relative greater increase in F due to a bigger selection pressure on MY. A proper breeding policy should account for aspects as r and F to make choices in a long-term perspective and limit the chance of F depression.

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C-116

Genetic diversity of Mediterranean cattle breeds related to geography and climate

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In recent decades, changes in climate have caused impacts on natural and human systems. Mediterranean countries will be particularly affected by this phenomenon, with growing temperatures and reduced rainfall. Understanding how species and ecosystems respond to climate change has become a crucial focus in biodiversity conservation and management. The genome-wide SNP panels allows providing background information on genome structure in domestic animals, opening new perspectives to livestock genetics. The International Project GALIMED "Genetic Adaptation of Bovine Livestock and production systems in MEDiterranean region", develops an integrated approach that combines the analysis of SNP markers, bioclimatic variables and farming system information to identify genotypes and breeding practices able to respond to climate change. Italian and Corsican local breeds are part of this project. The aim of the study is to identify genomic regions related to adaptation to climate change in these local breeds. Individuals of Cinisara (71), Modicana (72), Maremmana (25), Piemontese (21), Romagnola (21), Sarda (30), Sardo-Modicana (28) and Corse (31) breeds were genotyped using Bovine SNP 50k. Farming systems data were collected by interviewing breeders. Geographic coordinates and 19 bioclimatic variables were also available. Principal Components Analysis (PCA) was performed on SNPs data and climatic variables. Co-inertia (CIA) analysis was realized to detect a possible common structure between such different information. After edits, 43,625 SNPs were retained. The PC1 on individual genotypes differentiates Modicana from other breeds, and shows that Sardo-Modicana is close to Modicana, but is clearly admixed to another breed. The PC2 differentiates Cinisara from a cluster "Romagnola/Maremmana" and shows a large variability of the Modicana breed. The CIA coefficient between molecular data and both geographic and climatic information is equal to 0.38, (P-value=0.001), suggesting evidence of genetic adaptation to different climatic pressures. Further analyses are on going to identify the genetic regions with a potential adaptive role. The results will provide a solid scientific foundation to reconsider objectives and selection criteria and to improve farming practices to prepare livestock to new environmental conditions.

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C-117

Genomic inbreeding estimation in small populations: evaluation of runs of homozygosity in local cattle breeds

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The availability of high throughput genotyping has facilitated the quantification of inbreeding by genomic markers in farm animals. Run of homozygosity (ROH) are contiguous lengths of homozygous genotypes and represent an estimate of the degree of autozygosity at genome-wide level. The current study aims to quantify the genomic inbreeding derived from ROH (FROH) in three Italian local cattle breeds. Individuals of Cinisara (71), Modicana (72), Reggiana (168) were genotyped with the 50K v2 Illumina BeadChip. Genotypes from 96 animals of the Italian Holstein cattle breed were included in the analysis. The following criteria were used to define a ROH: two missing SNPs; one heterozygous SNP; minimum density of 1 SNP every 100 kb; maximum gap between consecutive SNPs of 1 000 kb. We used a definition of ROH as tracts of homozygous genotypes that were >4 000 kb in length with a minimum number of 40 SNPs included in the run. Across all four breeds, we identified 3,661 ROH. The Modicana breed showed the highest mean number of ROH for individual (11.03) and the highest value of FROH (0.053), whereas the Reggiana showed the lowest values (7.15 and 0.033, respectively). The three most homozygous animals present in our dataset were from the Cinisara (676.9 Mb), Modicana (681.2 Mb) and Reggiana (725.2 Mb), with almost a quarter of their genome classified as ROH. Differences among breeds existed for the ROH length. The individuals of Reggiana and Italian Holstein breeds showed high number of shorter ROH segments, whereas the Sicilian breeds showed ROH characterized by the presence of large segments. In fact, in all breeds, most ROH segment coverage was in the shorter length categories (4-8 Mb), but the Sicilian breeds presented the highest percentage of ROHs (12%) inside the ROH length category >24Mb. Therefore our results showed the presence of inbreeding due to recent consanguineous matings and a lack of gene flow from other herds and breeds. ROH analyses represent an important instrument that may be used in inference of population history and to associate with important production and disease traits. Considering that the increased of inbreeding leads to different negative effects, our results showed the necessity of implementing conservation programs to preserve the local breeds.

C-118

The distinctive mitochondrial DNA signature of Podolic cattle in Italy

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The domestication of wild aurochs (Bos primigenius), starting ~10,000 years ago, was an important step in human history with its cultural and socioeconomic implications for ancient populations. Nowadays, there are around two thousands cattle breeds recognized worldwide. An ancient group of breeds are known as Podolic cattle, which are thought to be descendants of the first domesticated animals or even directly of the wild ones. The name indicates a possible origin in the Podolia region of Ukraine, from where these animals spread in Europe. These grey cattle breeds were reared in Italy since the ancient times, but they have recently experienced a drastic demographic reduction in spite of their significant role in the cattle industry. The aim of this study is to assess the genetic variability of current Podolic breeds in Italy and possibly clarify their origin (at least from the maternal side) through the analysis of the mitochondrial sequence variation. A total of 932 mitochondrial DNA (mtDNA) control-region sequences from 9 different Italian Podolic breeds were retrieved from either GenBank or the published literature. This initial dataset was implemented by collecting and sequencing 87 novel samples (including 41 Bianca di Valpadana, 35 Maremmana and 11 Italian Podolian) and compared with 470 mtDNA control regions from non-Podolic breeds. In total, 405 distinctive haplotypes were identified and classified into haplogroups T1, T2, T3, Q1, Q2, R1, and R2. Our preliminary analyses highlighted significant differences between Italian podolic and non-podolic mitochondrial gene pools, which will be further investigated by carefully comparing them with the most closely related western Eurasian grey breeds (*e.g.* Hungarian Grey and Turkish Grey) and by extending the analysis to the entire mitochondrial genome. The main goal of this study is to focus attention and increase knowledge on these ancient autochthonous breeds, in order to preserve their tradition and develop appropriate strategies for biodiversity conservation.

C-119

Investigation of *Bos indicus* and *Bos taurus* admixture in Sanga cattle from Uganda by whole genome sequence analysis

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African cattle have a complex history that in part still needs to be elucidated. In particular, African "Sanga" cattle likely derive from a cross between taurine and indicine breeds introduced in Africa respectively 4000 and 2000-3000 years ago. However a genetic contribution from an independent domestication of aurochs in northern Africa is still under debate. Cattle crosses between Bos indicus (Bi) and Bos taurus (Bt) have composite chromosomes with Bt and Bi segments. Sorting out those segments would permit the independent reconstruction of African Bi and Bt genealogy, demography and history. Using whole genome sequences from 148 animals (Bt: Australian Angus (n=30), Jersey of Australian origin (n=30) and Australian Holstein (n=30); cross: Ugandan cattle (n=26)); Bi: Australian Brahamans (n=32)) we identified the Bt or a Bi ancestry of Sanga chromosome segments. Preliminary results suggest that 35% of the Ugandan cattle genomes have B. indicus origin, 23% have taurine origin and the rest was unassigned. Chromosomes 23 and 13, in particular, presented the highest (48%) and lowest (31%) percentage of segments of indicine origin and the lowest (15%) and highest (27%) percentage of segments of taurine origin, respectively. Chromosome segments will be pooled separately according to their Bt or Bi origin and used to infer ancestral demography with the method developed by Iona et al. This approach adopts a predictor of multiloci linkage disequilibrium (LD) based on a coalescent model that allows for changes in effective population size (Ne).

C-120

Fatty acids milk composition in Italian Simmental and Italian Holstein cows

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Increasing importance is given to healthy diets and to satisfy this demand, the benchmark of milk quality now includes also the specific composition of the lipid and protein fractions in milk. Milk fat has been criticized because of its higher concentration of saturated fatty acids (SFA) and low concentrations of monounsaturated (MUFA) and poly- unsaturated (PUFA), but fatty acids (FA) profile can be improved via genetic selection, which can provide a more permanent solution compared with management-related approaches. The aim of this work is to estimate the correlations among groups of milk FA, namely SFA, unsaturated (UFA), MUFA and PUFA, and EBVs (kg of protein EBVp, kg of fat EBVf) in Italian Simmental (IS) and Italian Holstein (IH) cattle. Milk sampling was conducted in 6 commercial farms of IS and 4 commercial farms of IH. Cows had free access to water and a *ad libitum* total mixed ration offered twice a day. The day of official milk recording, 100 mL of milk individual samples were collected and split in two 50 mL aliquots, one used for the determination of protein, fat, lactose and for somatic cell count (SCC) and the second frozen at -20°C within 2 hours for fatty acid analyses. Before statistical analysis, animals were classified for parity with ordinal value of 1 for first calving, 2 for second calving and 3 for cows with more than 2 calving. Analysis was performed with a mixed model, with fixed effects of Breed and Parity and random effect of Farm and the linear relationships with DIM and with EBVs within breeds. Results evidenced a significant correlation between SFA, UFA, PUFA, MUFA with EBVp and EBVf in both breeds. Relevant to highlight is the ratio rumenic/(vacenic+rumenic), resulted to be statistically significant considering breeds as well as EBVp and EBVf. This may be due to a divergent selection for the gene involved in vaccenic/rumenic methabolism in the two breeds while the significant association with EBVf and EBVp suggests the existence of a genetic variability of the trait within breeds. Daily CH4 emission (g/kg of FPCM), estimated on the basis of fatty acid profile, was also correlated with EBVf and EBVp. Although further investigations are required, these traits can be considered in breeding programs for the next generation dairy cows.

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C-121

Effect of an intensive silvopastoral feeding system based on *Leucaena leucocephala* on the physico-chemical and sensory characteristics of the artisan cheese from Michoacán, Mexico

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The purpose of the present study was to determine whether a diet of dairy cattle based on an intensive silvo-pastoral System, based on *Leucaena leucocephala*, (ISS), compared to a Traditional System (TS), can modify the physico-chemical and sensory attributes of the Tepeque Artisanal Cheese (TAC), including ripening process. The cheese is produced in the Valley of Tepalcatepec, located in western Mexico at an altitude of 300 m and average temperature of 24 oC. A batch of 48 cheeses of 3 kg of fresh weight each was made, from this batch, 24 cheeses were made with milk from the ISS and 24 were made with the TS milk; then they were divided into groups of six cheeses and subjected to four periods of ripening: 10, 45, 80 and 165 days. All cheeses were ripened under the same conditions (27+5°C, 72% relative humidity). At the end of each period the cheeses were analyzed in the laboratory. The moisture content was determined by a forced air oven at a temperature of 60°C for 24 h, the crude protein content was determined by the Kjeldahl method. The ash content was determined by incinerating the sample in a muffle furnace at 550°C for 4 hours. The fat content was determined by ether extract by the Soxhlet method. The pH was measured with a potentiometer (Orion M520A, USA) in a 9:1 homogenized dilution of cheese and distilled water. For the chloride content the Volhard method was used. A sensory analysis was performed by using a trained panel consisting of 12 panelists who were selected according to their ability to distinguish aromas, flavors and textures. Subsequently, all cheese samples were subjected to sensory analysis using a categorical scale ranging from 1 (no perception) to 5 (intense perception) for each of the descriptors. Principal Component Analysis (PCA) was used; and to evaluate the effect that derives from the feeding system, a Discriminant Analysis (DA) was used. No significant effect ($P>0.05$) was observed on the physico-chemical parameters of milk and cheese associated with the feeding system. The concentration of fat, protein and NaCl was increased during ripening, while the pH and the moisture decreased ($P<0.05$). According to the PCA, the TAC is a cheese with rigid texture, and little fracturability during ripening. The salt and fat are the predominant aromas. The DA did not identify any associated effect with the feeding system. It was concluded that the use of the ISS has no effect on the physico-chemical and sensory attributes of TAC.

C-122

Influence of monensin continuous-release intraruminal device on milk and whey starter quality used for *Parmigiano Reggiano* cheese production.

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Aim of the study was to evaluate the effects of treatment with monensin continuous-release intraruminal device (Kexxtone), on milk and whey starter quality used for *Parmigiano Reggiano* cheese production. For that purpose, 33 cows were treated with Kexxtone 3 weeks before the predicted calving date, as label indications. Around 10 days after calving, those cows became part of a treated group, while another control group was set up with untreated cows. Groups were housed in two comparable pens, fed the same diet and milked separately twice a day. From each group 2 cheese wheels were produced daily. Milk and whey starter of each group were maintained separated during every phase of the cheesemaking process and then compared. The experimental period lasted 7 weeks, during which groups were balanced for parity and average DIM. Morning tank milk and whey starter were sampled twice a week, for a total amount of 13 samples per group. Milk was analyzed for fat, casein, protein, lactose, titrable acidity, pH, clotting characteristics, Somatic Cells Count, urea and total bacterial count. Whey starter was analyzed for titrable acidity, fermentative activities at 45, 52, 54°C and total lactic acid bacteria count. All cheesemaking procedures were recorded by the cheesemaker. Data were analyzed using one-way ANOVA and t-test Tukey as post-hoc (SPSS 17.0, USA). Statistical difference was fixed for $P<0,05$. Milk composition and whey starter quality didn't show any significant difference between the groups. SCC in morning tank milk was significantly lower in treated than control group (116,69±16,01 *vs.* 228,85±29,79 *1000/mL) as well as Total Bacterial Count (4,08±1,27 *vs.* 6,08±3,07 *1000 UFC/mL) and titrable acidity (3,66±0,04 *vs.* 3,72±0,06 SH/50ml). Cheesemaking procedures didn't show any significant differences, except for the raw/skimmed milk ratio, lower for treated than control group (1,53±0,03 *vs.* 1,59±0,04) and 36 hours cheese yield, which was slightly lower for treated group (8,68±0,14 *vs.* 8,79±0,14 %). These results suggest that treatment with a monensin continuous-release intraruminal device in cows, doesn't affect milk and whey starter quality, as well as cheesemaking procedures. Besides, it seems to have a positive effect on somatic cells count in milk, probably associated to an improved general health status.

C-123

The inclusion of a by-product from myrtus communis in the diet of lactating ewes: effects on milk production traits

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The use of exhausted berries (EB) of *Myrtus communis* L., a by-product obtained after production of the characteristic myrtle liqueur typical of Sardinia (Italy), in ruminant nutrition could represent an alternative for their problematic management and disposal. This by-product contains polyphenols, which might have beneficial antioxidant, antibacterial and anti-inflammatory activities. To evaluate its potential use in sheep nutrition, we investigated the effect of dietary EB, on milk production traits in lactating ewes. Twenty-four Sarda dairy ewes were randomly assigned to three treatments consisting of a control (CON), a group supplemented with 50 g/d (EB1) or 100 g/d (EB2) per head of exhausted berries. The study lasted 11 weeks, with three weeks of adaptation period and 8 weeks of experimental period. Milk yield was measured and samples were collected weekly and analyzed for fat, protein, casein, lactose, pH, and somatic cell count (SCC). The milk coagulation properties (RCT rennet coagulation time, K20 curd firming time, A30 curd firmness) were determined with a Formagraph. Data were analyzed using PROC MIXED of SAS (SAS Institute Inc., Cary, NC). Dietary treatments affected milk yield and milk coagulation properties. Milk yield decreased and fat and protein contents increased in EB2 compared with CON and EB1 groups. The highest dose of EB caused an increased in the milk CCS and a worsening of milk coagulation properties compared with CON and EB1 groups. The RCT in EB2 group (13.9 min) was higher than CON (11.5 min) and EB1 (11.6 min), whereas A30 was lower (55.7 mm) than CON (58 mm) and EB1 (60 mm) groups. These results suggest that exhausted berries can be included in the diet of lactating ewes in a low dose to avoid adverse effects on milk yield, milk coagulation properties and mammary health status.

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C-124

Effect of PUFA supplementation on milk production and fatty acid profile in sheep under hot season

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The objectives of this study were to evaluate the effects of different source of polyunsaturated fatty acids (PUFA) supplemented to lactating sheep exposed to high ambient temperature, on milk production and milk fatty acid profile. The experiment was conducted during summer, involved 32 ewes divided into 4 groups. All groups were fed twice daily and received oat hay and pelleted concentrate; ewes in the experimental groups were supplemented with whole flaxseed (FS, 250 g/ewe/d), seaweed Ascophyllum nodosum (Tasco®, AG, 5% A. nodosum), or their combination (FS+AG). Milk samples from each ewe were collected at morning and afternoon milking every week, and analyzed for pH, total protein, casein, fat, lactose content, and SCC. At the beginning of the experiment and then at 4 and 6 wk each milk sample was analyzed for milk fatty acids using a gaschromatograph. Data were processed using ANOVA for mixed models using the MIXED PROC of SAS. During the trial, ambient temperature and relative humidity were monitored with thermo-hygrographs placed at 1.5 m from the floor, and the average temperature-humidity index (THI) was calculated. Maximum THI started from 85 during the first week of the experiment, and never dropped below 76 until the end of the trial. PUFA supplementation increased milk yield. The total content of saturated fatty acids (SFA) in milk was lower in FS group, followed by FS+AG group. Monounsaturated fatty acids (MUFA) increased in FS and FS+AG group. PUFA , PUFA/SFA and total n-6 FA significantly increased in FS group; the total n-3 FA was found to be higher in FS and FS+AG than in AG and C. C18:1t11 increased in flaxseed supplemented group by 57% compared to C, by 65% compared to AG and by 71% compared to FS+AG group. FS and FS+AG showed a major content of C18:2t9t12, C18:3n3 and C22:5n3. In particular, C18:3n3 increased in FS group by 168% compared to C group, and by 159% compared to AG group. Flaxseed supplementation increased total CLA isomers compared with the other PUFA supplement in the diet of ewes; the total CLA content was the highest in FS milk. Finally, flaxseed supplementation, also in combination with seaweed A. nodosum, decreased AI and TI indexes of sheep milk. As a conclusion, flaxseed supplementation alone, or to a lesser extent in combination with seaweed A. nodosum, can be suggested as an adequate supplementation to sustain milk production and milk fatty acid profile of sheep under hot season.

C-125

Effect of the inclusion of lemon citrus pulp in the diet of Valle del Belice lactating ewes. Milk production and composition

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Lemon citrus pulp residues from the citrus juice industry; it belongs to the citrus by-product that are more extensively used for livestock feeding. Furthermore, citrus pulp is a source of flavonoids such as hesperidin and naringin, which have antioxidant properties. This study aimed to investigate the effect of fresh lemon citrus pulp (LP) as natural antioxidant, on feeding behavior, milk yield and milk quality of Valle del Belice lactating ewes in the hot summer season in Sicily. A total of 15 second lambing ewes were individually allocated in 3×3 m box and fed with 3 diets in a 3 × 3 Latin square design, with 3 phases composed of 21 days. The diets were: LP0, mixed hay *ad libitum* plus 600 g/d of concentrate; LP1, mixed hay *ad libitum* plus 400 g/d of concentrate and 1 kg/d of LP; LP2, mixed hay *ad libitum* plus 200 g/d of concentrate and 2 kg/d of LP. The samples of offered and refused forage, concentrate and LP were analyzed for the determination of DM, CP, EE, ash, NDF, ADF and ADL. Individual milk samples were analyzed for lactose, fat, protein, casein, urea, SCC, pH, titratable acidity. At the end of the pre-experimental and experimental phases, total polyphenol intake, milk polyphenol content and milk antioxidant capacity were also determined. Statistical analyses were performed using the MIXED model where experimental phase (1, 2, 3) and diet (LP0, LP1, LP2) were fixed factors and the ewe was considered as random factor. On the basis of voluntary feed intake, the ewes of each group ingested the same dry matter, around 2 kg/d/head, but the LP2 group received lower net energy than other groups (1,27 *vs.* 1,33 UFL), in average slightly higher of their nutritional needs. The LP resulted 10% and 20% of the total DM intake, in LP1 and LP2 group respectively. The effect of the high temperatures determined average low milk productions. The LP2 ewes produced significantly lower daily milk yield than the other two groups (322 g/d *vs.* 369 g/d and 355 g/d for LP2, LP0 and LP1 groups respectively), probably due to lower net energy ingested. Milk fat did not differ significantly among groups, while milk protein and casein percentages showed higher values in LP2 than in LP1 group, probably due to higher availability of amino acids for the intestinal absorption. The milk urea content of LP0 resulted significantly lower than other groups. Polyphenol analyses are still under execution.

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C-126

Effect of olive pomace and extruded linseed on milk yield and fatty acid composition from dairy ewes

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Use of extruded linseed (EL) in the diet of dairy ewes is an effective strategy to enrich milk with omega-3 polyunsaturated fatty acids (PUFA n-3) and conjugated linoleic acid (CLA). However, since EL is largely biohydrogenated in the rumen, the amount of extruded linseed needed to obtain an effective enrichment of PUFA n-3 in milk fat may often result in an increase of feeding cost. The use of polyphenols in the diet of dairy ruminants has been proposed as strategy to perturb rumen biohydrogenation of dietary PUFA and to increase the passage rate of PUFA omega-3 from the diet to milk. Crude phenolic concentrate (CPC) obtained by membrane filtration of olive vegetation waters to reduce their pollutant charge could be considered a good source of polyphenols, mainly secoridoides derivatives. The aim of this study was to evaluate the effect of the inclusion of linseed and CPC in diets of dairy ewes on milk yield and fatty acid (FA) composition. Twenty pluriparous Comisana ewes were randomly allotted into four groups after a baseline period of three weeks, when the animals received the same dietary regimen based on alfalfa hay and a concentrate feed poor in lipids. Subsequently, the four experimental diets were based on alfalfa hay *ad libitum* administrated and 800 g/head and day of a concentrate feed containing: linseed (L diet) or linseed plus different quantity of CPC to provide 0.4 (L0.4), 0.8 (L0.8) or 1.2 (L1.2) g/kg DM of polyphenols. The trial lasted 5 week, individual milk yield was weekly recorded and analysed for proximate and FA composition. Data were analysed by a repeated measure model. The inclusion of EL and CPC in the diet of dairy ewes did not affect milk yield and proximate composition, however, at the maximum dose, CPC resulted in a significant increase of linoleic and alpha-linolenic acid by 18% and 24%, respectively, if compared to milk fat from ewe fed L diet. At the same time, milk fat from ewe fed L1.2 diet was lower in t11 18:1, CLA content (-38% for both FA) and other intermediates of the biohydrogenation process, probably as a consequence of a perturbing effect of OP on rumen bacteria responsible of the biohydrogenation of dietary PUFA. In conclusion, addition of OP to diet supplemented with EL enhanced the content of PUFA in milk fat from dairy ewes, without affecting productive performance of the animals.

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C-127

Growth response, plasma metabolites and intestinal brush border enzyme activity of sea bass fed diets including a blend of two marine microalgae

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Microalgae have attracted increasing attention as animal feed supplements since they are natural sources of bioactive compounds which give them nutraceutical properties in addition to their basic nutritional value. More recently, dry microalgae biomass have also been proposed as raw materials in partial substitution for fish meal and oil in aquafeeds. The aim of this study was to evaluate the effects of including a blend of dried marine microalgae in low fish meal/fish oil diets on growth performance, levels of plasma metabolites and activity of brush border intestinal enzymes in adult European sea bass (*Dicentrarchus labrax*, L.). Two test diets (A1 and A2) were prepared by including a blend of Isochrysis galbana and Tetraselmis suecica dried biomass in a 2:1 w:w ratio to replace 15 and 45% fish meal protein and 10 and 30% fish lipid of a control diet (C) with a 50:50 fish to vegetable-protein-lipid ratio. One hundred eight fish (mean body weight 204±12.7 g) were randomly divided among 9 groups kept in a marine recirculating tank system ensuring nearly optimal water condition to sea bass (temperature 21°C, salinity 28‰). Fish were fed the test diets to visual satiety over 15 weeks according to a randomized design with 3 replicates per dietary treatment. At the end of the trial, final biomass, specific growth rate (SGR) and feed conversion ratio (FCR) were calculated per group and 6 fish per dietary treatment were sacrificed and immediately subjected to blood and intestine sampling for further analysis of plasma metabolite levels (glucose, cholesterol, triglyceride, total proteins and albumin levels) and activity of mucosal brush border enzymes in different sections. No diet-induced effects were noted in the final individual live weight, 420±14.5g, SGR, 0.68±0.003 and FCR, 1.7±0.11. Amongst plasma metabolites, only cholesterol and total proteins were affected by dietary treatments resulting in reduced levels only in fish given the diet highest in microalgae relative to controls (347.2 *vs.* 276.6 mg/dL and 5.90 *vs.* 5.37 mg/dl; respectively, P<0.05). The activity of the intestinal mucosal enzymes varied according to the different intestinal tracts but did not show major diet-dependent changes. This study suggests that a blend of dried marine microalgae biomass could further reduce reliance on fish meal and fish oil in practical diets for adult sea bass without adversely affecting growth response and digestive-absorptive functions.

C-128

Dietary fish oil and stearate action on adipose lipid metabolism transcriptomics in periparturient dairy goats

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The aim of this study was to evaluate the effects of saturated or unsaturated fatty acids on adipose mRNA level of genes involved in the major lipogenic metabolic pathways. Twenty-three second parity alpine dairy goats were assigned to three treatments: (C; n=8) fed a non fat-supplemented basal diet, (ST; n=7) fed a basal diet supplemented with stearic acid and (FO; n=8) fed a basal diet supplemented with fish oil. The supplementation started from the last week of gestation and lasted 21 days after kidding. Treatments supplied 30g/head/d extra fatty acids during the dry period and 50g/head/d during lactation. Individual blood samples were taken at 14, 7 and 2 days before the expected kidding day as well as 0, 2, 7, 14 and 21 DIM. Liver and adipose tissue biopsies from each experimental subject were harvested on days -7, +7 and +21 relative to parturition. Quantitative real-time RT-PCR of ADIPOQ, LPIN1, LPL, PPARG, SREBF1 and THRSP expression was performed. Data obtained were analyzed using the MIXED procedure of SAS. No differences were observed for milk production, milk composition, body weight and body condition score. Differences were detected for EB between C compared with ST and FO at day 7 (0.34 *vs.* -1.12 and 0.91 respectively; P,â§0.05). Serum cholesterol concentration was significantly higher in FO compared to C (P<0.01) and ST (P<0.05) at day 21. Fat supplementation significantly affected subcutaneous adipose genes involved in TAG synthesis (LPIN1), in the regulation of lipogenesis (THRSP) and nuclear receptor (SREBF1). No effects were observed for genes involved in LCFA uptake (LPL) and on the expression of the adiponectin (ADIPOQ). LPIN1 expression was significantly higher in the FO group compared with ST (1.46-fold *vs.* 0.59-fold; P<0.04) suggesting increased lipogenesis. THRSP (P=0.003) and SREBF1 (P=0.04) were up regulated before kidding and increasingly down regulated from 7 to 21 days postpartum. In conclusion, this study highlighted the important role of saturated or unsaturated fat administration in dairy goat diets to modulate mRNA expression of genes involved in TAG synthesis, regulation of lipogenesis and nuclear receptor.

C-129

Effects of conjugated linoleic acids isomers on oxidative mammary gland metabolism

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The study aimed at investigating the effect of CLA isomers cis-9,trans-11 (c9,t11) and trans-10,cis-12 (t10,c12), on oxidative status of bovine mammary gland. For the study bovine epithelial cell line BME-UV1 and the aflatoxin B1 (AFB1) as oxidizing agent as experimental model have been used. The uptake rates of CLA in cells at 3 and 48h were tested using DAD HPLC analysis. The cells were pre-incubated with complete medium containing 50 μM c9,t11, 50 μM t10,c12 and 50 μM CLA Mix (50% c9,t11 and 50% t10,c12) and then AFB1 (20 μg/mL) was added. After 48h of incubation the cells treated in the presence or absence of AFB1 were collected for determining cell viability (XTT assay), oxidative markers such as nicotinamide adenine dinucleotide phosphate (NADP+/NADPH), glutathione (GSH/GSSG), thiobarbituric acid reactive substances (TBARS), protein carbonyl groups (CP), superoxide dismutase (SOD), glutathione peroxidase (GPx1), glutathioneS-transferase (GST) and glutathione reductase (GR). The mRNAs quantification of bovine GSHPx-1, GSR, GST and SOD, and milk α-CN, β-CN and α-Lalb was performed by rt-PCR. Data were analysed by ANOVA and differences were declared significant at $P<0.05$. The results showed that the uptake rates of CLA in cells increased from 3 to 48h. Cells exposed to AFB1 showed a loss of cell viability after 48h. CLA have increased ($P<0.05$) the concentration of reduced GSH and NADPH and decreased ($P<0.01$) the levels of GSSG, mostly in cells treated with t10,c12. The activity of GR and GST was decreased ($P<0.05$) in cells treated with CLA. Higher levels of GSPx1 and SOD ($P<0.01$) activities were observed in cells treated with CLA in presence of AFB1. Increase of TBARS levels was observed in cells treated with CLA in presence of AFB1, whereas CP content increased both in cells treated with CLA and with CLA in presence of AFB1. Regarding the mRNA's expression of GPX1, GR, GST and SOD no differences were observed among all treatments. No treatments, CLA alone or CLA plus AFB1, had a substantial effect on gene expression of milk proteins. Findings of the present study corroborate an antioxidant role of CLA by developing a significant improvement of redox status in cells, in particular t10,c12. CLA treatment might enhance the intracellular redox homeostasis and could be of help in improving physiological oxidative stress situations as the periparturient period in dairy cow.

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C-130

Effect of the ruminal slow-release of monensin during the transition period of dairy cows on health status, energy metabolism and inflammatory conditions

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Monensin is a ionophore which regulates the transport of ions across the cell membrane of microbes. In the rumen milieu, monensin selects for Gram- bacteria increasing the propionate and lowering the butyrate molar proportions in VFA, and produces an anti-ketotic effect with important benefits in early lactating cows. The transition period in dairy cows is also characterized by an inflammatory condition, but the influence of the monensin has not been investigated in this respect. Thus, 14 heifers and 32 multiparous cows were allocated in two homogeneous groups: MON, dosed with a slow-release bolus of monensin (32.4 g, Kexxtone®, Elanco Animal Health, UK) 4 weeks before the expected calving, and CTR used as control. Health status, body condition score, milk yield and blood inflammatory-metabolic profile were frequently monitored from -28 to 56 days from calving (DFC). During the experiment, MON had more cows without clinical symptoms (11 *vs.* 8) and a lower number of clinical cases (0.73 *vs.* 1.18 N./cow) in comparison to CTR. The milk yield resulted similar among the groups until 30 DFC, but in the second month of lactation it was higher in MON (+2.8 kg/cow/d). Non-esterified fatty acids (0.45 *vs.* 0.56 mmol/L) and β-hydroxybutyrate (0.57 *vs.* 0.65 mmol/L) in plasma were reduced by the treatment ($P<0.10$), particularly the week before and after calving. MON (vs CTR) also showed an higher uremia, mainly around calving, and a marked reduction of creatinine ($P<0.10$ *vs.* CTR from 7 and 21 DFC) concentrations. Indices of oxidative stress and inflammation showed the typical pattern of changes around parturition and did not differ between groups. Nevertheless, concentrations of zinc in plasma remained lower in MON until 28 DFC (11.8 *vs.* 13.1 mcmol/L, $P<0.05$). The rumen supplementation of monensin as slow-release bolus confirms its anti-lipolitic and anti-ketotic effects in the periparturient dairy cow, mitigating the severe negative energy balance. Moreover, the more marked reduction of creatinine deposes for an increased mobilization of the muscle tissue in early lactation. The higher uremia in MON confirms this circumstance and suggests the utilization of the amino acid for the gluconeogenesis. Despite the inflammatory and oxidative stress conditions were not affected by monensin supplementation, the lower ketonemia seems to improve the immune system activity, as confirmed by the lower incidences and severity of clinical diseases during the experimental period.

C-131

Alteration of bovine milk fatty acid profile caused by the dietary crude protein content supplemented or not with rumen protected conjugated linoleic acids

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Twenty cows housed in 4 pens, homogeneous for parity, days in milk (DIM), and milk yield (MY) received 4 diets with 150 (CP15) or 123 (CP12) g of CP/kg DM, supplemented or not with rumen protected conjugated linoleic acids (rpCLA) (6.34 and 6.14 g/d of C18:2c9,t11 and C18:2t10,c12, respectively), according to a 4×4 Latin Square design. Dry matter intake and milk yield were recorded daily and milk samples were collected during the last of 3 wks of each of the 4 experimental periods. Milk fatty acids (FA) were extracted with hexane-isopropanol and methylated with sodium methoxide. Commercial standards for FA molecules were used to identify milk FA and their detection was performed using a bi-dimensional gas chromatographer. Data were analyzed with a mixed model which considered period, dietary treatment as sources of variation and group as residual error term. The dietary CP had small influence on the FA profile, as only few FA, mainly some odd and branched FA, were altered. In particular, cows receiving the low protein diet produced milk with a significantly greater proportion of branched and odd FA ($P<0.001$) compared to control. Differently, rpCLA had a strong influence on the FA profile, as it reduced the proportion of short and medium chain FA and that of saturated FA ($P<0.001$) and increased the proportion of CLA ($P<0.001$).

C-132

Intragiugular infusion of long chain omega-3 fatty acids at calving: effect on metabolic profile, feed intake and milk yield

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Calving represents a drastic change in the metabolism of the cow, accompanied by a physiological inflammation, which if too intense could compromise the health status and the future performance of the cows. It is well known that omega-3 fatty acids can modulate the inflammation, thus with the aim to reduce the inflammation at calving, 5 cows were intragiugularly infused (OV) at 12, 24 and 48 h after calving with 15 g of purified fish oil containing 2.3 g of EPA and 3.4 g of DHA, while 5 cows were used as control (CTR). From 30 d before calving till to 28 days in milk, dairy cows have been checked daily for dry matter intake (DMI), rectal temperature (RT) and health-related problems. Blood samples were collected before the feed distribution every 3-4 days and daily close to calving. The samples were analysed to measure the indices of the energy and protein metabolism, of the liver functionality, and of inflammatory conditions. Milk yield at each milking was also recorded. During the week before calving all the cows were in optimal health condition, maintaining high DMI (on average value of 12.6 kg/d) and normal range of daily RT (on average 38.8°C, range 38.3-39.3°C). At metabolic level no differences attributable to the treatment have been observed. Haptoglobin was lower in OV, with significant difference ($P<0.05$) during the first 4 d after calving (0.35 *vs.* 0.58 g/L in OV and CTR, respectively). During the first week of lactation a higher level of albumin (37.0 *vs.* 35.2 g/L, $P<0.09$) and of Zn (11.4 *vs.* 8.32 mcmol/L, $P<0.05$) were observed in OV *vs.* CTR. These results indicate a reduction of inflammation in cows infused with fish oil. The better condition in OV cows was confirmed by their higher DMI during the first 12 DIM (17.2 *vs.* 16.0 kg/d; $P<0.1$). In turn the increased DMI allowed a higher milk yield in OV cows during the first 12 DIM (34.4 *vs.* 29.2 kg/d, $P<0.08$). Our results confirm that the restraint of the inflammation at calving by omega-3 fatty acids (mainly DHA and EPA) improves the welfare conditions even in apparently healthy dairy cows, which reward with higher performance. Actually, the post-calving infusion of omega-3 fatty acids is a technique difficult to implement in commercial herds, but analogues results can be achieved with the supplementation of appropriate rumen-protected products enriched with long chain omega-3 fatty acids.

C-133

Copy number variation in cattle breeds

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Detecting all classes of genetic variation in livestock species, such as cattle, is a pre-requisite to studying their association to traits of interest. Copy Number Variations (CNVs) are classes of polymorphic DNA regions including deletions, duplications and insertions of DNA fragments of at least 0.5 kb to several Mb, that are copy number variable when compared to a reference genome. CNVs can be identified using various approaches, among those the SNP array data are low cost, dense coverage, and high throughput. The aim of this study was to obtain a consensus genome map of Copy Number Variable Regions (CNVRs) in the Brown Swiss (dataset of 192 bulls), Red Pied Valdostana (dataset of 143 bulls) and Finnish Ayrshire (dataset of 243 bulls) cattle breeds all genotyped on the Illumina Bovine HD BeadChip, and two SNP based CNV calling algorithms. Brown Swiss cattle originated in the Swiss Alps, kept as a triple purpose breed. Once imported in the US, it was mainly selected for increased milk production. The Valdostana Red Pied cattle is the most common autochthonous dual purpose breed in the region Valle d'Aosta in Italy (13,000 animals in 2013, almost all of them registered in the Herd Book). The Finnish Ayrshire is the most common cattle in Finland. CNVs were called with the PennCNV and SVS7 software and were summarized to CNVRs at the population level as overlapping CNV calls within breed. PennCNV identified 2,377, 1,723 and 1,689 for the Italian Brown Swiss, the Red Pied Valdostana and the Finnish Ayrshire, respectively. SVS7 detected 370, 235 and 2,063 for the three cattle breeds. These regions were annotated with Ensembl v78 Bos taurus gene set (UMD3.1) and genomic regions harboring QTL for production and functional traits. The comparison among CNVRs here identified provided common regions in the breeds. The results of this study are a comprehensive genomic analysis of cattle CNVs derived from SNP data, which will be a valuable genomic variation resource and will enrich the bovine CNV map in the cattle genome, providing new information for association studies with traits included in the selection programs.

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C-134

Haplotype detection for monogenic factors in the Italian Holstein cattle breed

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Italian Holstein genomic data-base has more than 125.000 genotyped animals with several SNPs panels, and every month this number is increasing. These genotypes are not only used for genomic breeding values, but are used also as parental confirmation and detection for herd book animals. Moreover the availability of these enormous amount of SNPs allows the reconstruction of several haplotypes. Recently we have derived information on 11 haplotypes of which 9 contain a lethal disorder and the other 2 haplotypes are for two variations of the polled gene. The concept is to scan imputed chromosomes for a haplotype in common between carrier animals. If a disorder is lethal than the haplotype containing the mutated gene should never occur in homozygous form except when recombined. First we introduced the haplotype detection for recessive factors affecting fertility, these haplotypes are indicated with HH1, HH2, HH3, HH4, HH5. For each of these five haplotypes carrier females risk an embryonic loss during gestation. Lately carrier status for Holstein haplotypes with mutations for brachyspina (HH0), bovine leukocyte adhesion deficiency (BLAD), complex vertebral malformation (CVM), mulefoot (syndactyly), and polledness (2 carrier haplotypes) have been introduced. In total 125.498 unique genotypes were used. Also identical twins, imputed animals which are not genotyped but have several genotyped relatives and some ancestors get a haplotype assigned. This leads to 152.322 animals with information. Around 77% of the animals are free of a lethal genetic disorders; the remaining part have a small probability to have an abortion or a genetic disorder. For recessive disorders, problems occur only in 25% of the matings between carriers. Table 1 reports in detail the different carrier haplotype frequencies detected in the Italian Holstein population. A monitored mating plan like the one offered by Anafi Webpac would help farmers to avoid undesirable genetic disorders. Furthermore AI centers can use haplotypes as an additional early-stage selection tool.

C-135

A statistical learning approach to detect carriers of the HH1 haplotype in Italian Holstein Friesian cattle

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The increasing availability of SNP (single nucleotide polymorphisms) genotype data in livestock is stimulating the development of new data analysis strategies, which can be applied in animal breeding. One possible application is the prediction of carriers of specific haplotypes, especially if they impact animal health. It is therefore convenient to have a practical and easy-to-implement statistical method for the accurate classification of individuals into carriers and non-carriers. In this paper, we present a procedure for the identification of carriers of the haplotype HH1 on BTA5 (Bos Taurus autosome 5), which is known to be associated with reduced cow fertility in Holstein-Friesian cattle. A population of 1104 Holstein bulls genotyped with the 54K SNP-chip was available for the analysis. There were 45 carriers (5.3%) and 1045 non-carriers (94.7%). Two complementary multivariate statistical techniques were used for the identification of haplotype carriers: Backward Stepwise Selection (BSS) to select the SNP that best fit the model, and Linear Discriminant Analysis (LDA) to classify observations, based on the selected SNP, into carriers and non-carriers. In order to explore the minimum-sized set of SNP that correctly identifies haplotype carriers, different proportions of SNP were tested: 2.5; 10; 15; 30; 50 and 100%. For each proportion of SNP, BSS and LDA were applied, and the classification error rate was estimated in a 10-fold cross-validation scheme. Data were split in 10 subsets. The first subset was treated as validation set, while the model was fit on the remaining nine subsets (the training set). The overall error rate for the prediction of haplotype carriers was on average very low (~1%) both in the training and in the validation datasets. The error rate was found to depend on the number of SNPs in the model and their density around the region of the haplotype on BTA5. The minimum set of SNPs to achieve accurate predictions was 8, with a total test error rate of 1.27. This work describes a procedure to accurately identify haplotype carriers from SNP genotypes in cattle populations. Very few misclassifications were observed, which indicates that this is a very reliable approach for potential applications in cattle breeding.

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C-136

Fine mapping of loci on BTA8 associated to antibody response to *Mycobacterium avium* paratuberculosis in cattle

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Paratuberculosis (ParaTB) or Johne's disease, caused by *Mycobacterium avium* subspecies paratuberculosis commonly known as MAP in cattle, is a chronic gastroenteritis characterized by diarrhoea, decreased milk production and ultimately death. MAP is responsible for huge economic losses, particularly in dairy cattle herds. Susceptibility to MAP infection has been found to be heritable with heritability estimates ranging from 0.06 to 0.102. The definition of an infected animal can be based either on the presence of anti-MAP antibodies in the serum, or by direct demonstration of MAP in tissue or faeces by culture or PCR. Several studies have addressed the identification of genetic loci associated with MAP susceptibility. The objective of this study was to refine a locus associated with antibody response to *Mycobacterium avium* paratuberculosi (MAP). Using a genome-wide association analysis, a single nucleotide polymorphism on Bos taurus autosome BTA8 namely the SNP rs43161947 at position 35398490 with a p-value of 7.02 e-05, has previously been identified by the authors as associated with MAP infection. Fine mapping of the region was conducted with 100 single nucleotide polymorphisms spanning a region between BTA8: 34422912 and BTA8: 364553881 covering 2 Mega bases (Mb) designed in to cover 1 Mb ahead and after the SNP identified on BTA8. The 2 Mb region on BTA8 was evaluated within a group of 966 Holstein cows collected from routine ParaTB screening in the province of Lodi in Italy, in an area with a high prevalence of ParaTB. Animals were defined as ParaTB positive based on the detection of serum antibodies produced in response to MAP infection using the ID-screen® ELISA test (ID VET Montpellier, France). Of the 966 samples, 483 were MAP antibody positive (cases) and 483 MAP antibody negative (MAP negative controls). All animals were female, and cases and MAP negative controls were from the same farm tested on the same day.Using a single marker association analysis, conducted within the R statistical environment, we identified 3 different QTLs within the 2 Mega base region, under the main QTL on BTA8 associated with antibody response to MAP, in position 34.700.000, 35.800.000 and 36.400.000 bp. This reveals the complexity of the genetic architecture of the trait and confirms the need to further explore the genome with fine mapping approaches, or by the use of whole genome sequencing to investigate complex traits, such as disease resistance.

C-137

Heritability of milk b-hydroxybutyrate and its genetic association with milk yield and fat-to-protein ratio in Italian Holstein cows

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Ketosis is a metabolic disease occurring when the cow experiences negative energy balance, typically in early lactation. Cows are more likely to suffer from subclinical ketosis, which is characterized by increase concentrations of ketone bodies such as β-hydroxyburyrate (BHB) in blood and milk. Subclinical ketosis has a negative economic impact on the dairy herd, mainly related to the loss of milk production. Despite ketosis is typically influenced by environmental factors, recent studies have reported that a genetic basis for this disease exists. The aim of this work was to estimate heritability of milk BHB (mmol/L) and to assess its genetic correlations with milk yield (MY, kg/d) and fat-to-protein ratio (F:P) in Italian Holstein dairy cattle. Test-day milk samples analyzed by mid-infrared spectroscopy were retrieved from the laboratory of the Breeders Association of Veneto region and included 86,908 records from 19,980 cows of parity 1 to 3 and between 5 and 305 days in milk. Samples were collected between April 2013 and May 2014 in 299 herds. The average number of records per animal within each lactation was 3.5 (range: 1 to 10). To achieve normality and homogeneity of variances, values of BHB were log-transformed to lBHB according to the formula lBHB = ln(BHB+1). The pedigree file (58,687 animals) included individuals with phenotypic records and all their ancestors up to 4 generations back. Genetic parameters for lBHB, MY and F:P were estimated within each lactation using a random regression animal model. Average heritabilities of lBHB were 0.14, 0.13 and 0.08 for parities 1, 2 and 3, respectively. Heritability was quite stable during lactation, with a slightly different pathway between first and second lactation. Mean genetic relationships between lBHB and MY were -0.21, -0.09 and -0.13 for parities 1, 2 and 3, respectively, and the highest negative values (-0.59, -0.36 and -0.20, respectively) were found in early lactation. Finally, mean genetic correlations between lBHB and F:P were 0.33, 0.28 and 0.31, respectively, and the highest positive values (0.63, 0.41 and 0.67, respectively) were assessed at the beginning of lactation. Results suggest that milk BHB routinely analyzed during test-days in Italian Holstein cows is a heritable trait and thus breeding strategies to produce progeny less susceptible to ketosis can be adopted.

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C-138

Genetic and phenotypic relationships of milk coagulation properties and curd firmness modeling with the cheese yield and curd nutrients recovery in bovine milk

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The aim of this study was to elucidate the relationships of milk coagulation properties (MCP) and curd firmness (CF) modeling with the cheese yield (CY) and milk nutrient and energy recoveries (REC) in the curd, measured at the individual cow level. Data were collected from 1,167 Brown Swiss cows reared in 85 herds. A 2.0 L milk sample per cow was used for determining 10 phenotypes related to MCP, CF and syneresis as well as 7 cheese-making traits. The first set of traits comprised 4 traditional single point lacto-dynamographic properties [MCP expressed as RCT: rennet coagulation time, min; k20: time to a CF of 20 mm, min; a30 (a45): CF 30 (45) min after rennet addition], 4 parameters derived from modeling 360 CF data recorded over time (1 every 15 sec) for each milk sample (CFP: potential asymptotic CF at infinite time, mm; kCF: curd firming instant rate constant, %×min-1; kSR: syneresis instant rate constant, %×min-1; RCTeq: RCT obtained from modeling all CF measures) and 2 traits calculated from individual equations (CFmax: maximum CF, mm; tmax: time at CFmax, min). The second group of traits accommodated 3 CY traits expressing the weight (wt) of fresh curd (%CYCURD), curd dry matter (%CYSOLIDS), and curd moisture (%CYWATER) as % of wt of milk processed, and 4 REC traits (RECFAT, RECPROTEIN, RECSOLIDS, and RECENERGY calculated as the % ratio between the nutrient in curd and the corresponding nutrient in processed milk). Several multitrait analysis were performed, in a Bayesian framework, to estimate the phenotypic, additive genetic (rg), herd and residual relationships between the aforementioned traits. Results showed that a45, CFP and CFmax were genetically associated with all %CY (rg varying from 0.75 to 0.85 for a45; 0.49 to 0.58 for CFP; and 0.75 to 0.80 for CFmax) and REC traits (rg varying from 0.29 to 0.90 for a45; 0.43 to 0.69 for CFP; and 0.41 to 0.94 for CFmax), with the exception of RECPROTEIN. Based on our findings, it is proposed for RECPROTEIN (heritability of 0.36) to be incorporated in a genetic index for the improvement of cheese-making efficiency at population level in dairy cattle breeding. Moreover, our results demonstrated the weak genetic relationship of RCT with CY and REC traits.

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C-139

Effects of variation in milk coagulation properties on cheese yield, curd composition and recovery rates of the main milk constituents measured by micro-cheese making

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This study investigated the effect of variation in milk coagulation properties (MCP) on cheese yield, curd composition and recovery rates of protein and fat in the curd of soft model cheeses. Rennet coagulation time (RCT), curd firming rate (k20), and curd firmness measured at 30 min (a30), 45 min (a45), and 60 min (a60) from rennet addition were assessed in 1,004 individual milk samples of Italian Simmental cows. Raw cheese yield (RCY) was measured by micro-cheese making procedures using 25 mL of individual milk. The micro-curds obtained were analyzed for contents of moisture (MOI), protein and fat in cheese dry matter. Inference on effects of MCP on cheese yield, curd composition and recovery rates of protein and fat in the curd was based on Bayesian analyses. The linear model included the effects of the micro-cheese making batch (83 levels) the linear effect of fat, protein, lactose content, SCS and pH of milk, the linear effect of RCT, k20, a30, a45 or a60, and the additive genetic effect of the animal. Separate analyses were carried out for different MCP traits. Increased RCT was associated with increased RCY, which was ascribed to greater amount of water retained in curds yielded by late-coagulating milk compared to those of short-coagulating milk. Curd MOI increased linearly when RCT increased (0.64% per 1 RCT standard deviation). Low amounts of protein were recovered in curds obtained from milk having high k20, resulting also in a slightly reduced dry matter yield. Firm curds at 30 min yielded low MOI cheeses, gave great amounts of protein recovered in the curd (0.75% per 1 a30 standard deviation), and high dry matter cheese yield relative to weak curds. Curd firmness measured at 45 and 60 min since rennet addition was positively associated with protein recovery and protein yield. However, increases in RCY associated with increased a45 and a60 were mostly due to the increased amount of water in the curd. Heritability of the investigated traits ranged from 0.07 (for fat recovery rate) to 0.26 (for MOI). In conclusion, MCP marginally affected variation in cheese yield, cheese composition and recovery rates of protein and fat in the curd of soft fresh model cheeses. As a consequence, MCP are not expected to be effective indicator traits of the cheese making ability of milk.

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C-140

Amino acids as advanced technology to improve sustainable livestock production

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Livestock production is a major player in global environmental issues. It represents today up to 18% of the anthropogenic greenhouse gas emissions. The expected population growth and increasing demand for meat, milk and eggs will intensify the challenge in the future. The technology is the key driver to mitigate this environmental impact. This paper will review how the use of supplemental amino acids in animal feeding can benefit the long-term animal production and our environment. Different scenarios will be presented where the use of supplemental amino acids allow for a reduction in the animal emissions linked to a more efficient nutrition and usage of scarce land and crop resources.

C-141

Complete diet in pellet for ruminants nutrition

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The aim of this study was to evaluate nutritional and dietetic effects, rumen pH and rumination time of a pelleted forage based diet fed to growing heifers compared to TMR one. Eight tie-stall heifers (age 336±30d, BW 346±35 kg), were used in a 12 weeks study (4 periods of 3 weeks: 2 adaptive and 1 for data collection). Diets had the same ingredients (hay 41.8%, barley straw 27.4%, sunflower 13.7%, grain 16.4%, salt 0.7%), but fed in two different physical forms: TMR and PELLET (⌀=8mm), thus differing in fiber particle size (pef= 38,73% and 66.12% respectively). The animals, divided in two groups, were fed *ad libitum* with the two diets to alternate periods, and dry matter intake (DMI), DMI/BW (%), water intake, rumination time, rumen temperature and pH (average, pH area<5.8 and 5.5 and pH<5.8 or 5, in min/d) were evaluated daily. In addition, Average Daily Gain (ADG) was calculated at the end of each period. The data of the third week of each period were statistically analyzed with ANOVA for repeated measures using the software Statistica v.10. The DMI and DMI/BW% was higher (P<0.001) in pellet diet (11.49±1.72 *vs.* 9.03±1.33 kg) (3.05±0,33 *vs.* 2.40±0.24%) and also water intake increased (P<0.01) during pellet administration (52.00±13.00 *vs.* 41,00±9.00 liters/day). ADG at the end of the study was 1.0 (±0.45) kg/d. The data in pellet diet was higher than TMR but the difference was not statistically significant (1.07±0.32 *vs.* 0.90±0.54 kg/d). Rumination time was considerably lower (P<0.001) with pellet than TMR (256±58 *vs.* 521±66 minutes/day). Diet had no effect on rumen temperature and all the pH parameters were not statistically significant. The results of this study suggest that complete pelleted diet is well accepted by animals, and DMI was maintained on high levels, thanks to its reduced fill effect. In conclusion, a complete pelleted diet with those physical characteristics can be fed to growing ruminants without creating dietetic problems. In fact even if rumination time drops with this particular diet, rumen pH maintained same average values as that with TMR.

C-142

FEEDNEEDS: new prospective for R&D in the Italian and Serbian feed sectors

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FEEDNEEDS is an Italian-Serbian bilateral project, funded by the Italian Ministero degli Affari Esteri e per la Cooperazione Internazionale. The main aim of this project is to investigate potential areas for research and development in the feed sectors (i.e. research needs). For this purpose, a survey was conducted in 2014. The objectives of this survey-based study were to prioritize the main elements of the research and development (R&D) in the feed sector, and to obtain opinion of stakeholders on how to translate this concept into action by integrating its elements and components into practices. Accordingly a questionnaire containing 29 demands has been developed. It was based on 3 main sections, namely: i) Company Overview; ii) Products and Process Features; iii) R&D and innovation. One-hundred and thirteen feed companies has been contacted, of these 37% contributed by filling the questionnaires, which generated 464 records for Italian (IT), and 319 records for Serbian (RS) feed companies. Data collected have been processed in order to obtain simple frequency statistics and basic graphs of the data. Results obtained indicated that: RS industries are led by male (100%) and younger leader compared to the IT ones, instead IT industries are led by both gender (60% and 40% for male and female, respectively). In both countries feed production is based on use of raw material, even though IT consulted industries were manly focused on ruminant feed production, whereas RS were focused on pig and poultry feed production. Considering R&D in the past, 7% of IT responders industries have not planned any budged for R&D,

while for RS this percentage has reached 47%. Of note in both countries informal activities of R&D have been developed. When the main focus of R&D activities have been tested, 64% of IT responders mentioned industrial processes, while 82% of RS activities are focused on new product developments. Increase in product quality, search for new markets and reduction of energy consumption has been essential for R&D for both countries, in the near future. A further difference in the two countries was the main difficulties met: high cost for innovation and paperwork/bureaucracy for IT and bureaucracy and lack of government incentives for RS ones. Thus, it can be concluded that in both countries, feed sector is experiencing several changes, and there is considerable uncertainty as to how these factors will play out in the two regions in the coming years.

C-143

The inclusion of black soldier fly material in an experimental extruded feed: extrusion optimization and its impact on digestibility

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The aims of this study were i) to investigate the inclusion of black soldier fly (BSF) material in an experimental extruded feed, and ii) to evaluate the impact of extrusion on the nutritional value and digestibility. For this purpose, premixes of BSF larvae or prepupae (wet material) and wheat flour in a ratio of 25:75 with or without soy oil addition have been formulated as follows: prepupae + wheat 25:75 (no added oil); prepupae + wheat 25:75 (low oil); prepupae + wheat 25:75 (medium oil); prepupae + wheat 25:75 (high oil) ; larvae + wheat 25:75 (no added oil). Gross nutrient composition prior to extrusion of the respective formulations was: 24.21, 24.02, 23.84, 23.65 and 23.71% moisture; 11.48, 11.39, 11.30, 11.21 and 10.93% crude protein on wet basis; 3.2, 3.9, 4.6, 5.4 and 4.6% fat on wet basis. Feed mixtures were then extruded using a co-rotating, conical twin-screw mini extruder (HAAKE,™ MiniLab II). Net torque value (NTV) was measured in order to evaluate the extrusion features of the BSF-wheat based blends. The best performing mixture was tested at four barrel temperatures (60, 70, 80 and 90°C) and the same screw speed (100rpm). Furthermore, the effect of extrusion (versus non-extruded blend) and extrusion temperature on *in vitro* organic matter (OM) digestibility and protein digestibility (PD) of extrudates was measured. In the present experiment, increasing the blend fat content (up to 5.4%), the NTV was reduced by four times (<100 Ncm) compared to the 3.2 and 3.9% fat mixtures. The best performing mixture was larvae + wheat 25:75 (no added oil), containing 4.6% of fat. When different barrel temperatures were considered for the same blend, no substantial effect on water loss was observed. Processing the mixture by extrusion increased OM digestibility by 13%, but not PD, compared to the untreated (no extrusion) mixture. Extrusion temperature did not affect OM digestibility nor PD. Thus, it can be concluded that extrusion can contribute to increasing OM digestibility in insect containing feed blends. Fat content in the mixture is a key variable that should be defined in the process. The present results obtained on lab scale need to be confirmed in a large-scale pilot plant.

C-144

Morphological evaluation of *Nicotiana tabacum* plants transformed for the expression of verocytotoxic *Escherichia coli* antigens

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Two transgenic *Nicotiana tabacum* plants carrying respectively the F18 and the B subunit of verocytotoxin genes from O138 verocytotoxic E.coli serotype (VTEC), were developed by agroinfection as model of edible vaccine. VTEC causes severe enterotoxaemia in weaned piglets and is responsible of important economic losses. For the construction of transgenic plants, the bacterial genes were placed under control of GLOB for the seed specific expression. Previous studies demonstrated that the Vt2e-B and FedA fimbrial genes were stably incorporated into tobacco plant genome by being transcribed through the nuclear apparatus of the plant for specific expression in the seeds, and that these genes are inherited by the next generation. The dietary administration of transgenic tobacco seeds promoted a significant increase in the number of intestinal mucosal IgA-producing cells in mice and showed a protective effect against VTEC strain in piglets. Agrobacterium tumefaciens binary vector system is an efficient tool to transform plant cells; however, the exogenous gene integrates at semi-random into the nuclear chromosomes. In other words, the insertion of a transgene into the plant genome inevitably disrupts the sequence of the endogenous plant DNA and may be accompanied by other mutations. For these reasons, the aim of this study is the morphological evaluation of *Nicotiana tabacum* plants transformed for the expression of F18 and Vt2e-B proteins with respect to *Nicotiana tabacum* wild type (WT). Three lines of tobacco seeds (F18, VT2e-B and WT) were seeded in homogeneous conditions and were harvested simultaneously. Tobacco plants were analysed by optical microscope in different phases of growth. Germination of transgenic seeds was delayed of three and five days compared to WT in two replicated experiments, suggesting that genetic manipulation influenced mechanisms leading to germination. The analysis of F18 and VT2e-B seed polypeptides, following two different methods of protein extraction showed differences in the electrophoretic profiles with respect to WT. Furthermore, morphological observations using optical microscope, showed no difference in the embryos of tree samples. On the contrary, a large amount of storage material (oleosomes or aleuron grains) are observed in the endosperm of F18 seeds, with respect to WT endosperm, in which storage proteins and lipids were already mobilized. This data could explain the delayed germination of transgenic lines.

C-145

Proteomic characterisation of *Nicotiana tabacum* seeds expressing verocytotoxic *Escherichia coli* antigens

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Transgenic plants are becoming a common platform for the production of valuable oral vaccines. Seeds have already proven to be the ideal oral delivery system in animal models for clinical prophylaxis against different infections. In this study, we focalized our attention on tobacco seed-based edible vaccines against verocytotoxic *Escherichia coli* strains (VTEC), responsible for severe enterotoxaemiae in weaned piglets. Two independent tobacco lines were developed, by Agrobacterium-binary vector system, for the seed specific expression of the F18 adhesive fimbriae and the VT2e-B toxin, from O138 *Escherichia coli* strain. Previous studies demonstrated that the Vt2e-B and F18 proteins were expressed in tobacco seeds, and that transgenic tobacco seeds, upon oral administration, showed a protective effect against VTEC infection in piglets. Nevertheless, Agrobacterium-mediated transformation is a random process, leading to physical disruption in the genome or gene regulation. In the attempt to increase the chance of detecting unintended effects deriving from transformation events, the aim of this study was the evaluation of proteomic profile of *Nicotiana tabacum* seeds expressing VTEC antigens. Average samples of tobacco seeds, positive respectively for F18 adhesive fimbriae and for Vt2eB, were screened by polymerase chain reaction using specific oligonucleotides. The chemical analysis of tobacco seeds was performed for the principal components: crude protein, dry matter, fat, crude fiber, fiber and neutral detergent, ash. Since seed proteome is highly dynamic, depending on the species variability and environmental influence, we analysed the proteomic profiles of F18 and Vt2eB transgenic tobacco variety in double with their respective isogenic controls (WT) in different experimental conditions. No substantial differences were observed for principal components between transgenic and WT seeds. The analysis of F18 and VT2e-B seed polypeptides showed differences in the electrophoretic profiles with respect to WT. Proteins play an important role in an organ developmental process and their expressions were changed between transgenic lines and wild type. An exhaustive differential proteomic analysis allows determining similarities and differences between traditional and new plants, and a case-by-case assessment of the new product should be carried out in order to have a wide knowledge of its features.

Acknowledgments
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C-146

Chemical, amino-acidic and anti-nutritional composition of new sweet genotypes of narrow-leaf (*Lupinus angustifolius* L.) and white lupines (*Lupinus albus* L.) seeds

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Lupine has important nutritional qualities, including a high percentage of protein and fiber and a low fat content. Objective of the study was to assess the chemical, amino-acidic and anti-nutritional profile of seeds of novel sweet genotypes of narrow-leaf (NL) and white lupine (WL) created at ENEA research center (Rome, Italy). Plants were grown in central Italy (Rome and Viterbo, years 2007-2010), on acidic/sub-acidic soils. Harvesting was performed after physiological maturity. Seeds were analysed for moisture, crude protein, ether extract, crude fiber, ash, amino-acid profile and anti-nutritional compounds, such as quinolizidine alkaloids and their precursors, inositol phosphates, total tannins, soluble carbohydrates and α-galactosides. All data were subjected to ANOVA and differences between means were compared by Fisher's LSD test on a probability level of 95%. Results showed that all sweets genotypes have a high content of crude protein (30-35% DM) and a low content of fat (3-7% DM). The analysis and amino acid composition in the different seeds also revealed that all sweet genotypes are rich in lysine, arginine, histidine, asparagine, proline, glutamic acid and tyrosine, with considerable presence of glutamine and leucine-isoleucine-norleucine. We observed that sweet genotypes contained low quantities of alkaloids and a high amount of lysine. Almost all the alkaloids (albine, angustifoline, oxosparteine, lupanine and hydroxilupanine) were identified in bitter and semi-sweet genotypes, analysed as a positive control. The content of the other anti-nutritional compounds was, on average (on DM basis): 3% for total tannins, 87 mg/g for soluble carbohydrates and 9 mg/g for inositol phosphates (assessed only in NL lupine). Genetic improvement has allowed to obtain lines of sweet lupine with alkaloids content up to 35 times less compared to lines currently cultivated, without decreasing the presence of nutritional compounds (protein and amino acids, in particular). The cultivation of the most promising genotypes may contribute to the total or partial replacement of soybeans in animal feeding, increasing the sustainability of farms.

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C-147

Effect of different doses of cracked whole soybean on milk fatty acid composition in buffalo

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In order to improve the health characteristics of buffalo milk and Mozzarella, the effect of two concentrates differing in linoleic acid (LA) content on milk fatty acid (FA) composition was considered. Dietary LA supplementation, in fact, is the most effective strategy to increase milk content of conjugated linoleic acid (CLA), which has potentially positive effects on human health. Thirty-two buffaloes were randomly allotted in two groups: experimental (EG) and control (CG). During the first period (P1, from 26 to 82 days in milk) EG fed a total mixed ratio (TMR) composed by barley silage, alfalfa and grass hay, maize meal and 3 kg of a concentrate composed by 30% cracked whole soybean (WS) and 70% horse bean (HB), which provided 12.6 g/kg DM of LA. During the second period, (P2, from 97 to 152 days in milk), the experimental concentrate was 70/30 WS/HB that provided 25.4 g/kg DM of LA. During both periods CG fed a diet based on the same forage than EG and a concentrate composed by maize meal, soybean meal, and cotton seeds (7.2 g/kg DM of LA). Individual milk samples were weekly collected and data of milk FA composition were analysed by a repeated measures model. At the end of each period, milk from each group was separately processed and six Mozzarella cheese for group were sampled. During P1, LA content of milk from EG was significantly higher than milk from CG (1.85 *vs.* 1.52% of TL), whereas CLA and vaccenic acid (VA) content did not differ between groups. During P2, the LA content in EG milk was more than two times higher than that in CG milk (3.91 *vs.* 1.59% of TL). Also CLA (0.59% of TL *vs.* 0.38% of TL for EG and GC respectively) and VA (1.67%of TL *vs.* 1.25% of TL for EG and CG, respectively) milk content significantly differed between groups. Oleic acid (OA) content significantly differed between treatments, being higher in milk from EG (+17%). Interestingly, differences were observed also in the substrate/product ratio related to stearoil-CoA desaturase enzyme (SCD). This enzyme acts either on C18:0, that is converted in OA, and on C14:0, C16:0 and VA, which are converted in cis9 unsaturated FA. Results suggested a higher affinity of SCD for C18:0. The desaturation index of C18:0, in fact, was more than ten times higher than that of other substrates. The FA composition of mozzarella reflected that observed in milk, confirming that cheesemaking did not affect the transfer of FA from milk to cheese.

C-148

Plasma α-tocopherol content and its relationship with milk somatic cells count in Italian commercial herds

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In the last few years vitamin E supplementation in dairy cattle and its activity on udder health has been often controversially debated. This work was aimed to investigate relationship between plasma vitamin E concentration and milk somatic cell count in healthy cows in commercial herds. 49 multiparous cows from two commercial dairy herds in Milan area were monitored from the day of dry off until 90 DIM. BCS was assessed and blood samples were collected at dry off, day 0, 30, 60 and 90 postpartum. Plasma was analyzed for α-tocopherol, Zn and Se content. Quantification of NEFA and BOHB was performed in serum samples. Milk production and composition was obtained from routinely test-day of Italian milk producers association. Somatic Cell Score (SCS) was calculated and included in the dataset. Analysis of data was performed using MIXED repeated and CORR procedures of SAS. Vitamin E plasma concentration significantly decreased at day of calving and progressively increased in the first 90 days of lactation. A significant ($P<0.01$) negative (-20%) correlation was observed between NEFA serum content and α-tocopherol plasma concentration. Serum selenium content was positively correlated (+31%, $P<0.05$) to zinc concentration. Grouping cows on the basis of their plasma α-tocopherol content higher or lower than 3 μg/mL at dry off, SCS at 30 and 60 DIM tended to be higher in lactating animals with lower content of α-tocopherol (1.12 *vs.* 1.72, $P=0.18$ at 30d; 0.92 *vs.* 1.72, $P=0.07$ at 60d). No significant differences were observed on analyzed parameters when cows were grouped based on their plasma α-tocopherol content higher or lower than 2 μg/mL at day of calving. Overall low SCS values in investigated animals (mean values at days 30, 60 and 90; 1.42, 1.32, 1.58) could explain the lack of correlation between plasmatic vitamin E and somatic cell score. However, plasma α-tocopherol content at dry off could be usefully correlated with somatic cell count in fresh cows.

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C-149

Mealworm inclusion in Label chicken diet does not affect both performance and welfare parameters

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The use of insects as an alternative source of protein in animal feed is becoming more globally appealing, also in poultry. Soybean or fish meal in broiler chicken or laying hen have been successfully replaced at low inclusion levels by black soldier flies (*Hermetia illucens*), housefly (*Musca domestica*), mealworms (*Tenebrio molitor*, TM) and silkworm (*Bombix mor*i) pupae. In this experiment, female Label chickens were reared in free range condition (5 replicates per treatment and 12 birds per replicate). Birds were housed at the age of 42 days (average weight 715 g). Two experimental diets were designed: 72.0% corn, 17.0% soybean meal, 7.5% gluten meal (C-group) or mealworm meal (TM-group) and 3.5% mineral-vitamin premix. Diets were formulated using AMEn values calculated for broiler chickens by our research group (Schiavone *et al.*, 2015). C-group and TM group diets were isoenergetic (AMEn: 12.18 and 12.22 MJ/kg, respectively) and isonitrogenous (crude protein: 16.9% and 16.8%, respectively). The experiment lasted 60 days. Birds were weighted at the beginning and at the end of the experiment. At slaughtering, blood samples were taken and the main commercial cuts were evaluated (carcass for grilling, breast and thigh) as well as liver, spleen and Bursa of Fabricius weights. In addition foot pad dermatitis score was evaluated according to the EU Commission recommendations. Data were analysed by Student's t-Test for independent samples. C-group and TM-group displayed similar final body weight (2131 *vs.* 2162 g), feed conversion ratio (4.37 *vs.* 4.36 g), carcass for grilling (1459 *vs.* 1545 g), breast (347 *vs.* 371 g), thigh (497 *vs.* 503 g), abdominal fat (41 *vs.* 45 g), liver (37 *vs.* 39 g), spleen (4.0 *vs.* 3.8 g) and Bursa of Fabricius (4.3 *vs.* 4.2 g). By comparing blood analysis of C-group *vs.* TM-group data of red (2.4 *vs.* 2.6 millions) and white blood cells (9.4 *vs.* 9.9 thousands), H/L ratio (0.55 *vs.* 0.51), total protein (3.98 *vs.* 4.05 d/dl), AST (190 *vs.* 198), ALT (14.5 *vs.* 14.0 UI/l), uric acid (5.0 *vs.* 3.9 mg/dl), triglycerides (43.4 *vs.* 47.8 mg/dl) and glucose (262 *vs.* 244 mg/dl) no significant differences were found. Birds did not displayed any foot pad dermatitis lesion. In conclusion TM meal can be safely used as feed ingredient for Label chicken at the inclusion level of 7.5% without affecting growth and slaughter performance as well as blood and welfare parameters.

C-150

Haematological, biochemical and serum protein profiles during lactation in Modenese and Italian Friesian cattle breeds

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The aim of this study was to evaluate the variations of the blood parameters during lactation in the Modenese (MO) cow, a dual-purpose autochthonous cattle breed, included in the endangered-maintained breeds list of FAO (2000), for assessing its nutritional and health status compared to a specialized dairy breed, the Italian Friesian (IF). The study was carried out on 25 pluriparous lactating cows, 11 MO and 14 IF that were housed in a free-stall barn, under identical conditions of feeding and management, in a commercial farm. Blood samples from all cows were drawn from jugular vein at peak (8 wk) and mid lactation (21 wk) and analyzed for haematological and biochemical profiles, by automated analyzers, and serum protein profile, by agarose gel electrophoresis, using commercial kits. Body condition score (BCS) was evaluated after blood collection. The effects of breed, time (T8 and T21) and their interaction were assessed by a mixed model ANOVA. On average, MO cows showed higher BCS, plasma creatinine, potassium (K), alkaline phophatase (ALP), tartrate resistant acid phosphatase (TRAP), alanine aminostrasferase (ALT), hemoglobin (Hb), hematocrit (Hct), basophils (as % of total white blood cells, WBC), platelets count (PLT); but lower cholesterol, total protein, globulins, magnesium (Mg), and neutrophil (NEU, as % of WBC) compared to IF cows. The stage of lactation affected plasma glucose, non-esterified fatty acids (NEFA), urea, total iron binding capacity (TIBC), red blood cells (RBC), mean corpuscular volume (MCV), mean concentration hemoglobin (MCH), and serum β-globulin percentage in both the breeds. At T21 IF cows showed higher values of urea, phosphorous (P), aspartate aminotransferase (AST), ALT, WBC, and NEU count compared to T8, whereas MO did not. Breed x time interaction had no effect on any of the considered variables. We speculated that the observed differences were possible consequences of the different genetic selection criteria adopted for IF compared to MO cows, because some of these blood parameters are under genetic control. These results could help to gain a greater understanding of the physiological status of this local breed and of the mechanisms that characterize the MO for functional traits

and adaptability to the intensive management systems of the Po valley.

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C-151

Evaluation of the gene expression and Δ6-desaturase activity in different chicken strains

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In chickens, body fatty acids (FA) are derived from dietary uptake, *de novo* synthesis and/or bioconversion. Among the different fatty acid classes, n-3 long-chain PUFA (n-3 LC-PUFA) are of particular interest due to their positive role on human health. Domestic animals are unable to synthesize long chain-PUFA, but they can convert dietary linoleic and α-linolenic acid through a pathway catalyzed by elongation and desaturation enzymes. Meat from animal species is characterized by different FA composition; within the same species the FA profile reflects the endogenous biosynthesis as well as the composition of the diet. The relatively low efficacy of the desaturation enzymes in poultry allows for further consideration on the selection of genotypes able to synthesize a higher PUFA amount. In this study, the liver messenger RNA copies and enzyme activity of the Δ6-desaturase were evaluated in three chicken strains (slow-SG, fast-growing-FG and SG x FG crossing-SFG). Three groups of laying hens for each genotype were fed with a standard diet. Chickens were slaughtered at hatch, and liver was taken for analysis. A relationship between genotype and desaturation ability was evidenced. The FG strain showed a lower mRNA copies of FADS2 gene than the SG one ($P>0.01$). However, RNA expression was two or three-hundred times higher in SFG than SG and FG strains, respectively ($P<0.01$). Even the enzyme activity confirmed what shown by mRNA. The Δ6-desaturase activity was significantly higher in SG than FG (172.0 *vs.* 63.56 pmol in 30 min/mg prot), whereas in SFG was at intermediate level (136.66 pmol in 30min/mg prot). However, the enzyme activity of SFG was not significantly different from SG ($P>0.01$). SG chicken in comparison to FG strain showed a higher desaturase activity ($P<0.05$), whereas there were no significantly differences between Δ6-desaturase activity in SG and SFG. Even mRNA copy number was widely affected by genotype. Indeed, SFG showed a much higher mRNA abundance, whereas SG does not show significant differences. Data herein reported showed that the Δ6-destaurase activity is strongly affected by genotype. However, several other factors involved in the process of expression/translation, can be assessed. Gene expression may follow certain design principles for optimal evolutionary fitness as demonstrated by the high mRNA copies of the SFG.

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P-001

In field study to optimise doses of new synbiotic formulations for in ovo application

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Colonisation of the gastrointestinal tract by commensal microbiota substantially influences host immunity, nutrient absorption and digestion. The sooner the host microbiome affects physiology, health and production parameters, the more profits are expected throughout the host's lifespan. This work is a part of the ECO FCE project and aimed to assess the impact of different dosages of new synbiotic formulations for broilers in ovo. Synbiotics SYN1 (Lb.plantarum IBB3036 + lupin raffinose family oligosaccharides RFOs) and SYN2 (Lb.salivarius IBB3154 + Bi2tos, Clasado) were selected from *in vitro* bench studies and were delivered, in 3 repetitions on the 12th day of embryo incubation. Cobb 500FF eggs (n=4200) were divided into 12 experimental groups + control and were injected with 200 µL NaCl + negative control (without injection). Each synbiotic was given in 3×2 different modes of bacteria and prebiotic concentrations: 10^3-10^4-10^5 bacteria cfu/egg and 2 or 5 mg/egg, respectively. Incubation and hatch analysis were performed in a commercial production field of Drobex-Agro (Poland) and provided a basis to select the optimal doses for further treatment in ovo. The results were analyzed by one way ANOVA followed by Duncan post hoc test to compare mean values between the groups. The hatchability nor the health status of chicklets was significantly different among the groups when 2 mg/egg prebiotic concentration was applied. However, a significant decrease in hatchability was noted at 5 mg prebiotic concentration ($P<0.05$). This finding is consistent with earlier studies (Dankowiakowska et al. 2013) where the prebiotic solution applied at a concentration as high as 7 mg/egg decreased the hatchability below 50%. In this study, the hatchability exceeding 90% was observed in SYN1 at 105 bacteria cfu/egg + 2 mg/egg RFOs. The hatchability of the control groups were between 87-90% whereas it oscillated between 85-92% for SYN1 and 82-89% for SYN2 at 10^5 bacteria cfu/egg + 2 mg/egg prebiotic. Crippled or weak embryos (2-7%) were found in all the in ovo treated groups, but none was found in the negative control. In summary, prebiotic concentrations of 5 mg/egg applied in ovo were detrimental, but no data is available on recommended rates of probiotic for in ovo application. The optimal doses of synbiotics for further testing will consist of high concentration of bacteria cfu/egg combined with prebiotic at concentration not exceeding 2 mg/egg.

P-002

Chemical composition of caviar from farmed sturgeon: comparison between four different species

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In recent years the supplies of wild caviar have declined dramatically and have been replaced by aquaculture products. In this study the proximate composition and fatty acid profile of 23 caviar samples obtained from 4 farmed sturgeon species (*A. baeri* [Ab], *A. gueldenstaedtii* [Ag], *A. naccarii* [An], *A. transmontanus* [At]) were measured and compared with wild caviar data available in literature. Statistical analysis were performed by SPSS 22 statistical package. Except the protein content (23.9-25.3%), proximate composition presented significant differences among species. The most notable difference was the highest lipid content in caviar of At (18.0%) and Ag (16.9%). Fluctuations in fatty acid (FA) profiles were also observed in the interspecific comparison. Palmitic acid was the dominant saturated FA in all samples and it was significantly higher in An (19.1% of total FAs). Amongst the monounsaturated FAs, the most abundant was oleic acid (25.8-32.0%), which was in absolute the major FA of all samples tested. Regarding to n-6 polyunsaturated FAs, linoleic acid (LA) varied between 9.3 and 14.0% while arachidonic acid (ARA) ranged between 1.8 and 2.4%. LA and ARA contents were significantly lower in caviar of An and Ag, respectively. All species showed high level of n-3 polyunsaturated FAs, with docosahexaenoic acid being the dominant (DHA; 12.1-14.0%), followed by eicosapentaenoic acid (EPA; 4.4-6.3%). In literature it was reported that LA concentration was much higher in farmed caviar than in wild one whereas the opposite trend was found relative to ARA. High level of LA in farmed caviar could be attributed to the use of commercial feed containing vegetable oils and meals while the marine food chain contains more quantities of ARA. In contrast, EPA and DHA content in farmed caviar mirrored that of wild sturgeon, indicating a selective accumulation of these FAs irrespective of the diet. This study contributes to the chemical characterization of farmed caviar. Although it is well documented that diet composition can modify aquaculture products, it appears that farmed caviar maintains the nutritional quality of wild one. We found similar amount of n-3 polyunsaturated FAs (especially EPA and DHA) to those reported in literature for wild caviar and these FAs are well known to have several beneficial effects on human health. This leads us to assert that farmed caviar represents an effective alternative to wild products maintaining a good nutritional quality.

P-003

Prediction of eating quality of Italian Simmental beef from butcher's steak appearance judgment

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An expert is defined by the American Society of Testing Materials as someone with extensive experience in a product category who performs perceptual evaluations to draw conclusions about the effects of variations in raw materials, processing, storage, aging, and so on. As such, the butcher may be considered an expert on meat quality. Numerous systems of beef quality classification have been developed in different Countries that rely on appearance evaluation by experts. On the other hand, in Europe, reliable systems guaranteeing eating quality are still lacking, in spite of numerous private and official quality signs which exist at the consumer level. A European beef consumer survey found that consumers hold a strong interest in beef eating-quality guarantees. The aim of the present work, carried out in the framework of a CRITA-FVG project for the valorisation of Italian Simmental (IS) beef, was to define a quality meat grid system based on butcher's appearance judgment as a tool to predict beef eating quality. Four experts developed a grid system for the evaluation of the steaks quality: individual interviews were conducted about the quality of the Longissimus thoracis muscle (LT) of an IS steak between the 8th - 9th ribs. Seven meat characteristics, two descriptors of fat, the presence of any defects and global "overall quality" assessment were included in the quality grid system. Experts evaluated the quality of 29 young bull steaks. The partial least square (PLS) regression analysis was adopted to assess which experts' descriptors contributed to the overall quality, defining the criteria of the quality index calculation. Based on quality indexes, two levels of beef quality were highlighted: high and standard. A trained panel performed a quantitative descriptive analysis of LT from the same 29 carcasses. The panel evaluated the cooked muscle of each animal in duplicate. In order to identify any relationship between quality levels and sensory profile, the PLS discriminant analysis model was built between the sensory attribute matrix (X) and the quality class matrix (Y). Results showed that the differences in terms of quality highlighted by experts were also reflected in the cooked meat, when evaluated by a trained panel. The significant differences between the two quality levels in terms of sensory profile will be discussed. It seems that the developed quality index is a helpful tool to valorise the IS meat, guaranteeing the eating quality of beef.

P-004

Sensory properties of mozzarella cheese as affected by starter cultures and preservation liquid

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Industrial manufacturing of pasta filata cheeses with commercial starter cultures, despite a qualitative standardisation, may cause flavour flattening. In order to improve the sensory profile of mozzarella cheese, we used two starters: ST051 (commercial) *vs.* CL13A (natural milk culture) and two preservation liquids: TL (0.4% NaCl brine) *vs.* IL (0.4% NaCl, 0.67% CaCl2, 0.51% lactose, 1% Lact. lactis + Leuc. mesenteroides). Sensory analyses were performed on four products (two starters x two liquids) by a 10-member panel (7 females and 3 males) in triplicate, whereas 82 consumers evaluated product acceptability. ST051 products had higher brightness, colour uniformity, eye number ($P<0.001$) and buttermilk release ($P<0.05$). CL13A cheeses showed higher milk flavour, and higher tenderness ($P<0.05$) and grainy ($P<0.001$) mouth feel intensities, whereas sourness ($P<0.01$), bitterness ($P<0.05$) flavours and mouth feel of residual ($P<0.01$) were lower. IL products had a lower eye number ($P<0.001$), buttermilk release ($P<0.05$) in terms of aspect, lower fruity and bitterness flavours ($P<0.001$), increased butter flavour ($P<0.05$), and increased shear strength ($P<0.01$), moisture ($P<0.05$) and grainy ($P<0.001$) mouth feel. Consumers expressed higher overall and taste/flavour liking for CL13A ($P<0.01$), whereas IL products were preferred in terms of taste/flavour liking ($P<0.05$). The PLS regression of 82 judges on 23 sensory variables (38% and 33% of Y variance explained by the first and second component, respectively) allowed to cluster four groups representing 5, 28, 37 and 21% of the consumers, respectively. Group I preferred ST051 cheese preserved in IL liquid, with high surface uniformity, shear strength and butter flavour. Group II preferred both CL13A products with high milk and sweetness taste/flavour and grainy and tenderness textural attributes. Group III did not express a preference for a particular product, albeit showing a preference for attributes such as fruity flavour and colour intensity, while Cluster IV favoured ST051TL cheeses, characterized by brightness, eye number, and uniformity, in terms of aspect, bitterness, sourness and yogurt taste/flavour, and residual and oily mouth feel. The identification and selection of suitable cheese making technologies, involving the use of adjunct cultures or flavouring preservation liquid may be useful to the industry for product differentiation.

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P-005

Relationships between milk acidification rate with protein metabolism and diet characteristics in dairy cows

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The milk cheese-making aptitude depends on physico-chemical, bacteriological and fermentability traits, including the natural aptitude of milk to be fermented by lactic acid bacteria (i.e. acidification rate). The aim of our research was to study the relationships between diet characteristics and protein metabolism with the main milk cheese-making features, with particular attention to the milk acidification rate (AR). This study was carried out on 10 dairy herds identified through a preliminary investigation on 68 Italian Friesian herds located in BS, MN and PC provinces. Five of the selected herds were characterized by a slow (S) and five by a fast (F) AR of bulk milk. Besides AR measurement, the following analyses were performed on bulk milk of each farm: fat, protein, casein, lactose, urea, somatic cell count, titratable acidity and rennet clotting properties. The milk AR was determined by measuring the increase of the titratable acidity of pasteurized milk (80°C x 10') inoculated with natural lactic acid bacteria fortified with starter lactic acid bacteria and incubated at 45°C per 3 h. In the selected herds 14 healthy and multiparous lactating cows were chosen according to DIM (7 in early lactation: 30-90 DIM; 7 in mid-late lactation: 160-240 DIM). From each cow a representative milk sample from afternoon milking was collected and subjected to the same analytical measurements previously indicated. The next morning the same cows were bled and the urea concentration was measured on plasma. Furthermore, checks on feeds and diets were done. The diet of S compared with R herds was richer in energy and protein content, with greater concentration of non-fibrous carbohydrate and starch, and lower content of NDF. In milk, a lower fat concentration in S (3.6%) compared with F (3.8%) was observed. Conversely, the protein concentration was greater in S (3.50 *vs.* 3.33%, in S and F, respectively). Greater values of urea in blood in S (5.78 mmol/L) compared with R (4.90 mmol/L) were observed. A mild but significant negative correlation between blood urea and AR was observed ($r=-0.22$; $P<0.05$). The correlation between these two variables, taking into account also the herd effect, was highly significant ($r=-0.38$; $P<0.001$). The results seem to indicate that the conditions that result in an increase of urea in blood and milk are associated with a worsening of the acidification rate of milk.

P-006

Free amino acids in meat of buffalo young bulls fed faba bean as protein source

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Consumers perceive meat as a product with large variability in sensorial qualities. A tight control of meat quality requires understanding of all elements inducing this variability. Among the factors, that influence meat properties, recently free amino acids, peptides, and proteins have become of interest since they are important precursors of taste and flavor. The present study is concerned with the knowledge that free amino acid concentration contribute to understand the variability of the meat sensorial properties and that the animal diet effects on free amino acid profile of muscles. In this study, free amino acids were analyzed on *Longissimus thoracis* of 16 Italian Mediterranean Buffalo young bulls. Animals were divided into two groups and fed with iso-protein and iso-energy diets, characterized by different protein source in the concentrate: faba bean tannin-free variety (*Vicia faba minor* L.) *vs.* whole seed soybean (*Soja hispida*), FB and SB group respectively. Animals were slaughtered at 350 kg BW. Samples of Longissimus thoracis were collected for free amino acids analysis. The derivatized amino acids were analysed by reverse-phase HPLC in a C18 column (250x4.6 mm). The samples were monitored at 254 nm. The quantification of each amino acids was carried out by external standard method. The statistical analysis was performed considering the variables protein source effect (i = FB, SB). The protein source did not influence the muscle protein percentage (FB: 21.2 g 100 g^{-1} *vs.* SB: 21.5 g 100 g^{-1}). The free amino acid profiles of Longissimus thoracis of FB and SB group show that the different protein source significantly increased only the quantity of Glycine (FB: 43.2 mg 100 g^{-1} *vs.* 29.4 mg 100 g^{-1}; $P<0.01$). Among the amino acids with taste properties, Glycine, named for its sweet taste, has the lowest relative sweetness of the amino acids (in order of decreasing sweetness: Tryptophan, Histidine, Phenylalanine, Tyrosine, Leucine, Alanine, and Glycine). Results show that free amino acids had similar values in meat from animals fed faba bean *vs.* soybean; therefore, faba bean, that leads, by itself, to agronomical and economic advantages, can be used as a protein source alternative to soybean in the diet of young buffalo bulls without modify meat sensorial properties.

P-007

Microencapsulated fish oil to enrich yoghurt with omega-3 fatty acids: evaluation of fatty acid composition and volatile compounds

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According to the European Food Safety Agency, human dietary intake of long chain n-3 fatty acids (EPA and DHA) is lower than that recommended (250 mg/d), so the Food Industry is interested to produce functional foods enriched with these fatty acids (FA). However, enriching food with EPA and DHA may increase the risk of lipid oxidation and off-flavour development. Microcapsules (MC) obtained by spray-drying may help to avoid this issue, by including fish oil (FO) in a dry powder ready to be integrated into the food. Recently, several dairy products have been proposed as functional foods, by enriching milk with functional lipids or probiotic and prebiotic substances. The aim of this work was to evaluate the FA and volatile compounds (VOCs) profile and the lipid oxidation of yoghurt enriched with fish oil MC. Four types of MC containing FO were produced using whey protein isolate plus gum arabic (GA) or maltodextrin (MD), with (GAQ; MDQ) or without quercetin as antioxidant. MC were used to obtain four different types of yoghurt that were compared with non-enriched yoghurt (CY) or with yoghurt enriched with unprotected FO (CO), during a 21 days storage period at 4°C. FA composition of yoghurts was determined by gas chromatography (GC) analysis, while the oxidative stability was characterized through the analysis of thiobarbituric acid reactive substances (TBARS). Volatile compounds were determined by solid phase microextraction (SPME) coupled with GC/MS. The VOCs composition was evaluated by a multivariate analysis. Yoghurts enriched with 15 g/L of MC resulted in a significant increase of EPA (0.082 to 2.012 g/100 g total lipids) and DHA (0.011 to 1.649 g/100 total lipids), irrespective of the presence of quercetin as an antioxidant. At the end of the storage period, the value of TBARS significantly increased only in CO and CY samples, suggesting a protective role of MC against FA oxidation. Nevertheless, the MD yoghurt was associated with an oxidative aromatic profile,(2-hexenal, 2-methyl furan) whereas the presence of quercetin eliminated this aspect. GA, GAQ and MDQ yoghurt were associated with an acid flavour profile. CO and CY yoghurts were associated with sweet and fruit flavour profiles (2-nonanone). In conclusion, MC could be an effective strategy to enrich yoghurt with n-3 PUFA and simultaneously preserve FO from oxidation, however some differences in the oxidative stability and VOCs profile were observed depending on the properties of the MC wall.

P-008

Comparison of *Canestrato Pugliese* cheese quality between native and non-native sheep breeds

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Local breeds represent an important resource for a sustainable pastoral system in Mediterranean areas. The aim of the study was to investigate the effect of native and non-native sheep breeds on gross composition, fatty acid (FA) profile, health promoting index (HPI), thrombogenic index (TI) and volatile organic compounds (VOC) of Canestrato Pugliese cheese two months ripened. The experiment was carried out at the experimental farm of CRA-ZOE (Southern Italy) in spring. A flock set up of two native endangered breeds, Altamurana (A) and Leccese (L), and one non-native breed, Sarda (S), was used. All animals were fed on native pasture supplemented with concentrate at each milking. For each breed, three cheese-makings of Canestrato Pugliese were carried out for three consecutive days following the traditional technology. Cheeses were ripened for two months in a natural cave. All analyses were performed in duplicate. On cheese samples, gross composition was measured according to FIL-IDF standard procedures, FA profile was determined by gas chromatography and VOC profile and content by GC-MS analysis. The results were analysed by one-way ANOVA procedure with genotype (A, L and S) as the main factor. Means were computed by Fisher's LSD test. Cheese gross composition, nutritional indexes, FA and VOC profile were affected by breed ($P \leq 0.05$). L and S cheeses were characterized by higher dry matter content than A cheese (69.4 and 71.8 *vs.* 63% respectively; $P \leq 0.05$) due to their fat content (35.3 and 35.8 *vs.* 29.3%; $P \leq 0.05$), despite milk fat percentage was not significantly different among breeds. A and L cheeses exhibited significantly higher HPI (0.50 and 0.51 *vs.* 0.40; $P \leq 0.01$) and lower TI (2.64 *vs.* 3; $P \leq 0.01$) values than S cheese, due to their higher unsaturated FA (26.21 and 25.61 *vs.* 24.19 g/100 g FA; $P \leq 0.05$) and lower saturated FA (73.78 and 73.39 *vs.* 75.80 g/100 g FA; $P \leq 0.01$) contents. The class of n-3 FA was higher in A and S cheeses than L cheese (0.88, 0.73 *vs.* 0.69 g/100 g FA; $P \leq 0.05$). No significant differences in conjugated linoleic acid content were found. A and L cheeses showed higher total VOC content than non-native S breed (1,852 and 2,297 *vs.* 1,452 arbitrary unit; $P \leq 0.05$). Cheeses obtained from both native breeds were characterized by some peculiarities that are considered beneficial for human health. The results indicated that native sheep breeds had potentiality for adding value to cheese products for sustainable use.

P-009

Lipid sources influence on healthful indexes of lamb meat

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The research is intended for evaluating the influence of different lipid sources in lambs ration on health index of muscle lipids, to the purpose of human well being. 18 male lambs Gentile di Puglia were weaned at 40 days of age, divided into three homogeneous groups and fed with: concentrated feed (C); replacement of 1.5% of feed with lyophilized algae (A); replacement of 5% of the feed with camelina sativa seeds (CS). The diets were isoenergetic and isoproteic and the food was given *ad libitum*. The lambs were slaughtered at 70 days of age and the fatty acid profile was determined on intramuscular fat of Longissimus lumborum muscle by gas chromatography and polyunsaturated fatty acids (PUFA)/saturated fatty acids (SFA) ratio, n-6/n-3 fatty acid ratio, plasma cholesterol lowering (PCL)/plasma cholesterol elevating (PCE) ratio, atherogenicity index (AI) and thrombogenicity index (TI) were also calculated. The fatty acids values were expressed as percentage (wt/wt) of total fatty acid methyl esters. The data were subjected to statistical analysis, using the SAS procedure. The A group, compared to C and CS groups, increased the docosahexaenoic acid (DHA) content in the lipids muscle (0.72 *vs.* 0.32 %; $P<0.05$) and decreased the n-6/n-3 ratio (2.63 *vs.* 3.74 - 3.87; $P<0.05$). The CS group compared to the other two groups, showed a higher unsaturated fatty acid (UFA); 56.16 *vs.* 51.82 -52.13; $P<0.01$) proportion, and a better PCL/PCE ratio (1.30 *vs.* 1.12 - 1.09; $P<0.05$); also those, compared to subjects receiving the alga showed a better atherogenicity index (0.65 *vs.* 0.77 - 0.78; $P<0.05$). The thrombogenicity index resulted lower and, therefore, better for the CS group compared to A group (1.17 *vs.* 1.31; $P<0.05$) and, especially, with respect to C group (1.17 *vs.* 1.39; $P<0.01$). In addition, camelina sativa, compared to algae diet, increased the essential linolenic fatty acid proportion (1.06 *vs.* 0.82%; $P<0.05$). In conclusion, the algae diet enriched meat lipids with DHA and provided better value for n-6/n-3 ratio, while the camelina sativa diet, conditioned the best AI and TI. Further research is needed in order to optimize the percentage of integration of lipid sources and to assess the usefulness of their association also with other sources.

P-010

Chemical and technological traits of *Nero Siciliano* dry-cured ham

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Technological, and chemical traits of cured ham from Nero Siciliano pigs, were investigated. Hams were obtained from 20 Nero Siciliano (NS) pigs, reared on a traditional extensive system, 10 fed on acorn (Acorn) and 10 on barley and faba beans (Barley) during the fattening phase, as provided by the ‚ÄúConsorzio di tutela del suino Nero dei Nebrodi‚Äù. At the end of seasoning, hams were deboned and a sample-slice, about 3 cm thick, was taken transversally from the caudal portion of ham to the middle of the femur impression. The following analysis were performed on muscles Biceps femoris (BF) from a sample-slice: moisture, ether extract (EE), crude protein (CP), non-protein nitrogen (NPN), total nitrogen (TN) and ash; salt content was estimated as chlorides, salt is the key ingredient of dry-cured ham because it decreases water activity, and contributes to the development of texture, and characteristic taste and flavor. Proteolysis index, expressed as percent ratio between nitrogen soluble in 5% trichloroacetic acid and total nitrogen was also calculated. Proteolysis indices of cured products is influenced by several parameters, such as pH24, salt, water activity and temperature. In fact excessive proteolysis of dry-cured hams, whit related defects (softness or bitter flavour), is more common in low salt hams. In addition, fatty acid profile was determined. BF of Acorn pigs had higher percentages of intramuscular fat (4.72±1.73% *vs.* 4.03±0.50%) salt content (7.90±0.52% *vs.* 6.94±2.02%) and lower percentages of crude protein (35.77±1,79% *vs.* 37.20±4.16%). The proteolysis index was 27.43±2.40 and 25.54±3.63 for Acorn and Barley respectively. Dry-cured ham from Nero Siciliano pig show a salt content similar to Parma dry-cured (6.76) ham, furthermore proteolysis indices is slightly lower compare to Parma ham (29.4). No significant differences between the two different types of samples were found regarding total saturated fatty acids (SFA), 32.33±3.05% *vs.* 33.41±1.15%, monounsaturated fatty acids (MUFA) 57.81±2.33% *vs.* 59.16±1.32% and polyunsaturated fatty acids (PUFA) 9.87±1.81% *vs.* 7.43±0.16% respectively for Barley and Acorn. Overall, the two different feeding systems in finishing phase, did not significantly influence the hams composition. Furthermore, proximate analysis showed chemical traits comparable whit other typical PDO Italian dry cured hams.

P-011

Utilization of lemon molasses and dry orange pulp in dairy ewes feeding during the first stage of lactation: effect on milk quality

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The use of agroindustrial by-products for ruminant feed is an economical and environmentally sound way for food processors to reduce waste discharge and to decrease waste management costs. Large amounts of waste from citrus processing for juice extraction are available in Sicily, Italy. Aim of this trial was to study the effects of feeding lemon molasses and dry orange pulp on ewes milk yield and milk composition. For this, 48 Comisana lactating ewes were divided into two homogenous groups of 24 each, respectively, called Control (CTRL) and Lemon Molasses/Orange Pulp (LMOP). Five hundred grams of pelleted feed with lemon molasses (4% DM) and blond orange pulp (10% DM) integrated was offered daily to the ewes as experimental treatment (LMOP), replacing all the cane molasses and beet pulp and part of the maize and sunflower present in the pelleted ration given to the controls (CTRL). Every 15 days, the individual milk yield of the two daily milking was recorded. At the same time, individual milk samples of the complete morning milking were collected, for the chemical composition and the coagulation properties of the milk determination. Data were processed by GLM of SAS using the repeated measures procedure. Administration of experimental feed influenced the milk yield that was significantly higher in the LMOP (887 g per day *vs.* 655 g per day in CTRL group) ($P<0.001$). The significantly highest fat and protein percentages ($P<0.001$ and $P=0.017$) were observed in CTRL group. The lowest somatic cell count (SCC) was observed in the LMOP group. A significant ($P<0.01$) increase was observed for clotting time (r) in the LMOP group, while no significant differences were observed for curd firming time (k20) and curd firmness (a30) parameters. Milk urea content was lower for LMOP group (20.23±8.56 mg/dL) compared to CTRL group (29.26±12.76 mg/dL) ($P=0.004$) resulting in a better nitrogen utilization with the citrus by-products supplementation. The readily fermentable lemon molasses and orange pulp could have helped ewes to use the N diet more efficiently and increased the response. This gives the rumen microbes a possibility to match the inflow of protein with carbohydrates. The results showed the possibility of administration of mixed feed with citrus by-products integrated for feeding ewes, considering the positive effects on the milk yield and no effects on the clotting properties.

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P-012

Effect of cooking on fatty acid composition, lipid oxidation and cholesterol content of hunted wild boar (*Sus scrofa*) meat

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Meat composition, as well as its physicochemical properties, undergoes significant changes during cooking, that affects the final quality of meat products, especially the fatty acid content, and modify the nutritional value of cooked products if compared to raw samples. Scarce data on quality characteristics of wild ungulates meat are published although its increased availability, both in the market and home consumption, due to the numerical increase of these species and consequent hunting harvesting. The lipid oxidation, occurring during cooking of meat, is the most important source for taste and flavour compounds formation but also induces the deterioration of meat, particularly for meat rich in polyunsaturated fatty acids as for wild boar. The aim of this study was to evaluate the influence of grill treatment on the fatty acid composition, lipid oxidation and cholesterol content of meat from 10 wild boar, hunted in some areas of the province of Rome. Cooked meat showed a lower content of saturated (30.47 *vs.* 32.13 %; $P<0.01$) and a higher level of polyunsaturated fatty acids if compared to raw meat (27.02 *vs.* 23.03 %; $P<0.05$). This observation may be explained by the fact that saturated fatty are largely represented in neutral lipids and are more prone to migration during cooking. The increase in PUFA after grilling has been also reported by other authors in beef, buffalo and hamburgers. From the nutritional point of view, the cooking treatment improves wild boar meat fatty acid composition, lowering the saturated myristic (0.83 *vs.* 0.93 %; $P<0.05$) and palmitic fatty acid content (18.39 *vs.* 20.13 %; $P<0.01$) responsible to raise total serum cholesterol levels in humans, and increasing the content of eicosapentaenoic acid (0.31 *vs.* 0.21 %), that has an important role in the prevention of cardiovascular diseases. The high values of malondialdehyde MDA (1.38 *vs.* 3.21 mg/kg meat; $P<0.001$) detected in cooked meat could be due to the high temperature that promoted lipid peroxidation. The cholesterol content showed no significant differences between raw and cooked wild boar meat (28.3 *vs.* 39.9 mg/100g of meat). In conclusion, the cooking treatment affects fatty acid composition and lipid oxidation and consequently nutritional value of cooked meat from free-living wild boar.

P-013

Effect of different breed on Ragusano cheese quality

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Specific objectives. Ragusano PDO cheese is a traditional dairy product of Sicily, the aim of the study was to characterised parameters of milk and Ragusano PDO cheese in different breeds. Experimental methods. The research was carried out in two farms in province of Ragusa, samples of Bruna (BR) and Modicana (MD) milk were taken during winter and stored prior to analysis. Chemical composition (dry matter, fat, protein, ash, casein, NNC) and lactodinamographic parameters of milk were determined. Ragusano PDO cheese was obtained after ripening period (four months) and chemical analysis (dry matter, ash, ether extract, crude protein) were carried out. Statistical analysis. Chemical and technological parameters of milk, chemical composition of cheese were examined by one-way ANOVA. When significant effects were found ($P \leq 0.05$), differences were determined using t-student. Data analyses were performed using JMP software of SAS (SAS Instit., NC, USA). Results and conclusions. The moisture of milk was higher in BR than MD (87.19% *vs.* 86.20%), although the ash content was greater in MD (0.82% *vs.* 0.76%). Crude fat showed a higher result in BR as the casein content (3.61% *vs.* 3.12%) and the casein/crude protein ratio (85.01% *vs.* 80.86%). Non-casein nitrogen was higher for MD than BR (0.74% *vs.* 0.64%), while crude protein didn't show differences between the breeds. The technological parameters of milk showed differences for k20 and a30 parameters. The rennet clotting time (r) didn't show significant difference for the breeds (13:30 min *vs.* 15:15 min). Curd firmness (k20) evidenced a longer period for BR than MD (06:15 min *vs.* 02:55 min), although final curd firmness 30 min after rennet addition (a30) was found higher in MD (28.96 mm *vs.* 21.74 mm). The chemical composition of cheese showed differences in dry matter and ash content. BR showed higher moisture level than MD (34.09 % *vs.* 32.59 %) while the ash level were higher in MD breed (5.95 % *vs.* 5.28 %). The difference of dry matter and ash level of the breeds can be assessed to the effect of seasoning, instead no correlation were found for protein and fat. It's know how MD has lower productive performance than BR, although in this trial the quality parameters of milk and cheese of autochthonous breed, as Modicana, is comparable with Bruna. Moreover samples of cheese didn't show differences for fat and protein content; for these reasons MD seems to be more carefully to be chosen for the production of Ragusano PDO cheese.chosen for the production of Ragusano PDO cheese.

P-014

Technological and quality characteristics of Bresaola from Cinisara cattle breed

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Recently in Sicily is increased interest for the Cinisara breeding, due to interest in the good quality of meat. The aim of this investigation was to give greater value to Cinisara meat by using it to produce typical bresaola called Busambrina. Ten Cinisara cows slaughtered from 2 to 4 years of age were utilized for Busambrina processing. For the Busambrina processing were used the semitendinosus muscle. A total of 20 Busambrina were produced. The meat was trimmed by fat and tendinous external parts, the raw material showing abnormal colour or that was too fatty was discarded. Then meat was covered with salt, pepper, nitrates and nitrites for at least 10-15 days at 2–4°C to allow the mixture to penetrate the muscle and to give it flavour. At the end of the salting phase, each Busambrina was washed and dried and stuffed into casing and enclosed in a net at 20–24°C and R.H. 65–68% for 5-7 days. After the drying phase, the temperature of the chamber was reduced to 3°C every day and the relative humidity was increased until it reached a temperature of 13–15 °C and a R.H. of 75–80%, the samples were ripened for 50 days. Finally, after the curing time, the casing and the net were removed and the Busambrina washed and vacuum packed. Commercial products, using by comparison, obtained with similar processing techniques and ripening times were bought from a local supermarket. The pH, colour, texture properties and chemical composition of cured Busambrina were determined. All data were subjected to ANOVA. Cinisara bresaola showed higher content of protein (Busambrina 36.40 *vs.* Commercial 31.45g 100g-1) and fat (Busambrina 7.46 *vs.* Commercial 4.51g 100 g^{-1}; $P<0.01$) and lower values of cholesterol content (Busambrina 43 *vs.* Commercial 67mg 100g-1; $P<0.01$) than the commercial beef bresaola. No significant differences were found between Busambrina and Commercial bresaola for the pH and colour parameters As regards technological properties, Busambrina showed less tenderness values than those of the Commercial bresaola (Busambrina 9.87 *vs.* Commercial 4.76 kg/cm^2; $P<0.01$). Our investigation demonstrates the possibility of processing Cinisara meat into bresaola with a good nutritional value to meet the consumers' growing demand for healthy products. Thus Cinisara meat can give an additional resource to local farmers in addition to milk production and typical products with the opportunity to create their own niche in the marketplace.

P-015

Productive performance and egg quality of an endangered Siciliana poultry breed

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Egg production in Italy, as well as in the other developed Countries, is obtained by commercial strains of hens, selected for improved performance and reared indoor. Approximately, 61% of the Italian autochthonous avian breeds are considered as extinct, 13% threatened, 17% scarcely diffuse and 9% diffused. With the aim to contribute to the safeguard of one endangered local breed the following research deepens the study of egg production and internal and external quality characteristics of the Siciliana chicken breed, so to obtain useful data for their eventual reintroduction in the productive system and, therefore, for an *in situ* safeguard. Thirty-nine Siciliana hens were raised in paddocks (about 4 m^2/hen) and fed with pasture and a commercial pelleted feed (crude protein: 16.5% as fed, *ad libitum*; water was available *ad libitum*. From September to February 2013, the production cycle was controlled. Daily, for each group the egg production was recorded and the average production/head was calculated; all eggs were individually weighted and measured to calculate the shape index. Moreover, the breaking strength was evaluated. The percentage of egg components (yolk, albumen and shell), their chemical composition and Yolk Colour were determined. All data were compared to those of commercial white eggs andd subjected to ANOVA. Egg production was 170 egg/head with an average weight of 54.93±4.39 g; in absence of past selection for these traits, the eggs can be classified in the medium category of marketable eggs. As regards the external quality traits, the Shape Index (71.07±2.20%) was very similar to that of the commercial eggs, while the Breaking strength (4.4±0.66 kg/cm^2) was higher in Siciliana breed than that of commercial eggs. As regards the internal traits, albumen (53.76±2.83%), yolk (32.35±1.80%) and eggshell (13.70±1.71%) were very similar to those of the Commercial eggs. The chemical composition of the yolk showed higher content of protein (16.75±0.47g 100 g^{-1}) and lower content of lipids (26.49±1.73g 100 g^{-1}) than those of the commercial eggs, while the chemical composition of the albumen showed similar protein content (9.94±0.32g 100 g^{-1}) to that of the commercial eggs. The Yolk colour (Roche scale) showed values (9.49±1.07) very similar to the biological eggs, while lowers than the commercial eggs.

P-016

Determinants of consumer choice for mozzarella cheese

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This study aims to assess determinants of consumer demand for mozzarella cheese. Eighty-two consumers recruited in Potenza (Italy) received a questionnaire consisting of two sections: the first part concerned socio-demographic characteristics and consumers' habits; the second section aimed at attaining specific information about the consumption of pasta filata products (*e.g.* frequency of purchase and consumption and place of purchase). In this section consumers also had to express their beliefs about items affecting purchasing and consuming pasta filata products (*e.g.* appearance, colour, ethical aspects, brand name, geographic origin, etc.) using a 7-point scale: from 1 (unimportant) to 7 (very important). Consumers participating to the questionnaires were equally distributed for gender, mostly comprised in a range of 20 - 30 years of age (57%), they were partially involved in purchasing food and meal preparation and 63% had a university degree. Most of the respondents preferred a 150 g round size to 50 g and 20 g round sizes and 70 g braided size. They largely purchased these products at supermarkets. In order to identify the different items affecting purchasing and consumption of pasta filata products, the value of least significance difference (LSD) was calculated (significance level set at 95%). Thus variables were grouped into four significantly different groups: the first group showing the highest consumers' scores included taste/flavour, raw material origin, absence of preservatives, and texture, thus confirming the fundamental role played by sensory and safety dimensions in affecting consumer choices. In the second group, animal welfare was a central aspect affecting consumer decision and indicating their ethical involvement. In this group were also listed healthiness, colour, production area and nutritional characteristics. In the third cluster items related to appearance, dietetic features, ethical aspects (*e.g.* sustainability of the production chain), rearing system, animal diet and the place of purchase were all grouped. Conversely, packaging and brand were the least important for this sample of consumers. These results may help producers identifying the most important characteristics of mozzarella affecting consumer choice.

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P-017

Quality characterization of products from Romagnola chicken breed: 1. Carcass and meat

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In Italian poultry market, there is a deepening interest in the recovery and valorisation of local breeds. Among them, the dual purpose Romagnola chicken breed has been recently recovered and used to produce both meat and eggs. Therefore, this study was aimed to characterize carcass and meat quality of free-range Romagnola chickens (RMG) as compared with conventional rotisserie-type broiler hybrids. For this purpose, 24 carcasses (12 males and 12 females, 180 d, weighing around 0.85 kg, respectively) from RMG and 24 carcasses (35 d, 0.9 kg) from Ross 308 broilers (C) were obtained from two local producers. Skin colour was measured in pterylae tract and cut-up yield (breast, wings, thighs and drumsticks) was determined. Technological properties (pHu, colour, drip loss, cook loss and AK-shear force), chemical composition (moisture, protein, fat, ash and collagen) and fatty acid profile were evaluated on breast and leg meat. As expected, RMG carcasses exhibited a decrease in breast yield (28.7 *vs.* 36.1%; $P<0.01$) and higher thigh and wing yield (16.3 *vs.* 14.4% and 12.1 *vs.* 10.9%, respectively; $P<0.01$). Besides, RMG showed significantly lower skin lightness (L*) and yellowness (b*). With regard to meat quality, both leg and breast meat from RMG birds exhibited higher water holding capacity and AK-shear force values ($P<0.01$). In addition, even if there was no difference in breast colour, lower lightness (L*, 49.9 *vs.* 54.7; $P<0.01$) associated with an increased redness value (a*, 9.7 *vs.* 12.9; $P<0.01$) was observed in RMG leg meat. As for breast meat (without skin) composition, RMG showed lower moisture (73.8 *vs.* 75.8%; $P<0.01$) and intramuscular fat (2.19 *vs.* 2.99%; $P<0.01$) coupled with significantly higher protein (25.6 *vs.* 23.3%; $P<0.01$) and collagen (1.15 *vs.* 1.04%; $P<0.01$). In addition, RMG exhibited lower PUFA content (32.6 *vs.* 37.1%; $P<0.01$). With regard to leg meat (with skin) composition, RMG revealed higher moisture (74.2 *vs.* 71.6%; $P<0.01$) and protein (22.2 *vs.* 18.5%; $P<0.01$) content together with a reduced fat level (5.1 *vs.* 9.5%; $P<0.01$). Furthermore, higher SFA (32.7 *vs.* 24.1%; $P<0.01$) coupled with reduced PUFA content (26.5 *vs.* 35.9%; $P<0.01$) were detected. In conclusion, in respect to broiler hybrids, RMG meat exhibited inherent quality traits that need to be exploited using special marketing channels (*i.e.* small butcheries, typical restaurants *vs.* large scale retailing) and culinary preparations (*i.e.* roasting *vs.* pan cooking).

P-018

Investigation on milk yield and composition of Sarda donkeys

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Donkey milk is growing interest as an alternative milk for infants with intolerance to cow milk protein. This study investigated the yield and chemical composition of milk from donkey of Sarda breed. A total of six asses were used. Three asses belonged to the wild population of the Asinara island, which were never subjected to milking; for this reason they were confined in a large enclosed area and were fed hay *ad libitum* and concentrate in dose of 500 g/head/d. The others 3 asses from a private farm, were domesticated and free to graze. Body weight and milk yield of mare were measured and milk samples were collected at 0, 15, 30, 45, 60, 90 and 120 days after foaling. On sampling days, asses were hand milked after 2 hours of physical (not visual) separation from their foals. Individual milk samples were analyzed to determine the contents of fat, protein, casein, lactose, urea, somatic cells and total bacterial. Data were analyzed by linear model using population and sampling as fixed factors. Body weight was higher in domesticated asses than wild population (kg 146±2 *vs.* 117±2) and its variation on first 120 days of lactation was similar in both population (about -132–134 g/d). Milk yield was higher in domesticated than wild asses (g/2h 219 *vs.* 169; $P<0.05$), and it increased from 0 until 15 days of lactation, remained stable until 60 days and then decreased. Milk composition of domesticated asses showed lower fat (% 1.3 *vs.* 0.8; $P<0.01$) and higher urea (mg/dl 26 *vs.* 21; $P<0.05$) contents than wild group. No differences in lactose (6.3–6.4%), protein (2.1–2.2%), casein (1.8–1.9%), somatic cells (8–135 x1000/mL), and total microbial (8.7–9.7x1000/mL) were observed between the two groups. The results evidenced that the Sarda ass is capable to produce interesting amount of milk, considering its small body size and the fact that were not trained at the milking. On the basis of the data, it is possible to hypothesize that, under standard rearing condition, Sarda donkey would be able to produce even 600–700 g/d of milk, with two daily milking and after at least 3h of foal separation. The survey provided useful data in view of the potential use of Sarda donkey in the production and commercialization of donkey's milk.

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P-19

Functional food in mass catering: methodological approach

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The science of nutrition is moving from the concept of proper nutrition to the one of optimal nutrition. The so-called functional foods are products that have the characteristic of improving health and potentially reduce the onset of certain diseases. Functional foods are also very important in the competitive strategies of firms, due to the change in the consumers' choice and in the life style. In this context, strategies for business growth should be directed towards the most dynamic components of food demand, or towards the additional attributes of the products. In particular, meat and meat products represent an important component of European diet (average consumption 81 kg per capita). Animal nutrition is a key factor for improve meat nutritional quality. The competitive challenge for companies will be to allow the consumers to follow the most appropriate nutritional regimen, with particular attention to improving the health status, without changing their eating habits. The aim of the project is to set up a methodological approach to test the feasibility for functional food inclusion in mass catering, measuring the effect on the consumer behavior. Different steps include: change in consumers' habit, assess consumers' attitude towards functional food, evaluate the influence of functional food information in mass catering on consumers' food acceptability and expectation, evaluate the nutritional quality of the diet, nutrients deficiency, social role of mass catering, identification of animal source food, functional food design. The approach included the administration of a questionnaire planned to explore: consumer's general knowledge, consumer behaviors, attitudes and beliefs related to mass catering combined with the hedonic evaluation of meat functional food in different situation. The purposes of the questionnaire were to collect information about general attitude and awareness about meat and functional food and to group subjects according to their sustainability level.

P-020

Algerian dairy policy: causes weakness of milk production

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The dairy sector remains unstructured, with a collection rate brushing against the 10% of national production, and integration rate in the transformation process, around 5% only. Preliminarily, we can consider that the development of agriculture in general and especially of dairy cattle is strongly associated with water policy. The shortcomings in the mobilization and distribution of this essential factor is the length of drought cycles have that efforts remained in favor of this sector and insignificant to the eyes of consumption needs. This paper aims to identify the causes that have driven extroversion of this sector. This extraversion, which can enter through the total dependence of the global market dairy industry in terms of basic raw materials, is generally due to the lack of dairy policies in the various plans set work, and the existence of two driving modes that is the semi-intensive and extensive system. The dominance of the husbandry system in family and traditional character, with herds of small sizes, hindering the introduction of technical innovation. The little incentive price for milk production. Indeed, the raw milk is sold for direct consumption and small artisans at a price varying between 35 and 40 AD liter. The low milk yield ranging from 1 500 to 3000 liters between farms, which is of the wrong combination of different factors of production and the low level of guidance and technical support to farmers. Overall costs remain high and vary between 15 and 35 DA / liter; food costs dominate the cost structure of production. The contribution of these charges in the cost of production represents 60 to 70%. With a diet that consists largely of 50% concentrate. The dairy industry is completely disconnected from the agricultural sector to the extent that a very small part of its requirements is covered by the production of dairy farms. Domestic production continues to suffer from major handicaps.

P-021

Linseed supplementation in Pezzata Rossa Italiana lactating cows: effect on milk and Cacioricotta cheese quality

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The study aimed to evaluate the effects of linseed supplementation in Pezzata Rossa cows on fatty acid profile of milk and Cocioricotta cheese. The experiment of 40 days involved 24 cows divided into 2 groups; one group (Control group, C) received a diet based on concentrate, corn grain, and vetch and oat hay; the other group (Linseed group, L) in which 1 kg of linseed replaced an equal amount of concentrate. Individual milk samples were collected weekly and analyzed for pH, principal composition, SCC and fatty acid profile. At the end of the experimental period cow milk was collected for Cacioricotta cheese production. Cheese production protocol provided for: raw milk heating at 90 °C; adding of microbial rennet (150 g /100 l); cutting of the coagulum, draining off the whey and manual molding. The curd was held at ambient temperature for 12 h and then dry-salted for 1 day and ripened at 15°C and 95% relative humidity for 1 week. Ripened Cacioricotta cheeses were analyzed for chemical composition and fatty acids analysis. Data were processed by ANOVA using the GLM procedure for repeated measure of SAS (SAS Institute, 2011). The average values of the main components of the individual milk samples of Pezzata rossa cows were within the reference range for this breed. Milk nutritional composition did not differ significantly between groups however animals fed linseed supplement showed higher levels of fat and protein in milk than control milk. The effects of linseed supplementation were beneficial on the nutritional profile of bovine milk with lower values of saturated fatty acids (SFA) and higher levels of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) in milk from cows fed linseed than in control milk. Moreover 9 cis,11 trans conjugated linoleic acid (0.28 and 0.13±0.04% in L and C respectively) and n-3 fatty acids were higher in L than in C group. The differences in the fatty acids profile led to improved atherogenic and trombogenic indexes which showed lower values in milk from animal fed linseed. The nutritional value of the experimental Cacioricotta cheeses confirmed the results obtained in milk, in particular 9 cis,11 trans CLA and n-3 FA were increased by 50% and 32% respectively in L cheese in respect to control cheese. Atherogenic (2.98 and 2.13±0.1 for C and L) and trombogenic (3.37 and 2.68±0.09 for C and L) indexes of cheese underwent a significant reduction in cheese made using milk from cows fed linseed. Feeding linseed to Pezzata Rossa lactating cows led to a desirable enrichment of milk fat for human health.

P-022

Preliminary study on the carcass and meat characteristics of two free-range reared Italian local hen breed: *Bianca di Saluzzo* and *Bionda Piemontese*

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The aim of this study was to describe carcass and meat characteristics of two free-range reared Italian local hen breeds: Bianca di Saluzzo hen (BS) and Bionda Piemontese hen (BP). Eighty birds (female, 40 per breed) were periodically (5 animals per breed every two months) collected in two commercial slaughterhouses. Birds, aged between 5 and 8 months, were individually weighted and slaughtered. The day after, head and legs were removed and net carcass mass, breast, tight, and leg bone were weighed. pH (24h) and colour (CIE L*a*b*) were measured on breast and tight muscles. Colour was also measured on breast skin. Water, protein, fat and ash of breast and thigh muscles were determined by AOAC methods. Data were analysed by GLM procedure, considering strain, muscles and strain by muscle interaction fixed effect. No statistical difference between BS and BP was found for final live weight (BS = 1757 g; BP = 1778 g), carcass weight (BS = 1008 g; BP = 998) and slaughter yield (BS = 58.5%; BP = 57.6%). BS showed higher breast weight (205.8g *vs.* 185.5g; $P<0.05$), while no difference was found with respect to the tights weight. In relation to proximate analysis, BS in comparison to BP showed higher water content (74.57% *vs.* 71.71%; $P<0.01$). Regarding colour, BS presented higher lightness (L*; 50.63 *vs.* 46.69; $P<0.01$) and lower redness values (a*; 5.95 *vs.* 6.99; $P<0.01$). Marked differences were found in breast skin colour. BP showed higher yellowness and redness index than BS (b*; 53.59 *vs.* 21.79; $P<0.01$; a*; -0.24 *vs.* -2.83; $P<0.01$). With regard to muscles, breast in comparison to tight, showed a higher protein (24.36% *vs.* 20.60%; $P<0.01$) and a lower fat content (0.57% *vs.* 4.02%; $P<0.01$). Higher lightness and lower redness values were observed in breast (L*; 53.72 *vs.* 43.60; $P<0.01$; a*; -1.95 *vs.* 14.89). Significant interaction between strain and muscle for pH, ash, and yellowness were found. As regard the breast, BP in comparison to BS had a lower pH, while for tight no differences were found. BS breast in comparison to the tight showed a higher ash content while in BP no differences were found in ash of the two muscles. Yellowness index was higher in the two muscles of BP in comparison to BS and lower in the breast of BS and in the tight of BP. In general, this study gave an overview on the meat quality of these two local breeds. Further studies, in a controlled condition, are needed to better investigate any possible difference.

P-023

Nutritional quality and aromatic profile of *Caciotta* cheese in cows fed with *Linum usitatissimum* L.

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The increasing attention for functional foods and sustainable agriculture leads to assess diets that can naturally improve dairy products. Aim of this study was to evaluate the nutritional indexes (Health Promoting Index – HPI, Thrombogenic index – TI), the single fatty acids (α-linolenic acid -ALA, Conjugated linoleic acid - CLA) and the aromatic profile of cheese of dairy cows supplemented with Linum usitatissimum L., extensively cultivated in the past for fibre flax production. Ten dairy cows were allotted into two homogeneous groups: Control and *Linseed flour* (L). Animals received the same diet, while in the experimental group 20%/DM of concentrate supplementation was replaced with L for 15 days, after two weeks of adaptation. During the experimental period, the milk was collected and cumulatively processed in Caciotta cheese (a soft cheese, 25 days ripened) for three times. The Fatty acids profile of cheese was performed by gas chromatography and HPI was calculated according to Chen *et al.* (2004). Two techniques were performed for the headspace aroma profile analysis: thermal desorption by GC-MS for Volatile Organic Compounds (VOCs) determination and the Electronic Nose for fingerprint characterization. Statistical analysis of FAs (g/100 g FA) and VOCs, allotted in compound classes and expressed in arbitrary unit (a.u. = peak area 10^{-6}), was carried out by ANOVA. Principal Component Analysis (PCA) was used for the evaluation of VOCs and data by Electronic Nose. FAs profile and HPI were affected by the supplementation. The ALA content of cheese from L group showed a significant increase compared to Control group (0.68 *vs.* 0.41; $P\leq0.001$). The same trend was observed for CLA content (1.60 *vs.* 1.34; $P\leq0.01$). Significant differences were also observed for the nutritional indexes ($P\leq0.05$). The highest HPI value was observed in cheese from L group (0.63), while the TI showed an opposite trend, as expected. The lowest n-6/n-3 ratio was detected in cheese from L group (1.07 *vs.* 2.4; $P\leq0.01$). No significant differences were found between the two groups using the GC/MS method and Electronic Nose. The two techniques seemed to lead to the same result. The results showed that L supplementation increased the beneficial fatty acids content of Caciotta cheese improving its nutritional and nutraceutical quality, without affecting aromatic profile. Moreover, the reintroduction of the flax in Campania region might represent a valid tool for sustainable agriculture.

P-024

Use of multivariate procedures for classification of Toscano dry-cured ham according to the volatile composition

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The Prosciutto Toscano is a traditional Italian dry-cured ham that obtained the Protected Designation of Origin, (Regulation (EC) No. 1263/96) in 1996. The curing time, in the production of dry cured ham, is becoming increasingly important being consumers currently oriented towards products with a longer maturity. Several studies have demonstrated that a prolonged ripening time has significant effects on the aromatic composition of dry-cured hams. The aim of this study was to develop a method to assign the hams to the proper class of seasoning on the basis of their volatile composition. The volatile compounds of 10 hams were extracted by the HS SPME-GC-MS technique. From each ham, samples of Subcutaneous fat and Semimembranosus muscle were taken at 0, 1, 3, 6, 12, 14, 16 and 18 months of seasoning. Ninety seven volatile compounds were identified and submitted to three different multivariate statistical techniques. The Stepwise Discriminant Analysis (SDA), performed to select the most discriminant compounds, the Canonical Discriminant Analysis (CDA), used to test, on the basis of the selected compounds, the effective discrimination among seasoning classes. Finally, the Discriminant Analysis (DA) was used to assign the samples to the proper seasoning class. The discriminant procedures were firstly applied to the whole data set to assign samples at two different seasoning classes: "fresh" (0, 1, 3 and 6 months) and "mature" (12, 14, 16 and 18 months). The SDA selected 2 and 3 compounds for fat and lean samples, respectively. The CDA, by using the selected variables, significantly separated the two classes, whereas the DA correctly assigned all samples. The discriminant procedures were then performed within "mature" class to assign samples to the own class of seasoning. With only 14 compounds, the CDA significantly discriminated fat samples in the four classes and the DA correctly assigned all samples. For lean samples, SDA selected 27 compounds and the CDA significantly separated the classes. The DA, however, correctly assigned 75% of samples. The combined use of the three multivariate discriminant techniques was able to select a reduced number of volatile compounds for correctly assign hams to the different seasonal classes. The procedure was more efficient for fat than lean tissue.

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P-025

Effects of slaughter weight on meat quality in two pig genotypes

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The present work was a preliminary study for the determination of the chemical-physical characteristics of the raw meat to produce a seasoned product called *Cuore di Spalla*, resulting from the pig boneless shoulder. Twenty-eight pigs, belonging to Duroc X Large White (DxLW) and Cinta Senese X Large White (CSxLW) genotype, were reared in the same conditions. The animals were slaughtered at the same age and at an average live weight of 143±15 kg for DxLW and 122±15 kg for CSxLW. At slaughtering, samples from *Triceps brachii* muscle were submitted to physical (shear force on fresh and cooked meat; cooking loss) and chemical (moisture; crude protein; ether extract; ash; total lipids; fatty acids) determinations. The live weight at slaughtering (l.w.) showed a great variability between and within the genotypes so that the results were analysed as trend on the l.w.. As regards the physical traits, changes in shear force and cooking loss with increasing l.w. were not found for both crosses. For DxLW the chemical analyses showed an increase of fat content and a relative decrease of protein and of moisture as l.w. increased . For CSxLW no change were found. As for fatty acid content (g/100 g of dry matter), the results showed a more complex pattern: saturated (SFA) and monounsaturated (MUFA) increased in DxLW genotype; polynsaturated fatty acids (PUFA-n3 and PUFA-n6) showed no variation as l.w. increased. As regards the qualitative analysis of acidic profile (% on total fatty acids), both genotypes showed no changes as l.w. increased. In conclusion, genetic effect was evident for slaughter l.w.. This result, together with the effect of the paternal line, were the characterizing factors of the recorded values on meat. Differences were found especially in the fat component, a very important factor in the final product quality.

P-026

Adult ovine meat marketing in Tuscany

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This paper presents the results of a market study on the adult sheep meat trade in Tuscany considering the Conventional and the Halal (Islamic slaughtering) slaughter products. 55 questionnaires were administered to Conventional butchers, and 17 questionnaires to Halal butchers. The latter were mainly in Pisa, Livorno, Firenze, and Prato. In the Conventional market, the ovine meat consumption seasonality was confirmed; lamb is traditionally eaten at Christmas and Easter. The sheep meat sold percentage never exceeded 5% of the total: the main product was the lamb. In the 7.3% of cases, the adult ovine meat was sold. During the year, an average of 203 lamb carcasses was sold. 270 sheep carcasses, which derived mainly from a Campi Bisenzio (FI) sheep meat butcher, were annually sold; in this town there is a tradition for the sheep meat consumption. The lamb carcasses mean weight was 9.0±1.9 kg, while the sheep carcasses mean weight was 23.4±1.9 kg. In the 78% of cases the ovine carcasses derived from the Garfagnana, Casentino, Mugello, and Maremma Tuscan areas. In Garfagnana the consumers mainly preferred the local Garfagnina bianca and Massese breed lambs. The lamb carcass average price was 9.00 €/kg, while the lamb meat average price was 17.50 €/kg. The adult ovine average prices were respectively 3.5 €/kg for the carcass and 7.0 €/kg for the meat. The lamb carcasses were normally presented with head, heart, trachea, lungs, and liver, which were sold by butchers at very low prices (*e.g.* 1.00-2.00 €/kg for the head). The Islamic consumers appreciated more adult sheep meat than the Italian consumers. 50% of Halal meat came from France and Ireland, because the consumers considered the Islamic Italian meat, which came mainly from Brescia, Lucca, and San Miniato, too red and tough. Islamic consumers preferred male sheep and the front half of carcass. The Islamic butchers had not difficult to find Halal meat in Italy. The Halal sheep carcasses average weight was 20.6 ± 3.0 kg. During the year, the Islamic butchers sold an average of 163 lambs and 153 sheep. The Halal butchers on average paid € 3.00/kg carcass, and sold the meat on average € 5.00 €/kg. This survey confirmed a lack of interest in adult sheep by Tuscan consumers, while Halal consumers have shown increased interest.

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P-027

Whey proteins as functional food affecting metabolic health

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Whey proteins have been raising interest for their nutritional value and for their impact on metabolic health. Considerable attention is being paid to the effects of whey protein supplements on glycaemic control for their possible application in the prevention and treatment of type 2 diabetes. GIP and GLP-1 are gut derived peptides (incretins) that are released after food ingestion and potentiate insulin secretion from the islet beta-cells in a glucose-dependent manner. Actually, incretins are rapidly inactivated by the enzyme DPP-IV. DPP-IV inhibition increases incretin half-life and improves glycemic control. The products of whey protein digestion can increase both GIP and GLP-1 secretion and counteract their rapid inactivation by inhibiting protease DPP-4. In recent years, our research group contributed to the *in vitro* and in silico characterisation of short peptides with DPP-4 inhibitory activity among the products of enzymatic hydrolysis of beta-lactoglobulin (beta-LG) and alpha-lactalbumin (alpha-LA). Our studies also suggest that the hydrolysate of beta-LG obtained by using digestive proteases (pepsin and trypsin) may be more effective at inhibiting DPP-IV than alpha-LA hydrolysate. Actually, the effects of whey protein intake on the enteroendocrine system is not limited to the incretin release. Like other dietary proteins, whey proteins stimulate the secretion of Cholecystokinin (CCK) in the gut. CCK is known as satiety hormones. Its secretion into blood in response to food intake, delivers satiety signals from the gut to the central nervous system. In agreement with data in the literature, we confirmed that the alpha-LA hydrolysates are effective at increasing colecystokinin (CCK) release from *in vitro* cultured enteroendocrine STC-1 cells (50%±12% of increase *vs.* basal secretion). We compared the effects of the hydrolysates from alpha-LA to those of beta-LG hydrolysates and we did not find any significant differences. As to the molecular basis for the interaction between whey proteins and enteric hormone activity, no short amino acids sequences within whey proteins that could be related to their stimulatory effect on enteric hormone secretion, including CCK secretion, have been identified, yet.

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P-028

Evolution of fatty acid profile of gilthead sea breams during the decade 2005-2014

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The aquafeed industry is facing the progressive lack of availability and high price of fish meal and oil, in the formulation of feeds. Nowadays the increasing use of plant derived oils and oils obtained from by-products of farmed fish have modified the fatty acid (FA) composition of sea bass and sea breams feeds. To investigate how this process is changing the lipid profile of farmed fish we compared the FA composition of sea breams collected on the Italian market during last year with a similar carried out in 2005. The sampling covered sea breams of different origin, mirroring the presence of different country products in the Italian market. In 2005 we sampled 46 fish, 18 farmed in Greece, 15 from Italy, 10 from Croatia and 3 from Turkey. In 2014 we sampled a total of 44 sea breams, 18 farmed in Italy, 14 from Greece, 6 from Turkey, 3 from Croatia and 3 from Malta. In addition to we sampled also 21 wild sea breams: 19 during the 2005 and 2 during 2014. Lipid content of fillets was determined by chloroform/methanol extraction, FA methyl esters were analyzed by gas-chromatography. In order to compare different samples and to detect the most important fatty acids affecting the distribution of fish samples, data were processed by principal component analysis using FA. Lipid content of farmed sea breams has proven quite stable in the period of the trial, 2.8% in 2014 and 2.6% in 2005. Farmed sea breams, showed a higher lipid content respect of wild ones, where the lipid of muscle were found at 0.7%. Concerning FA, sea breams appeared well separated according of their year of farming and type of production. The 2014 sea bream were more characterized by linoleic and oleic acids, and they showed a smaller spatial dispersion compared with the 2005 sea breams, which were placed in the intermediate position between wild fish and 2014-farmed ones and resulted less homogeneous concerning their spatial distribution. Wild fish were characterized by the presence of a higher amount of n-3 polyunsaturated fatty acid and arachidonic acid without differences between 2005 and 2014. Our study confirms that the increase of plant derived oils and fish by-products oils is modifying the FA profile of sea breams. The 2014 fish present the higher difference from wild ones. The small spatial dispersion of 2014 fish suggests a tendency to a standardization of the fish feeds, which was lower in 2005, when probably there was more variation of the lipid sources used in feed formulation.

P-029

Microbiological quality of milk obtained by machine and manual milking

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The milk is an important source of food because it is rich in nutrients; therefore may be an excellent culture media for microbial growth. The way that the milking is performed influences the final contamination of the product, once is the first stage in which environmental bacteria may contaminate the milk. In this context, the aim of this work was to evaluate the total bacterial count (TBC), total coliforms and thermotolerant coliforms in refrigerated raw milk obtained by machine and manual milking. To this, nine dairy farms were randomly selected in the city of Papanduva, Brazil: in four of which the milking were performed by machines and in five the milking were performed manually. The cows were maintained in an extensive system, from Holstein and Jersey breeds and were milked twice daily. In each farm the samples of raw milk were collected four times separated by a one-week interval, in a total of 36 samples. These samples were taken from the cooling tank in the beginning of the morning and were immediately transported in cool boxes to the Laboratory of Food Quality at University of Contestado. The TBC was estimated by the standard plate count method and were calculated as colony-forming units per mL (CFU/mL) and for the total and thermotolerant coliforms the multi-tube fermentation technique was used to determine the more probable number per mL (MPN/mL). The TBC data were submitted to Mann-Whitney (Wilcoxon) test and a descriptive analysis was performed for total and thermotolerant coliforms, being considered the following standard (MPN/mL): low: 0 to 100; medium: 101 to 600; high: 601 to 1100; very high: above 1101. It was not observed difference in TBC between manual and machine milking in the four replicates ($P>0.05$), showing that the way of milking did not affect the amount of bacteria. The frequency of results low, medium, high and very high in total coliforms in machine milking was 50.0, 31.2, 6.25 and 12.5% and in manual milking was 95.0, 0.0, 0.0 and 5.0%. In thermotolerant coliforms the frequency of results low, medium, high and very high in machine milking was 50.0, 43.7, 0.00 and 6.25% and in manual milking was 60.0, 20.0, 5.0 and 15.0%. We conclude that, in the evaluated dairy farms, the way of milking is not a determinant factor for the amount of bacteria. However, the total coliform contamination tends to be lower when the milking is performed manually.

P-030

High throughput SNP discovery in cultivated European sea bass (*Dicentrarchus labrax*) and comparison of genetic stocks of two hatcheries

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The European sea bass (*Dicentrarchus labrax*) is a common and cosmopolitan species. In the wild, it is present in the Altlantic Ocean (from Canary Islands to Norway), in the Mediterranean sea and in the Black sea with two naturally hybridizing lineages. D. labrax is a rustic fish that can tolerate high ranges of temperature and water conditions. The European sea bass was one of the first fish species, after salmon, to be farmed in Europe. Sea bass breeding started in France and Italy in the '60 with the development of a few hatcheries. As sea bass is an economically relevant species, several studies started to decipher its genome. At present a partially assembled genome of *D. labrax*, constituted by a total of 36,166 contigs that represent about 16% of its estimated 600 Mbp genome, is publically available. In this work we applied a next generation sequencing approach to investigate differences between two sea bass populations. Two equimolar genomic pools composed each by six fishes belonging to i) a hatchery located in Agrigento and ii) a hatchery located in Lampedusa (Italy), respectively, were sequenced with the Proton Torrent next generation sequencer, producing a total of 10.78 Gbp. The sequenced reads produced from the two sequenced pools were aligned to the available sea bass partial reference genome obtaining a total of 36.8 million of aligned reads. The variant caller produced a total of about 313.5 k high quality single nucleotide polymorphisms (SNPs). Despite the low number of animals that composed the genomic pools, we could detect about 44 k SNPs in which there are clear allelic differences between the two hatcheries. These differences could be a first evidence of divergent selection (including bottleneck and/or founder effects) between the two hatcheries due to artificial selection. To our knowledge this is the first experiment of genome wide SNP discovery in cultivated sea bass. This information will contribute to plan new breeding strategies in sea bass including genomic information.

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P-031

Next generation semiconductor based sequencing of the donkey (*Equus asinus*) genome

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The estimated donkey (*Equus asinus*) population of about 43.5 million of heads is mainly distributed in developing countries in which this species is employed as pack and working animal. In Europe, the number of donkeys is rapidly decreasing, raising concerns about conservation of animal genetic resources represented by numerous small donkey populations or breeds that are listed as endangered or close-to-the-extension populations. The paucity of genomic information available in this species limited the genetic investigations in *E. asinus* to the description of genetic variability using microsatellite markers and mitochondrial DNA. A preliminary recent study conducted by others reported a draft assembly of the donkey genome using Illumina short reads. In our study, we applied the next generation semiconductor-based sequencing technology that we have available with the Proton Torrent platform to re-sequence the donkey genome and to identify single nucleotide polymorphisms (SNPs) as a first step to define useful tools and information to monitor the loss of genetic diversity in this species. A total of five Ion PI™ Chip Proton Torrent runs were used to sequence the genome of two Grey x Ragusano male donkeys. About 237 million of reads were generated and aligned to the reference genome of the horse (EquCab2.0) and to the draft genome of the donkey. Aligning performances were better against the EquCab2.0 genome (97.5% coverage) than those against the draft donkey genome (94.8% coverage) confirming the better quality of the horse genome assembly. Using the horse genome information and the newly generated sequences for the donkey genome we estimated that the differences between the two species were 0.80-0.95%. Comparing sequence information available from the three donkeys (one obtained with Illumina by others and two obtained with Proton Torrent in this study) we identified about 1.2 million of autosomal SNPs validated across the different animals/sequencing platforms. In addition, a few hundred of Y specific polymorphisms, that could be useful to track the paternal lineages in this species, were identified. This study might represent a starting point for a more comprehensive description of genomic variability of the donkey.

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P-032

Transcriptome-Wide comparison of sequence variation in skeletal muscle of Chianina and Maremmana

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Identification of genes controlling meat tenderness and correlated metabolic pathways can be easily and straightforwardly addressed by high-throughput mRNA sequencing, which assesses transcripts quantities and features. Through deep mRNA sequencing, we aimed at analyzing gene expression in muscle tissues of two Italian cattle breeds, Maremmana and Chianina, to uncover genes encoding meat tenderness, which is considered an important trait for meat quality. On the basis of phenotypic analysis of the sample tenderness, which includes the Warner-Bratzler shear force, the index of myofibrillar degradation, the proteolytic activity and the insolubility of collagen, samples were classified according to their degree of tenderness, from tough to tender. Our deeply sampled RNA-Seq generated 38,904,560 short sequence reads. We used Bowtie to align reads on the genome, TopHat pipeline to map splice junctions, Cufflinks to estimate transcript abundances and to test for differential expression in muscle samples and an in house software to classify alternative splicings. SNPs were called using VarScan software from the .bam files from TopHat, using the UMD_3.1 assembly as reference genome. The selected SNPs were annotated by means of SnpEff using the available UMD_3.1 annotations as reference. Using all the aligned RNA-seq reads and the UMD_3.1 assembly as reference genome, a total of 27,466 putative SNPs were identified. Furthermore, we considered only homozygote SNPs which are opposite in each category. Comparing tender and tough meat we found 1,462 homozygous SNPs showing the alternative allele with a 100% frequency; 1,595 SNPs were found comparing tender CN and tough CN meat; 610 SNPs, comparing tender MM and tough MM meat; 2221 SNPs, comparing MM and CN meat. SNPs were then classified into several categories on the available UMD_3.1 annotations, by their position in intergenic, up- or down-stream regions of a gene, and in the primary transcript (in this case the mapping is further refined as in intronic, exonic, 3' or 5' UTR regions) and also by their effect on gene coding (synonymous or non-synonymous polymorphisms, affecting 5' or 3' splice site recognition and insertion of stop codon). We could also identify new stop codons which may shorten the proteins and affect their activities. We have found several SNPs that potentially affect the gene expression of key proteins in meat quality features and particularly in tenderness.

P-033

Genetic evaluation of behavioural and morphological traits in Murgese horse

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Murgese horses can be used for many purposes: dressage, therapeutic and educational aids, equine tourism, mounted police, etc.. This study aims to assess the hereditability of morphological and temperamental traits to be included in future genetic selection programmes. A database concerning 711 males and females Murgese subjects, born from 1927 to 2013, and their morphological traits (height at withers, chest girth, and cannon bone circumference) assessed at 3 years of age was used. In addition, for 103 out of 711 animals, a questionnaire on temperament with 15 items was submitted to 11 caretakers and owners. The respondents rated each item on a 9-point scale labeled at the left end with "attribute absent" (score=1), at the right end with "attribute very strong" (score=9). Data were analysed using the Restricted Maximum Likelihood methodology applied to a multiple-traits animal model. A VCE software was used. The fixed effects were: sex, year of birth and caretaker; the inbreeding coefficient was estimated with SAS proc inbreed and used as covariate (SAS, 2007); random effects were the animal and the residual error. The mean inbreeding coefficient of our sample (animals born from 2010 to 2013) was 6.3±2.42% and 7.51±1.13% for males and females, respectively. Hereditability ranged from 0.24 to 0.38, and was highest for concentration (0.35), trainability (0.36) and consistent emotionality (0.38). Morphological traits were not significantly correlated with temperamental traits. However, significant and positive correlations were found between chest girth *vs.* cannon bone circumference (0.60; $P<0.001$), and between height at withers *vs.* chest girth and cannon bone circumference (0.54 and 0.48, respectively; $P<0.001$). Genetic correlations between temperamental traits were all positive although they were moderate (with value under 0.42). In particular, friendliness towards people, low reactivity to noise, responsiveness to commands, ease of gate entrance were positively correlated with learning ability (0.27, 0.25, 0.35, 0.30, respectively). Concentration was positively correlated with responsiveness to commands (0.36), friendliness towards people (0.28), trainability (0.29), learning ability (0.42) and consistent emotionality (0.40). The high heritability values observed in this study suggest that temperamental traits could be used as selection targets to specialize the animals according to different breeding purposes.

P-034

Genetic variability of the Braque Français type Pyrénées dog breed assessed by pedigree data

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The Braques français are hunting dogs, from a very old type of gun dog used for pointing the location of game birds for a hunter. There are two breeds of Braque français, both from the South of France, the Braque français type Gascogne (larger size) and the Braque français type Pyrénées (smaller size). The original Braque français type of pointing dog has existed since the fifteenth century. The first breed club was formed in 1850, and the standards for both breeds were written in 1880. Both types belong to group 7 of ENCI classification. The complete electronic record of the Braque français type Pyrénées was downloaded from the ENCI database with the aim to estimate the genetic variability of the breed. In Italy 921 puppies (479 males and 442 females) were registered from 2003 to 2014 (average value: 76.6±40.24 dogs per year). Pedigree records of all registered animals (Reference Population=RP) were considered. The whole population (WP=RP and its genealogy) included 1,250 dogs (826 males and 622 females). 783 dogs were inbred. Up to 89% of the individuals had registered parents and 83% registered grandparents. To explain 50% of the genetic variability, a total of 9 and 7 ancestors were enough, respectively in the WP and RP. The average inbreeding coefficient in the RP resulted 4.3%, while the average inbreeding of the inbred was 5.19%. The inbreeding coefficient was <0.05 in 511 dogs (65.3% of inbred) whereas it was >0.20 in only 13 dogs (1.66% of inbred). Inbreeding coefficient per year ranged from 0.98% for dogs born in 2003 (27 dogs) to 7.05% in 204 dogs born in 2009. Puppies born in 2014 had an average inbreeding coefficient of 1.78%. Ten traced generations were highlighted; the maximum average inbreeding value (6.62%) was observed in the dogs with 10 traced generations (inbred: 93.15% with an inbreeding average value of 7.11%) while Ne for RP computed via individual increase in inbreeding was 42.42. A regular monitoring of genetic variability of the breed is important and must be adopted, in order to avoid the danger of an excessive increase of inbreeding in the future, which would result in significant inbreeding depression and in significant loss of genetic variation.

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P-035

Genetic structure and admixture in Western Balkans and Central European sheep: preliminary results from 50K SNP genotypic data

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The availability of affordable genome-wide SNP genotyping technologies prompted in the last years a wide collaborative study, which provided unprecedented insights into the phylogeographic structure of the world-wide sheep population. However, the original sheep sample collection did not include Western Balkans and Central European sheep populations, which may represent a valuable resource to understand historical pattern of migrations from the domestication centre and to fully reconstruct global sheep genetic structure patterns. We genotyped 96 animals from 19 different sheep populations, mainly belonging to the Pramenka group, from Slovenia (Bovec sheep, Jezersko-Solcava sheep, Bela Krajina Pramenka), Croatia (Cres Island sheep, Krk Island sheep, Lika

Pramenka, Pag Island sheep, Rab Island sheep, Dalmatian Pramenka), Bosnia and Herzegovina (Privorska, Dubska), Serbia (Lipska), Montenegro (Pivska Pramenka, Zuja, Sora), Republic of Macedonia (Ovchepolean pramenka, Karakachanska pramenka) and Czech Republic (Sumavska, Valachian) using the Illumina OvineSNP50 BeadChip. The Neighbor-Net clustering pattern generally reflected geographic origin, with clusters of populations from Croatia, Bosnia and Herzegovina, and Montenegro, respectively. On the other hand, Southeastern Slovenian Bela Krajina Pramenka is separate from the two other Slovenian breeds. Similarly, the two breeds from Czech Republic are separate, supporting the hypothesis that Valachian sheep were introduced between 15th and 16th century by immigrating Valachs. A preliminary MDS analysis based on the updated world-wide sheep population sample highlights the almost central position of Western Balkans and Central European sheep populations at the crossroad between breeds from Middle East/Far East/Africa and Northern European breeds. The Czech Sumavska is positioned relatively close to North-European breeds. Relative positions of Italian and Balkan breeds indicate a latitude gradient (northeastern Adriatic breeds being related to North Italian breeds and, southeastern Adriatic breeds to South-Italian breeds), resulting from historic gene-flow across the Adriatic Sea.

P-036

Relation between chromosome stability and rob(1;29) in Agerolese cattle

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In autochtonous Italian cattle breed rob(1;29) is the most frequent chromosomal rearrangement. Aim of this work was to verify the chromosome stability of rob(1;29) in Agerolese cattle. Chromosome Aberration (AC) and Sister Chromatid Exchange (SCE) tests were performed in 6 Agerolese cattle heterozygous carriers of rob(1;29) (group 1) and Agerolese cattle with normal karyotype (control group). Moreover aphidicolin-induced fragile sites (FS) were analyzed in group 1. 300 metaphases each group have been studied for AC test. Statistical analysis carried out with contingency table showed that total ACs in group 1 (59) is statistically higher (P=0.005; OR=1.9) than in control group (35) and that X-chromosome has an higher probability of breakages (P=0.04; OR=5.2) in group 1 (10) than in control group^2. The number of ACs observed on the rob(1;29)^4 corresponds with that expected (3.62) on the basis of relative chromosome length. Mean values of SCE/cell were 6.08±2.71 and 5.44±2.50 in group 1 and control respectively, being the difference statistically significant (P<0.05); mean values of SCE/X chromosome were 0.41±0.65 and 0.27±0.59 in group 1 and control group respectively being the difference statistically significant (P<0.05); mean values of SCE in t1 and t29 were 0.23±0.49 and 0.02±0.14 respectively. Although mean number of SCE/cell is higher in Agerolese cattle carrying rob(1;29) than in the control, the number of SCEs (53) observed on rob(1;29) was considerately lower than that expected (78.4) on the basis of relative chromosome length. On the contrary, the SCEs number observed on the X-chromosome (80) was much higher than expected (60.38). 300 metaphases have been scored for FS test in group 1 and 138 break points were observed, 16 of which are FS according to statistical analysis (P<0.05). No FS is located on rob(1;29) while BTAX shows 4 FS (Xp21, Xq12, Xq22 and Xq23) thus confirming the instability found in AC and SCE tests. According to these data, chromosome rob(1;29) in Agerolese cattle shows the expected chromosome stability rate but heterozygous carriers of this translocation have higher chromosome instability in particular on X-chromosome. To confirm this relation it is necessary to analyze an higher number of cattle carrying rob(1;29) and to perform a fragile sites analysis in Agerolese cattle breed.

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P-037

Study of the doublesex and Mab-3 related transcription factor 3 gene in Italian trotters

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HHorses show a large variability in their pattern of locomotion. The three natural gaits are walk, trot and canter/gallop. Most breeds are called “non-gaited”, including horses that are able to perform just the natural gaits. But some breeds, called “gaited”, can also use alternative gaits. Some authors showed that the doublesex and mab-3 related transcription factor 3 (DMRT3) gene plays a key role in this attitude. They demonstrated that a single nucleotide substitution (C>A) in this gene, causing a premature stop codon, is essential for the ability to perform alternate gaits and has a favorable effect on harness racing performance. Trotters are used for harness racing thanks to their ability to trot at high speed without falling into gallop. In fact, the “gaited” pattern of locomotion is under selection in trotter breeds. The DMRT3 gene has never been studied in the Italian Trotter, even though this breed includes horses that are among the best trotters in the world. Given the importance of this gene, our aim was to analyze some of the best Italian Trotters by a sequencing analysis. We analyzed 32 Italian Trotter horses from five stud farms well known in Italy for this breed. In addition, we analyzed 8 horses belonging to different "non-gaited" breeds, two samples per breed, as a control. The latter included two cosmopolite breeds, Arabian horse and Thoroughbred, and two Italian native breeds, Maremmano and Murgese. DNA was extracted from whole blood and the last exon of DMRT3 gene was sequenced. Sequences were aligned, confirming the SNP at position chr23:22999655. No other polymorphisms were found in this region. The SNP was then genotyped on the sample of 32 Italian Trotters. The wild CC genotype seems to be fixed, as expected, in all the breeds other than the Italian Trotter. But all the Italian Trotter horses analyzed were homozygous for the mutant allele A, except for two heterozygous animals. The genotype of these two horses at the analyzed locus confirmed what expected. In fact, they were signaled by the breeders for genealogy and attitude. Our results confirm the importance of the polymorphism in DMRT3 gene also for the Italian Trotter population. The analysis of further samples of this breed, including animals with low performance, may be useful.

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P-038

Genomic diversity and population structure of Ugandan taurine and zebuine cattle breeds

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An extensive sampling of Ugandan cattle was carried out in the course of the European project Nextgen to identify possible associations between genotypes, livestock endemic diseases and environmental variables. A total of 915 animals, representative of the Ankole group (crossbred between Bos indicus and Bos taurus), Zebu and Ankole-Zebu crosses, were sampled over the whole country and genotyped: 815 with Illumina BovineSNP50 BeadChip (ca. 54K SNPs) and 102 with BovineHD Genotyping BeadChip (ca. 800K SNPs). Prior to association studies, both population structure and possible introgression from non-native gene pools (*e.g.* industrial breeds) have been extensively investigated by comparing the Ugandan dataset to publicly available SNP data from previous research projects or public databases. After quality control and merging procedures, the final working set included Ugandan cattle together with 112 reference cattle breeds (taurine, indicine and crosses) sampled from all over the world: 28 from Africa, 26 from Asia, 49 from Europe and 14 from the Americas. The different approaches adopted for data analysis included Bayesian clustering procedures (Admixture software) and multivariate statistics (Multi-Dimensional Scaling plot). The results obtained with Admixture software showed that Ugandan cattle are characterized by two major genomic components roughly corresponding to Ankole and zebu ancestries, and which respectively cluster with Ankole animals sampled outside Nextgen project and Boran, Serere/Teso and Sahiwal indicine breeds from Africa and Asia. Also some minor genomic components were revealed, one of which is partly shared with East African Shorthorn zebus. The MDS plot highlighted a clear subdivision of three groups of individuals, namely European taurine cattle, African taurine cattle and Asian zebu. Turkish breeds, the closest to the center of domestication, are positioned near the origin of the axes, while Ugandan cattle are scattered on a wide surface overlapping to Ankole, African taurine x zebu crosses and African zebu. Some Ugandan individuals, also, separate from the majority of the samples and spread towards the center of the graph. Further investigations will be necessary to understand if this behavior mirrors an actual similarity to the Turkish breeds or merely an introgression from the European taurine gene pool.

P-039

Genetic variants of *CSN1S1* gene in Italian Mediterranean river buffalo: an association with milk yield

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Similarly to other species of ruminants, the river buffalo

(2n=50) is characterized by the presence of all four casein fractions (αS1, β, αS2, and κ) encoded by the four linked autosomal genes (CSN1S1,CSN2,CSN1S2 and CSN3, respectively) mapped on chromosome 7. In particular, the CSN1S1 gene is characterized by an extremely split architecture with 19 exons, many of which of small size (24 bp). It encodes for a precursor of 214 amino acids with a signal peptide of 15 amino acid residues.In recent years, several polymorphisms at milk protein loci associated with traits of economic interest like milk coagulation properties or milk composition have been intensified.Despite that, so far, not any SNP within the milk protein loci was found to be associated with an important trait like milk yield.The aim of this study was to evaluate possible effects of the SNP c.628C>T, identified at position 89 of 17th exon of the CSN1S1 gene and responsible for the amino acid change p.Ser178Leu (A and B allele, respectively) (EMBL n° HE573919-20), on milk yield in Italian Mediterranean river buffaloes. A total of 7547 records for milk yield measured monthly on 1096 lactations of 552 buffaloes belonging to different farms located in Salerno and Caserta province (Southern Italy) were analyzed. Sampling and phenotypic collection data were carried out in collaboration with the Italian National Association of Buffalo Breeders (ANASB).The genotyping of examined animals was carried out by a method based on MboI-ACRS.The major allele (B) had a relative frequency of about 0.6 and χ^2 values showed that there was no evidence of departure from the Hardy-Weinberg equilibrium ($P \leq 0.05$). Association between CSN1S1 polymorphism and milk yield was investigated with a mixed linear model that included effects of parity, calving season and month of production. A significant association between the SNP c.628C>T and milk yield was found ($P<0.05$).In particular, the BB genotype showed an average daily milk yield approximately 0.61 kg higher than AA buffaloes.The results reported in the present work represent the first example of association between a genetic marker in a milk protein encoding gene and milk yield for the Mediterranean river buffalo.Such association, if confirmed on larger population, should be evaluated in order to supply useful indications for the application of marker-assisted selection programs in river buffaloes.

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P-040

Imputation of microsatellite from dense SNP in the Valdostana Red Pied cattle – A Master thesis in Animal Production

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Microsatellite markers (MS) have been used efficiently for parentage verification in various livestock species and their impact on the industry to certify exact pedigree information has been massive for long time. In cattle, the International Society of Animal Genetics (ISAG) recommended a panel of 12 bovine MS markers for the individual parentage verification testing and a large MS database contains the historical data of populations. Recently, there is an increasing interest from the stakeholders in agriculture and the research community to use Single Nucleotide Polymorphism (SNP) for parental verification due to their higher genotyping accuracies, speed of genotyping, lower overall cost per genotype, and simplicity of automation. Thus, ISAG opened the parentage testing in cattle to SNP chips methodology. A tool to link the MS database to SNP markers tool have been developed in USA for main populations such as the Holstein and Brown Swiss cattle. The objective of the thesis is to develop a SNP-MS haplotype reference panel set in the Valdostana Red Pied cattle (the most common autochthonous dual purpose breed in the region Valle d'Aosta in Italy). The information on MS alleles recognized from ISAG are available from the National Association of Valdostana Breeders (A.N.A.Bo.Ra.Va.) and the genotypes obtained from the llumina BovineHD BeadChip (777,962 SNPs) array for 143 bulls are already accessible at UNIMI. Specific imputation software (*e.g.*: Beagle) and pipelines will be used for the haplotype estimation. This strategy may be employed in any species that has dense SNP genotypes and MS alleles information on a subset of the population large enough to define phase associations among MS alleles and SNP haplotypes. Moreover, this methodology will validate the parentage among individuals when different genotyping platforms have been used through the generations and will assess the sensitivity of such a conversion system using HD SNP data.

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P-041

Molecular tests for horse coat color determination

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Wild animals often have muted colors that allow them to blend into the background. During the domestication, people delight in selecting for color variants, so that domestic animals, including cats, dogs, cows, sheep, horses and goats exhibit a wide range of color patterns, even though they belong to the same species. The horse basic coat colors (chestnut, bay and black) are controlled by the interaction between two genes: Melanocortin-1-Receptor [MC1R; Extension (E,e)] and Agouti Signaling Protein [ASIP; Agouti (A,a)]. The Extension gene (red factor) controls the production of red and black pigment. Agouti controls the distribution of black pigment either to a point pattern (mane, tail, lower legs, ear rims) or uniformly over the body. Ten other genes may modify the distribution, production and quantity of these pigments and are responsible for the large variety of horse coat colors. Most of coat colors may be detected based on physical appearance or phenotype alone. The aim of this study is to develop molecular tests in order to define phenotypes that are visually ambiguous and identify the correct coat color phenotype for the four dilute phenotypes palomino, buckskin, cremello and perlino due to the effects of Membrane Associated Transporter Protein gene [MATP/SLC45A2; Cream (Cr,cr)] on basic colors. We demonstrate the effect of polymorphisms of MC1R, ASIP and MATP/SLC45A2 genes on 26 Akhal-Tekè horses bred in Italy with defined phenotypes bay, chestnut, black, buckskin, cremello and perlino. The 38.46% of horses tested, show a discrepancy between the phenotype reported on the certificate and the result of analyses carried out. On 11,54 % of the horses certificate is indicated any color. With the genetic analysis we can determine the correct genetic basis of the coat color and thus complete in a correct manner the certificate with the phenotype description.

P-042

Genetic diversity in three Italian donkey populations assessed by microsatellite markers

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We analysed the genetic variability of three Italian donkey populations, Ragusano (RAG), Grigio Siciliano (GSI) and Calabrese (CAL). Calabrese breed is mainly reared in the Natural Park of Aspromonte (Calabria) and represents a rare example of semi-feral autochthonous donkey. The genetic diversity of these populations has been investigated using 12 microsatellite loci approved for identification and parentage test by International Society for Animal Genetics (ISAG). We tested 100 unrelated animals: 40 Ragusano, 20 Grigio Siciliano and 40 Calabrese. Genomic DNA was amplified for 12 microsatellite loci (HTG10, VHL20, HTG7, HTG4, AHT5, AHT4, HMS3, HMS6, HMS7, HMS2, HTG6 and ASB17) in two multiplex PCR reactions. The PCR products were mixed with GeneScan 350 ROX internal size standard. Gel electrophoresis and genotype determination were performed on an 3500 Genetic Analyzer. We calculated: - the allele number and the allelic frequency; - the Polymorphism Information Content (PIC); - the heterozygosity; - the deviation from Hardy-Weinberg equilibrium; - the Inbreeding coefficients (FIS); - The Wright diversification (FST) according to Weir and Cockerham, using the computer packages GENEPOP and FSTAT. Data were analysed using different software (MICROSATELLITES ANALYSER, FSTAT and MICROSAT) to calculate basic population parameters, including genetic distances among breeds/populations. GENETIX and STRUCTURE software were used to evidence potential population structures among the Calabrese population and the other considered breeds. All microsatellites were polymorphic in each breed. The total number of alleles was 118. Considering the three tested donkey populations, allele number varied from 4 for AHT4 locus to 16 HTG7 and HTG5 loci; PIC varied from 0.809 for AHT5 to 0.174 for HTG6. The mean heterozygosity for each population was 0.436 (Ragusano), 0.545 (Grigio siciliano) and 0.503 (Calabrese). The inbreeding coefficient (FIS) was 0.0576±0.02, while the Wright diversification (FST) was 0.0862±0.02. Most loci did not show a significant deviation from HWE. We observed a considerable genetic variability even if the three populations are similar and show reciprocal influences, considering all tested parameters.

P-043

Effect of genetic polymorphisms at stearoyl-CoA desaturase and fatty acid synthase loci on subcutaneous adipose tissue of Casertana pig ancient autochthonous genetic type: preliminary results

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The fatty acid profile of animal products is affected by several exogenous (rearing techniques, etc.) and endogenous (genetic type, genotype, sex, etc.) factors. The fatty acid composition has always attracted considerable scientific interest for its effects on human health. The Fatty Acid Synthase (FASN) and Stearoyl-CoA Desaturase (SCD) loci have a key role in the synthesis of fatty acids: the FASN enzyme is involved in the synthesis of fatty acids with no more than 18 carbon atoms, while SCD is responsible for the conversion of saturated fatty acids in monounsaturated acids. The aim of this study is to investigate the effects of two SNPs (AY487830: g.641C >T in SCD and AY183428: g.265T >C in FASN) on fatty acid composition of subcutaneous adipose tissue of 36 Casertana pig AAGT. The lipid fraction was extracted from subcutaneous adipose tissue by Folch method (1957), and the fatty acids were analyzed by gas chromatography. DNA was extracted from blood and the two SNPs were typed by PCR-RFLP. The association between the two SNPs and the fatty acid composition was performed by analysis of variance (SPSS, 2001). In the model the genotype was included as a fixed factor and the net live weight and age at slaughter as covariates. The results of this preliminary study show that: 1. g.265T>C SNP in FASN may influence the content of linoleic, γ-linolenic and linolenic acid ($P<0.05$); TT and CT subjects, compared to CC have a higher content of linoleic, γ-linolenic and linolenic acid; this SNP shows also a suggestive level of association ($P<0.16$) with palmitic acid. 2. g.641C>T SNP in SCD has a weak association with the content of palmitic acid ($P=0.052$). The significant level of the association for oleic ($P=0.083$), eicosenoic ($P=0.10$), eicosadienoic ($P=0.08$) and eicosatrienoic ($P=0.10$) acid content is $P\leq0.10$: subjects with TT genotype have a higher content of palmitic acid than CC and a lower content of oleic, eicosenoic, eicosadienoic and eicosatrienoic acid than CC genotype. These obtained results, if verified on a larger sample, indicate that the genes FASN and SCD could be useful markers affecting subcutaneous fatty acid composition.

P-044

Towards molecular traceability of *Tinca Gobba Dorata del Pianalto di Poirino*

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The *Tinca Gobba Dorata del Pianalto di Poirino* (Golden humped tench of Poirino highland, PO), has long been farmed in Piedmont region, always playing an important role in the local economy. In 2008 the PO tench was registered as PDO product, but an effective system of identification does not exist yet, with the consequent risk of food fraud. Our previous sequencing data on ND1, ND6, cyt b and D-loop mtDNA segments in several tench populations (Lo Presti et al., 2014) detected one haplotype (H1e) possibly private of PO. It differed from the RefSeq (NC_008648) for two SNPs, in ND1 (3842 A>G) and ND6 (14210 G>A) segments. Since only one individual from PO was sequenced, the present study was carried out in order to verify in a larger sample whether H1e haplotype is really exclusive of PO population, hence useful in the framework of product traceability. A total of 276 samples of fin were collected from PO and other 9 populations located in different areas of Italy: North (Iseo and Valagola lakes), Centre (Bolsena, Bracciano and Trasimeno lakes) and Sicily (Alcantara and Prainito rivers, Cesarò and Santa Ninfa lakes). A PCR-RFLP assay was set up to analyse the SNP 3842 A>C as diagnostic for H1e. DNA was amplified using the primers For CGATTCCGATACGACCAACT and Rev TCTACTGCTCGTGGGTGATG, and the amplicons were digested with SfcI. The screening revealed that the H1e haplotype is present in PO with quite a high frequency (0.36) and absent in all the other examined populations. The data on the PO population were also examined separately by pond, showing that H1e was present in all ponds but two, and in same cases it was the predominant one. Therefore, the tench in Poirino highland might derive from a maternal lineage evolved independently from the original strain. These results further support the genetic diversity of PO tench, already demonstrated by the presence of another private haplotype, H2. Moreover, the Median-Joining network of the haplotypes found in Italian populations, constructed on the basis of the restriction sites, highlighted that H1e and H2 are the most divergent ones, confirming that the PO tench highly contributes to the species biodiversity. In conclusion, the exclusive mutations detected are worthy of note, underlying the genetic originality of PO, but they are only partly useful for traceability. This result suggests the need for further investigations aimed at detecting genetic labels able to univocally discriminate the PO tench.

P-045

Association study of *FASN, ACLY, ACACA, SCD, ELOVL6* genes with backfat fatty acid profile and intramuscular fat in Italian Large White pigs

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Fatty acid composition (FAC) of backfat tissue in pigs is an important trait in pig industry mainly for dry-cured ham production. In pig adipose tissue, variations in FAC contributes to qualitative characteristics of fresh meat and seasoned products. FAC is influenced by diet, genetics, breed, sex and environmental factors. Several genes affecting fatty acid composition were highlighted but the regulation of fatty acid (FA) metabolism and fat deposition is complex and not plenty known yet. For the current study we considered eight known SNPs of 5 candidate genes Acetyl-Coenzyme A Carboxylase Alpha (ACACA), Fatty Acid Synthase (FASN), ATP citrate lyase (ACLY), fatty acid elongase 6 (ELOVL6) and stearoyl-CoA desaturase (SCD) chosen for their functions in lipid metabolism and for the genome positions to perform an association study with FA composition in Italian Large White (ILW) breed. A sample of 536 ILW pigs was genotyped using PCR-RFLP or High Resolution Melting (HRM) PCR. FAC was determined by gas chromatography after after direct trans-esterification of subcutaneous lipids. The results allowed to determine that 37.58% of the total FA of backfat was represented by saturated FA (SFA), 43.70% by monounsaturated FA (MUFA) and 18.29% by polyunsaturated FA (PUFA). The content of individual SFA, MUFA and PUFA of the analyzed tissue was measured. The association between FA and the genotypes of the 5 candidate genes was assessed using a MIXED model and SAS software. Moreover, the coefficient of correlation between the analysed FA was calculated. Genotyping results revealed that the polymorphisms ACACA NM_198837:c.4899G>A, ACACA NM_198837:c.5196T>C, and ELOVL6 XM_003357048:c.533C>T were monomorphic in the analyzed samples. Significant association with FAC were obtained for the SNP FASN AY183428:g.265T>C with C14 (P=0.005), C18 (P=0.008), C20 (P=0.0006). The SNP SCD AY487830:g.2228T>C was associated to C18:1 (P=0.03). and MUFA (P=0.05). Moreover ACLY NM_001105302:c.2956T>C presented a significant effect on C20 (P=0.05) and C18:2 cis9,trans11(P=0.05). These results highlighted that the key genes for lipid metabolism considered in the study can regulate backfat FAC of ILW and the analysed polymorphisms could be markers useful to modulate the subcutaneous FA profile in pigs.

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P-046

Differential expression analysis for fertility in Italian Rep Pied cattle - A Master Thesis in Veterinary Biotechnology

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The decline in reproductive efficiency in dairy cattle has become a worldwide challenge to the dairy industry and scientific community. An efficient production is of great importance for farmers revenues and fertility in dairy cattle is a crucial aspect. Reversing this decline in dairy cow fertility, while simultaneously sustaining high yields, will be challenging. Fertility is a quantitative trait that is difficult to measure and little has been understood concerning the mechanisms underlying its genetic control. Quantitative Trait Loci (QTL) for fertility and functional genomics studies have revealed several genes that have a relationship with fertility traits; however many early embryonic failures are often related to poor oocyte quality due to the predominant role of maternal transcripts in early embryonic development. Gene expression in early stage embryos relies mostly on post-transcriptional control of maternal transcripts accumulated during oocyte maturation. The objective of the study is to compare gene expression in oocytes collected from fertile heifers (only one AI for pregnancy) and adult repeat breeders using RNAseq. A repeat breeder is a cow that is cycling normally, with no clinical abnormalities, but has failed to conceive after at least three successive insemination's, repeat breeding is often defined as a syndrome with many possible etiologies, including genetic or acquired defects of the oocyte. Oocytes were collected by OPU from 5 heifers of 11-15 months of age that became pregnant at the following estrus and from 4 adult cows with an age of 4 to 7 years, classified as repeat breeders after they failed to become pregnant for a minimum of 3 consecutive AI. Oocytes from each animal were pooled and sequenced as a single sample. A total of 9 pools were sequenced. Total RNA was extracted from pools by RNeasy Micro Kit (Qiagen). Amplified cDNA, from mRNA, was prepared starting from total RNA using the Ovation

RNA-Seq System V2 (Nugen). After library preparation using TruSeq DNA Sample Prep kit (Illumina), sequencing was performed on Illumina HiSeq2000 in the 50bp long single-read setup, at a 4-plex of multiplexing level, producing 30-40 million reads per sample. We will develop a differential expression analysis pipeline using a dedicated software through the iPlant collaborative web resource and the annotation data analysis will be performed with online open source tools.

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P-047

The founder signature: sequencing the genome of "Olimpo", the most important boar of the Italian Large White selection program

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Olimpo was a boar that is considered the founder of the Italian Large White breed. This boar was born in 1998 and since then its semen was extensively used in artificial insemination within this breed and in crossbreeding programs. Moreover, its thousands of descendants (boars and sows) further contributed to shape the genetic structure of this pig breed. The large use of Olimpo (and its descendants) was due to the favourable correlations between its estimated breeding values for meat production and meat quality traits, reversing their classical antagonisms. For these reasons, with the final objective to characterize the uniqueness of this pig and to track its contribution in the breed, we first genotyped this animal for polymorphisms in several candidate genes and for about 60,000 single nucleotide polymorphisms (Illumina Porcine BeadChip60K array). Then we linked genotype information with whole genome sequence data obtained by using a next generation semiconductor sequencing technology. A total of 130.77 million of reads (mean read length = 142 bp) were sequenced obtaining about 19.6 Gbp of sequenced DNA. Mean coverage was about 5X. Aligning these reads to the Sscrofa10.2 pig genome version we identified about 2.3 million of SNPs. Sequence and genotype data were used to impute and infer haplotypes and evaluate their frequency in the Italian Large White breed. These data will be used to design genomic selection programs in collaboration with the National Pig Breeders Association (ANAS), adding genome sequence information to classical SNP based genomic selection approaches.

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P-048

Identification of polymorphisms in the *GHR* gene and association with finishing weight in a commercial meat rabbit line

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Finishing weight is an easy-to-measure parameter that is usually used in rabbit breeding programs as a parameter of post-weaning growth rate. The identification of DNA markers associated with this trait or other growth rate related measures could open new possibilities to improve selection responses and efficiency in rabbit breeding programs. In this study we applied a candidate gene approach to identify markers associated with finishing weight in a meat rabbit commercial line. The selected candidate was the rabbit growth hormone receptor (*GHR*) gene. *GHR* mediates the action of the growth hormone and for this reason is a key component of the somatotropic axis that is well known to regulate growth in animals. Mutations in this gene have been already associated with several production and growth related traits in different livestock species. All 10 exons and part of intronic and flanking regions of the rabbit *GHR* gene were sequenced in a panel of 10 rabbits (six rabbits of the commercial meat line used in the association study, two Checkered Giant rabbits, one Champagne d'Argent rabbit and one Thuringian rabbit) by Sanger sequencing. Alignment of the obtained sequences identified 10 single nucleotide polymorphisms. A missense mutation identified in exon 3 of the gene was genotyped by PCR-RFLP in 298 rabbits of a paternal meat line. Association study between this mutation and finishing weight in the genotyped rabbits was carried out using the procedure MIXED of SAS, with a model that included the buck as a random effect and the fixed effects of sex, year, litter, and genotype. The analysed missense mutation was associated ($P<0.05$) with the investigated parameter. These results confirm that a candidate gene approach could successfully identify markers associated with production traits in rabbits opening new possibilities to design rabbit breeding programs including DNA marker information.

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P-049

Genetic variability of the Sardinian goat population assessed by the GoatSNP50 BeadChip

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The current Sardinian goats derive from a crossbreeding between the native population and the Maltese breed, made to improve milk production. This unplanned crossbreeding was performed with different intensities depending on the area. The aim of this work was to compare a breed assignment of Sardinian goats based on the morphological profile and on the breeding history of the whole herd, with the genetic variability at a medium density SNP chip. The herds were divided into native Sardinian (S), crossbred with Maltese (C) and Maltese (M), based on a phenotypic classification of the genetic type, assessed by the farmer and by a classifier specifically trained. The morphological traits considered were: type of ears, presence of horns and type of coat. The lop ears are the most distinctive trait for identifying the presence of Maltese crossbreeding in a herd, because native Sardinian goats usually have small upright ears. 15 animals were assigned to M group, 40 to S group and 41 to C group. DNA samples of these 96 animals were genotyped using the GoatSNP50 BeadChip devised by the International Goat Genome Consortium (IGGC). After standard quality check (SNP call rate > 0.95; Minor Allele Frequency < 0.01; individual genotype call rate < 0.95). Multidimensional scaling plot (MDS plot) and Reynold's genetic distances between groups were calculated using Plink and JMP®Genomics 6.0 software. The genetic distances of the three groups confirmed that the native Sardinian and the Maltese groups are the most distant, while the crossbred with Maltese is closer than Maltese to native Sardinian. The crossbred goats overlap to native Sardinian animals in MDS plot, suggesting that crossbred herds still contain a high portion of native Sardinian genome. These results show that this method of breed assignment can be used in conservation programs to identify native Sardinian herds. However, the high portion of native Sardinian genome observed in the crossbred herds, indicates that these population should also be considered to preserve the genetic variability of the Sardinian goat.

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P-050

mtDNA analysis for the characterisation of Sumerian equids

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Two equines teeth of Sumerian age were collected in the archaeological site of Abu Tbeirah with the aim of extracting and characterising nucleic acids. The site is located near Nasiriyah, 16 Km south-east from Ur, in Southern Iraq. In this locality, since 2012, an Iraqi-Italian Archaeological Mission excavated the Tell bringing to light a Sumerian city of about 42 ha, dated to 4500 B.P. The morphological characteristics of recovered teeth, as well as the postcranial skeleton, are deemed to belong to an asinine type rather than a wild ass or horse, whereby they should putatively be attributed to the domestic donkey. We would like to confirm these data and also test if there are evidences of hybrid form donkey-onager as hypothesized by cuneiform texts and archaeozoological metric data. In order to achieve the purpose, we performed a DNA extraction. We quantitated the extracted DNA of the two teeth of different samples and found respectively 2.5 and 4 ng/uL of DNA. A preliminary amplification of mitochondrial aDNA fragments using primers designed to obtain a polymorphic site able to discriminate between *E. asinus, E. caballus* and *E. hemionus onager*, was successful giving about 20 ng/uL amount of amplified DNA. Partial specific sequences of mitochondrial D-loop were obtained and we discuss the results achieved.

P-051

mtDNA analysis of medieval sheep (*Ovis aries*) in Central Italy and comparison with modern breeds

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The domestication of wild sheep occurred about 11,000 years ago in Southwestern Asia, as documented by archaeozoological and genetic evidence. Initially, sheep were used mainly for their meat: only about 4.000-5.000 years ago they were reared more intensively also for wool and milk. After the domestication, sheep have spread rapidly in the West and in the East, following human migrations. Particularly the Mediterranean Sea played the role of natural corridor between the Near East and North Africa and South West Europe especially for domestic sheep and goats easy to carry because of their small size and adaptable to various environments. This trade was present also in the Middle Age. We extracted DNA by 34 medieval ovicaprine remains, retrieved in tree archaeological sites of central Italy, quantified and analyzed it using mitochondrial DNA (mtDNA).We compared ancient samples with modern ones from Europe and Middle East in order to identify similarities between them and reconstruct migration routes. Our results suggest that the genetic variability of ancient sheep is comparable to modern one. A percentage of haplogroup A is found in the ancient samples. This haplogroup in Europe is rare and confined in countries belonging to the Mediterranean basin. We hypothesize that the B haplogroup in Central-North Europe originates through an ancient bottleneck of slow migration with founder effect of a few colonizers having only this particular haplogroup. Meanwhile, the presence of the A haplogroup in Mediterranean countries already established around year 1000 is due to fast migration from countries near the domestication centre (Mid-East), facilitated by sea routes.

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P-052

Microsatellite genotyping of medieval cattle from central Italy suggests an old origin of Chianina and Romagnola cattle

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The study of DNA from archaeological remains offers a valuable tool to interpret the history of ancient animal populations. The majority of studies conducted on ancient populations at molecular level mostly concerned mitochondrial DNA (mtDNA). However, mtDNA is maternally inherited and shows lower variability in comparison with highly polymorphic nuclear DNA as microsatellite. The objectives of this study are: identifying suitable microsatellites markers that can be used in ancient livestock remains; performing a comparative analysis between ancient cattle remains and extant breeds. Fifteen microsatellites markers with an allelic length shorter than 160bp, therefore suitable for degraded DNA, were selected from FAO list and amplified on medieval cattle bone collected from Ferento, an archaeological site in Central Italy. Allelic data from four successfully amplified markers, were compared with those obtained from extant cattle breeds. Genetic distances suggested a position intermediate between (1) Anatolian, Balkan, Sicilian and South-Italian cattle and (2) the Iberian, North-European and Central-European cattle, but also a clear relationship with two central-Italian breeds, Chianina and Romagnola. This suggests that the these breeds are derived from medieval cattle living in the same area. Our results indicate that microsatellite markers may be useful for the study of ancient livestock remains and could give information about the breeding purposes and livestock farming adopted in the ancient society.

P-053

Genomic selection of Marchigiana carcass traits

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The evaluation of the genetic merit of individuals for traits of economic importance based on pedigree and phenotypic records has resulted in extraordinary results especially in dairy traits. However, in beef the progress has been less pronounced particularly for some phenotypes as carcass traits that cannot be measured directly in live animals. The recent availability of high-density Single Nucleotide Polymorphisms (SNP) panels provides the opportunity to accurate estimate genomic breeding value of the animals at the time of birth also for carcass traits. Objective of

this work was to estimate Direct Genomic Values (DGV) for a sample of Marchigiana Bulls using medium density SNP panels. DNA samples were collected from 409 Marchigiana young bulls. Sampled bulls were genotyped at 54001 loci using medium density SNP chip (Illumina 54K BeadChip, www.illumina.com). Meat and carcass quality traits were recorded at slaughtering (bulls were between 16 and 24 months of age). Traits considered were: body weight (BW), average daily gain (ADG), carcass weight (CW), dressing percentage (D%), pH at slaughtering (pH), pH 24 hours after slaughtering (pH24h) and carcass conformation (CC) under SEUROP grid. A linear mixed model including the fixed effect of the herd, the age at slaughtering as covariate and the random effect of the sire was fitted. For each trait the residuals of this model were then used as new phenotypic values. Genomic BLUP were used to simultaneously estimate the effects of all SNP on meat traits in a set of training animals (350 bulls born before 2004). The remaining animals (the youngest 59 bulls) were then used to validate the genomic predictions evaluating the Pearson correlation between phenotypic values and DGV (rDGV). In the training bulls the rDGV ranged from 0.95 (D%) to 0.99 (ADG) as expected. Conversely, the rDGV spanned from 0.18 (D%) to 0.33 (CC) in the validation set of youngest bulls. The accuracy of DGV in the analyzed sample were substantially low probably due to the small size of the training population. In literature, the accuracies obtained using training population of similar size were comparable to those obtained in the present study. The use of genomic tools may speed up the achievement of the selective goals, in particular those more related with the commercial value of the animal product.

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P-054

A preliminary survey on distribution of runs of homozygosity in Italian Water buffalo

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Among DNA structural features that can be detected using tens of thousands of SNP genotypes generated by high throughput SNP platforms, stretches of DNA with a consecutive sequence of homozygous genotypes named Runs of Homozygosity (ROH) are of great interest in cattle for their relationship with inbreeding and selection history, and for estimating effects of inbreeding on production traits. The Buffalo genome is currently under sequencing by an international consortium, but a medium density (90K) SNP panel still exists based on the genomic map of Bos taurus. In this work, the distribution of ROH in a sample of 599 Italian water buffalo genotyped with the Affymetrix 90K bead-chip is presented. Markers with more than 1,000 missing data were discarded. No other edits were performed. Criteria for detecting ROH were: i) length ≥ 1 Mb; ii) number of SNP in a ROH ≥15; iii) distance between consecutive SNP in the same ROH ≤1 Mb; iv) neither heterozygous nor missing genotypes allowed in a ROH. According to their length, ROH were grouped into five classes (1-2Mb, 2-4Mb, 4-8Mb, 8-16Mb, 16Mb). The number of ROH (NROH), the average length of a ROH (LROH), and the total summation of ROH lenght genome wide (SROH) were calculated for each animal. Moreover, ROH based inbreeding coefficients were calculated as the ratio between SROH and the length of the genome; for this calculation, only ROH longer than 4Mb were considered. A total of 71,001 ROH were detected. Some of them were shared by different animals; for example a ROH of approximately 3.2 Mb was shared by 111 buffaloes. The average NROH was 215±15, higher than previous reports in cattle, and the average LROH of 21.6.±2.2 Mb. The distribution of ROH was 68%, 24%, 6%, 15 and 1% in the five length classes, respectively. Average Froh was 0.04±0.03. The peculiarities of buffalo ROH distribution that have been detected in this preliminary study, although affected by the fact that SNP position is still tied to the Bos taurus map, could be intepreted as a result of the the genetic history of this species. Comparison with other buffalo populations may offer further insights for evaluating possible effects of artificial selection and environmental adaptation.

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P-055

Application of human-based exome capture method to analyze the pig exome

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Discovery of a new protein-coding DNA variants related to carcass traits is important for the Italian pig industry pointed to POD productions. Selection schemes are focused to produce heavy pigs with higher amount of fat than pigs for meat production. Exome capture techniques offer the opportunity to focus on the regions of DNA potentially related to the gene and protein expression. To evaluate the performance of pig exome capture and to identify DNA variants, in this study was applied a human commercial target enrichment kit. DNA extraction was performed on two pools of 30 pigs, crosses of Italian Duroc X Large White (DU) and Commercial hybrid X Large White (HY). The NGS libraries were prepared following the protocol specifications of SureSelectXT Target Enrichment System for Illumina Paired-End Sequencing Library (Agilent) with the starting point of 3 µg of pooled DNA,. Sequencing was performed on Illumina's HiSeq 2000 at IGA Technology Services (Udine, Italy) using a paired read run of 100 bp. Thereby, 140.2 M and 162.5 M of raw reads for DU and HY respectively were generated. Mean coverage of all exonic regions for Sus scrofa (ENSEMBL Sus_scrofa.Sscrofa 10.2.73.gtf) was 89.33X for DU and 97.56X for HY, with the mean coverage of exons that contain only protein-coding sequences (CDS) – 129.97X for DU and 141.77X for HY. Thirty-five percent of aligned bases uniquely mapped to off-target regions, however, since the analogous exome enrichment (human-on-human) yields ~20% of off-bait bases, the efficiency of heterologous enrichment (human-on-pig) was considered satisfactory. Comparison of sequencing data with the reference Sscrofa10.2 assembly allowed us to identify several hundreds of thousands of putative DNA variants and, after applying some hard filtering criteria, a total of 232.530 single nucleotide variants (SNVs) of which 20.6% were exonic and 49.5% intronic, about 2.8 % of SNVs were located in 5' UTR, 2.7 % in 3' UTR and 0.3 % resulted as splicing variants. The remaining polymorphisms (24.1%) were mapped in intergenic regions. Totally 213 SNVs and the relative allele frequencies in the two pools were validated using the Sequenom MassARRAY® system. In conclusion, this approach offers new potentiality for the identification of DNA variants in protein coding genes which can be used for biodiversity studies and for the selection of phenotypic traits of relevance.

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P-056

An overview of Maltese breed of cattle through mitochondrial DNA

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Effective management of farm animal genetic resources is one of the most important concerns in modern livestock breeding. The strong economic emphasis focusing on a limited number of cattle breeds is leading to a dramatic and unrecoverable loss of diversity. Local breeds represent an important source of genetic variability that may also contribute to the selection of peculiar phenotypes. The Maltese cattle breed (vernacular Gendus) is considered an ancient breed that might be traced back to the prehistoric era, as it is suggested by pre-Neolithic temples carvings of bovines attributed to primitive societies. The conservation program started in the early nineties and nowadays Malta has a herd of about 20 animals. The objective of the present study is to explore the mitochondrial genetic diversity of this breed in order to reconstruct its maternal origins and develop appropriate tools and strategies to safeguard the current genetic diversity. A sequence analysis of the mtDNA control region (from np 15823 to 215 np) was performed on a total of 19 samples. Data analyses identified only two unique mtDNA haplotypes belonging to haplogroup T3. These haplotypes stand uniquely in the currently known cattle mtDNA control region gene pool, thus confirming the importance of monitoring geographically isolated genetic groups. Moreover, a comparison with the most important Mediterranean breeds and a parallel analysis of the Maltese population might be helpful in shedding light on the demographic history of the island.

P-057

Ethnological survey on autochthonous goat populations in Umbria

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In the most hard areas of the Umbria Appennini mountains two ancient goat populations named Grigia and Facciuta still survive. Their traditional management system was connected to the transumant migrations that interested also ancient sheep breeds such as Gentile di Puglia and Sopravissana. In order to define their morphological patterns (http://dspace.inea.it/bitstream/inea/744/2/Piano_biodiversita_animale_RRN_2_Alleg_Descrittori.pdf) on 73 Facciuta and on 19 Grigia heads from 9 flocks some biometrical measures were taken: wither heights, trunk length, chest girth, thorax width, thorax height, shoulders width, shin bone circumference. On the qualitative traits the frequencies were computed; on the quantitative characters the main inference parameters were estimated and a principal component analysis

was carried out (PROC PRINCOMP, SAS). Both the two populations can be assigned to the Mediterranean ethnological group. In the Grigia goat the size was defined as large and medium respectively in the 94% and 67% of the population; in Facciuta the size was large (61%), medium (35%) and small (4%). The coat in Grigia was always bicolor (mainly black and white), but in Facciuta was bicolor (brown and black) in 90% and brown in 10%. Only solid color was observed in Grigia, spotted coat was found in the 89% of Facciuta. The head profile was linear in 65% of Grigia and in 9% of Facciuta; the other animals had a convex profile. The horn and ear shape and direction were highly variable in both the breeds. Almost all the sampled goats had the beard, but wattles were present only in the 18% of the Grigia and in 42% of Facciuta. In Grigia the hairs were classified as long (76%) or medium (24%) and in Facciuta as long (79%), medium (1%), short (13%) and asymmetric (7%). The biometric measures were rather larger in Grigia than in Facciuta such as wither heights (70.18 *vs.* 68.87 cm), trunk length (71.56 *vs.* 70.76 cm), thorax height (30.79 *vs.* 29.25 cm), shin bone circumference (9.09 *vs.* 8.84 cm), but the thorax width was larger in Facciuta (16.41 *vs.* 15.44 cm). The more variable measures were in Grigia thorax width and height (CV=16.68 and 13.90), and in Facciuta thorax width and chest girth (CV=14.68 and 16.30). The principal component analysis didn't show any appreciable morphological separation between the two population.

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P-058

Identification of putative adaptive variability in the bovine genome using whole genome resequencing data of Cinisara cattle

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Local cattle populations have evolved their genome according to human directional selection and environmental pressures. Cinisara is a Sicilian autochthonous cattle breed reared mainly in Palermo province, in particular in the municipality of Cinisi from which its name comes from. This breed is included in the Podolic cattle group and is well adapted to marginal, harsh, wind-swept and high-summer temperature climates and challenging endemic disease-rich environments. Animals of this breed have an instinctive grazing ability on marginal sloped meadows with poor quality feeds. This breed is under conservation programs and its dairy products (in particular Caciocavallo cheese) are particularly appreciated for the richness in flavours. The characterization of the Cinisara genome might identify adaptive signatures providing useful information for more precise conservation and exploitation programs of this Sicilian animal genetic resource. In this study we sequenced the genome of eight Cinisara cows using the Illumina technology. A total of about 3.98 billion of reads were obtained, producing on the whole 402.7 and 299.1 Gbp of raw and trimmed sequences, respectively. Mean depth coverage of the UMD3.1 bovine reference genome was about 6.5X. Single Nucleotide Variations (SNVs) and indels were called only on four cattle with coverage >8X using Mpileup of SAMtools, setting stringent quality scores. A total of 656,075 variants were identified compared to the reference bovine genome (91.8% were SN*vs.* and 8.2% were indels). Homozygous short variants (337,252) were then analysed using the ENSEMBL Variant Effect Predictor tool. A total of 362,305 variant effects were identified, involving 15,977 distinct genes coding for 17,526 distinct transcripts. A total of 1,517 (0.45%) variants with 1,648 distinct effects were detected in 1,242 distinct genes coding for 1,362 distinct transcripts, annotated with the BAR+ (Bologna Annotation Resource platform) tool. A Protein-Protein-Interaction network with 1,121 nodes was obtained using STRING database. Gene enrichment analysis identified several processes and pathways that might characterize adaptive peculiarities of the Cinisara cattle breed.

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P-059

The Sicilian cattle breeds in a global context: genome-wide relationship with other worldwide cattle

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Genomic technologies, such as high-throughput genotyping based on Single Nucleotide Polymorphism (SNP) arrays, provide background information concerning genome structure in domestic animals. The aims of this study were to cluster animals, to explore the relationships among and within breeds, and to place the Sicilian breeds, Cinisara and Modicana, in a global context. The Illumina Bovine SNP50K v2 BeadChip genotyping data from 144 animals of Sicilian breeds and from other 1,543 animals belonging to 134 domesticated bovid breeds (DRYAD) were used. These breeds arose from 3 domesticated (sub)species: Bos javanicus, B. taurus indicus, and B. t. taurus. Principal Component Analysis (PCA) generated using PLINK and model-based clustering algorithm implemented in Admixture were performed. SNPs that did not satisfy the following quality criteria were discarded: SNP located on autosomes and common to all breeds; MAF ≥ 0.0005; SNP with genotyping rate ≥ 0.90. A total of 40,958 SNPs shared among all 136 breeds were analyzed. The Sicilian breeds showed outlier behavior for the values of PC2, with all individuals spread over the entire range of variability pertaining to the second component, therefore results of PCA were plotted using PC1 and PC3. The results showed the separation between B. t. taurus and B. t. indicus breeds and the divergences between African and Eurasian taurines. PC1 separated the individuals into several non overlapping clusters that corresponded with (sub)species and the geographical origin of each breeds. Using PC3, the Sicilian cattle breeds were closer to individuals of B. t. taurus from Europe that included some Italian cattle breeds, according to their geographic distribution, and to individuals of B. t. taurus from Asia. In fact, European cattle breeds were exported to Asia and admixed with Asian taurines. Ancestry models with 4 ancestral populations showed the 3 major groups of Asian indicine, Eurasian taurine, and African taurine; Sicilian cattle breeds formed a separate cluster, according to the findings based on the PCA using the first 2 components. These results reflected the differences between breeds resulting from separate domestic events, geographic dispersal and isolation, and breed formation.

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P-060

Italian autochthonous Grey Cows (BovGrAI) yet Podolian: factors affecting milk production and composition

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The study was carried out on 118 cows 'BovGrAI' machine milked and subjected to milk recording, according to the A4 method approved by the International Committee for Animal Recording (ICAR). The aim was to evaluate the effect of genetic polymorphism of 7 loci [αsl-casein (CSN1S), β-casein (CSN2), κ-casein (CSN3), α-lactalbumin (LALBA), β-lactoglobulin (LGB), Growth Hormon (GH) and prolactin (PRL)] as well as the effect of the order and the season of calving on milk production at different distance from calving. Data were analyzed by analysis of variance (ANOVA) and, only for milk chemical components by analysis of covariance (ANCOVA) using as covariate the distance from calving. DNA was analyzed by PCR-RFLP. The milk yield per cow (for each test day) was standardized (as a milk with 3.7% of fat, 3.5% of protein and 4.9% of lactose, according to Bettini's method,1972). ANOVA showed that milk production (evaluated at 30, 60, 90, 120, 150, 180, 210 and 240 days of lactation) was significantly influenced by: (a) genetic polymorphism of β-cn locus (BB genotype gives a higher milk yield than AB and AA genotype, $P<0.01$, $P<0.05$, respectively); (b) order of calving ($P<0.001$) multi-calving cow gave a higher milk yield than first-calving cow, as expected; (c) 'season of calving', in spring the milk production was significantly higher than other seasons ($P<0.001$). ANCOVA showed that: 1) a higher protein percentage characterizes the milk produced by cow with: CC (4.14) and BB genotype (4.10) respect to BC genotype (4.02) ($P<0.01$) at αs1-cn locus; BB genotype (4.14) respect to AA (4.02) ($P<0.01$) at k-cn locus; BB and AB genotype (4.11) respect to AA (4.04) ($P<0.05$) at β-lg locus; LV (4.17) and LL (4.16) genotype, respect to VV (3.94) ($P<0.001$) at GH locus; as well as a higher protein percentage characterizes the milk produced in spring respect to milk produced in winter (4.13 *vs.* 4.01; $P<0.05$); 2) a higher fat percentage characterizes the milk produced by cow with: BB genotype (4.36) respect to AB (4.18) ($P<0.05$) and AA genotype (3.90) ($P<0.01$) at k-cn locus; BB (4.27) and AB genotype (4.19) respect to AA (3.99) ($P<0.05$) at β-lg locus; AA genotype (4.22) respect to AB (4.08) ($P<0.05$) at PRL locus; LL (4.28) and LV genotype (4.26) respect to VV (3.91) ($P<0.01$) at GH locus.

P-061

Estimates of litter size traits in two local pig populations in the Mediterranean region

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Local pig populations such as Black Slavonian (BS) and Nero di Parma (NP) contribute to genetic diversity and represent a unique genetic material of the Mediterranean region. The objective of this study was to evaluate differences between the two genotypes in reproductive efficiency, as one of the most important factors for biological and economic sustainability of breeding. Data were collected from 1835 parities of 685 sows (264 BS and 421 NP). The following parameters were included in the analysis: total number of born (TNB), number of born alive (NBA), number of stillborn (NSB), number of weaned (NW) piglets. The effects included in the model were analyzed (GLM procedure in SAS), separately for each parity (from 1st to ≥4th) and for all parities taken together. The following fixed effects were tested: breed (BR), herd (HD), interaction breed*herd (BR*HD), year-month of farrowing (YMF) and parity order (PO); boar (BO) was added as a random effect. Primiparous BS *vs.* NP sows showed 5.9±1.9 vs.7.3±2.1 TNB and 5.6±2.1 *vs.* 6.9±2.3 NBA, with 0.25±0.96 *vs.* 0.38±1.20 NSB and 5.0±2.4 *vs.* 5.5±2.7 NW, respectively. BR, BO and YMF had a significant effect ($P<0.05$) on litter size traits. Higher values in litter size traits were recorded in 2nd parity sows, with 6.7±2.4 *vs.* 8.3±2.1 TNB, 6.3±2.4 *vs.* 7.9±2.2 NBA, 0.34±1.20 *vs.* 0.32±1.80 NSB and 6.1±2.4 *vs.* 6.3±2.6 NW for BS and NP, respectively. A significant effect ($P<0.05$) was found for BR, BO, BR*HD and YMF. No significant effects ($P>0.05$) were found in 3rd parity sows, with 7.1±2.1 *vs.* 8.1±2.1 TNB and 6.6±2.4 *vs.* 7.8±2.4 NBA, 0.40±1.13 *vs.* 0.29±1.10 NSB and 6.4±2.4 *vs.* 6.3±2.6 NW, for BS and NP, respectively. Fourth and >4th parity sows showed 7.4±2.4 *vs.* 8.3±2.4 TNB, 6.9±2.6 *vs.* 8.1±2.5 NBA, 0.53±1.31 *vs.* 0.31±0.91 NSB and 6.2±2.9 *vs.* 6.8±2.7 NW, for BS and NP, respectively, with a significant effect ($P<0.05$) of YMF and BO. The analysis of all parities together yielded significant effects ($P<0.05$) of BR, BO, BR*HD, YMF and PO. Reproductive parameters of the two local populations showed interesting results, probably related to both genetic and environmental effects. Future investigations of prolificacy are expected to establish genetic variation between the two local populations of pigs by use of the genetic markers that are involved in physiological process controlling reproduction. Knowing the variability between the two local populations of pigs will contribute to better preservation of local pig genetic types.

P-062

Phenotypic characterization of the Italian chicken breed Milanino

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In Italy, 90 local avian breeds were described, the majority (61%) were classified extinct and only 8.9% still present in rural farms. Therefore, efforts for conservation of Italian avian breeds are urgently required. Milanino is a chicken composite breed selected at the beginning of the 20th century in Lombardia region and was included in a conservation project run by the University of Milan since 2012. This study was aimed to characterize the phenotypic features of Milanino chickens in a small breeder population according to FAO guidelines for phenotypic characterization of animal genetic resources. Ten cockerels and 47 hens were kept in indoor floor pens with controlled environment at the Poultry Unit (University of Milan, Lodi) during the reproductive season (January-June) in 2014. The following phenotypic traits have been recorded at 36 weeks of age: colour of plumage, skin, eye, earlobe, tibia tarsus and comb type. Body weight, body length, wing span, tibia tarsus length and circumference, and chest circumference have been measured. Egg production and egg weight have been recorded daily. The birds had beautiful plumage plain white and soft, simple comb and orange eyes. The birds had white skin, white tibia tarsus and white or red earlobe. Phenotypic quantitative traits showed a great variability. Milanino breed is characterized by sexual dimorphism; males had heavier body weight and higher chest circumference compared to females. Mean body weight recorded in males was 3562 ± 358 g and in females was 2545 ± 373 g. The mean egg production was 3.24 eggs/female/week and mean egg weight was 58.9 ± 3.4 g. The results of this trial will be fundamental to include Milanino into "Registro anagrafico delle razze avicole autoctone", established by Ministero delle Politiche Agricole, Alimentari e Forestali in Italy on 2014 (MIPAAF, prot. 1st October 2014 n. 0019536).

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P-063

Genetic variability at the FASN locus in the Italian Mediterranean river buffalo (*Bubalus bubalis*, 2n=50)

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Fatty acid synthase (FASN) is a enzyme complex that plays a central role in *de novo* synthesis of long-chain satured fatty acids (SFA). The structural and functional characterization of the

FASN gene was reported in several species and genetic diversity has been found in cattle. For instance, the SNP g.763G>C has been associated with milk fat content in dairy cattle, whereas other SNPs have been associated with the FAs composition of adipose and milk fat. Despite these information in cattle, no studies have been carried out in a closely related ruminant, the river buffalo. In Italy this species is important in economic terms, being connected to the production of Mozzarella PDO. Although the buffalo genome project indicated the FASN sequence (EMBL ID AWWX01575345.1), this is not annotated and no data on genetic diversity are available so far. Therefore, the aim of this study was to explore the natural genetic variability existing at FASN. Genomic DNA was isolated from 17 individual blood samples collected in different farms of Campania region. A preliminary investigation was carried out by PCR on the region spanning the exons 6-9 by using the following primers (For: 5'-CTGACCAAGAAGTCCCTG-3' and Rev: 5'-AAGGAGTTGATGCCCACG-3') designed on the homologous bovine gene (EMBL ID AF285607.2). PCR products were analysed in 1.5% TBE agarose gel, purified and sequenced. Sequence analysis was performed by DNAsis software. A DNA fragment of 1345 bp including the exons 6-9 of the buffalo FASN gene was amplified and sequenced. The analysis of the sequences showed 7 SNPs. Five polymorphisms were found at intronic level, whereas 2 SNPs were exonic and both responsible of amino acid change. In particular, the SNP g.5976A>C at the 99th nt of the exon 6 is responsible for a Asn256>His replacement, whereas the SNP g.6100A>T at the 15th nt of the exon 7 is responsible for the change Thr265>Ser (numbering is relative to the homologous gene in cattle). These SNPs are the first polymorphisms reported at FASN locus for the Italian river buffalo. Considering the influence of the FASN on the FA composition and given that the average fat content in buffalo milk was 8.16% in 2013, the observed variability is interesting because it offers potential tools for the set up of future association studies with fat traits and for the application of marker assisted selection programmes to improve the productivity of this species.

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P-064

A high-resolution CNVR map in brown Swiss dairy cattle according to PIC and LD with SNPs

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Copy number variants (CNVs) are known to be a substantial source of genetic variation encouraging studies that measure their impact on phenotypes. The aim of this study was to create a high resolution map of CNV regions (CNVRs) in Brown Swiss cattle and the characterization of identified CNVs as markers for quantitative and population genetic studies. A total of 192 commercial semen samples were provided by the Associazione Nazionale Allevatori Razza Bruna Italiana (ANARB). Genomic DNA was extracted and genotyped with the Illumina BovineHD BeadChip and CNVs were called in a set of 164 sires with PennCNV and genoCN. PennCNV identified 2,377 CNVRs comprising 1,162 and 1,131 gain and loss events, respectively, and 84 regions of complex nature. GenoCN detected 41,519 CNVRs comprising 3,475 and 34,485 gain and loss events, respectively, and 3,559 regions of complex ones. Consensus calls between algorithms were summarized to CNVRs at the population level. GenoCN was also used to identify total allelic content in consensus CNVRs. Haplotypes of SNPs within consensus CNVRs and 10 adjacent SNPs each up and downstream of each CNVR was determined using polyHap software and to predict CNV allelic configurations. Moreover, population haplotype frequencies were calculated. Lastly, linkage disequilibrium (LD) was established between CNVs and SNPs in and around CNVRs. Polymorphic information content (PIC) was considerably higher in polymorphic CNVRs in comparison to normal state. Higher LD values were observed in gain CNVRs compared to normal state. In contrast, lower LD values were detected in loss CNVRs compared to normal state. Low LD was observed between SNPs inside CNVRs and underlying CNVs with neighboring CNVRs as well as for SNPs inside CNVRs and SNPs neighboring the CNVRs. In this study the potential contribution of CNVs as genetic markers for genome wide association studies (GWAS) has been assessed thanks to the PIC and LD values.

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P-065

Spermatozoal transcript profiles as estimated at thawing and after thermal stress in bulls of contrasting field fertility

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The *in vitro* assessments of sperm quality represent an important, but limited, approach to evaluate ejaculated fecundant capacity. To date, the most popular method worldwide for fertility estimation is related to non-return (NR) rate which makes it possible to estimate fertility in vivo only after the widespread use of a bull. This work is aimed at evaluating possible existing differences in trascriptomic profile of spermatozoa of equal motility in bulls of contrasting proved fertility in order to produce more insights into the very origin of hypofertility. Ten Holstein Friesian bulls were selected on the basis of their 56-day NR to estrus and assigned to two groups of low (ERCR < -1.5) and high (ERCR $> +1.5$) estimated relative conception rates (90% reliability). Motile fraction of the frozen/thawed sperm was selected by discontinuous Percoll gradients (t0) and an aliquot was incubated at 37°C for one hour and then subjected to Percoll (t1). The spermatozoal RNA was extracted and purified from t0 and t1 motile sperm, reverse transcribed to ds-cDNA and subjected at Illumina sequencing. Unigene expression was calculated using the reads per kb per million reads method (RPKM). The total spermatozoal transcripts (FPKM>0) were classified into biological processes, cellular components and molecular functions, and 6 genes were found to be differentially expressed ($P<0.05$) between high and low fertility bulls both at t0 and t1. At to, actin beta (ACTB), the mitochondrially encoded NADH dehydrogenase subunit 1 (ND1) and subunit 2 (ND2), phospholipase C epsilon 1 (PCLE1) and the tumor protein translationally-controlled (TPT1) were up-regulated while the mitochondrial voltage-dependent anion channel (VDAC2) was down-regulated in the high with respect to the low fertility groups. At t1 out of the same 6 genes PCLE1 was found to be still up-regulated whereas ACTB, ND1, ND2, VDAC2 and TPT1 were oppositely down-regulated in the high with respect to low fertility groups. These data provide a specific pattern of expression of these genes in motile spermatozoa of high and low fertility bulls at thawing and after incubation which conceptually reproduces the latent period of sperm in the female genital tracts after insemination. The analysis of gene expression would represent a new instrument of investigation that could potentially identify molecular markers for fertility to integrate conventional methodologies currently used to assess sperm fertilizing potential.

P-066

Analysis of polymorphisms of MC1R gene in Santa Inês sheep

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The Santa Inês breed sheep was naturalized in Brazil from the crossing of Morada Nova and Bergamacia breeds, presenting variations in coat color. This fact makes them an interesting model to evaluate the effects of polymorphisms in genes that affect this phenotypic trait. The MC1R gene is responsible for variations on this characteristic. The aim of this study was to describe the variation in three loci of gene MC1R aiming at correlation studies with phenotypes (coat color). We studied 121 Santa Inês breed sheep, multicolored and monocolored, raised in São Paulo state, north of Rio de Janeiro state and southern Espírito Santo state. The DNA was extracted from the hair follicle and collected from the final portion of the tail. The genotyping of three Single Nucleotide Polymorphisms (SNPs) was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) evaluated with restriction enzymes, (AciI, NlaIII and BmgBI) in the MC1R gene. The identification of alleles was made by capillary electrophoresis in advanCE FS96 equipment (Advanced Analytical). The allele frequency and heterozygosity rate were described from GENEPOP 3.4 and HARLEQUIN programs, respectively. The homozygous genotype for the allele without SNP (common) was 75% in Aci1-MC1R locus and 25% heterozygous, only one chromosome presented the SNP (mutant). The frequency of the mutant allele in this locus was 12.6%. The Nla1-MC1R locus demonstrated 37% of the common homozygous genotype, 23% of homozygous mutant genotype and 40% of the heterozygous genotype, with the frequency of 42.8% for the mutant allele. The Bmg3-MC1R locus showed 20% of the common homozygous genotype, 12% of homozygous mutant genotype and 68% of the heterozygous genotype, with the frequency of 45.8% for the mutant allele. It was possible to verify the presence of mutant alleles in three studied loci and it was suggested that there are associations in three loci of the gene MC1R with the different phenotypes of coat color.

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P-067

Genetic aspects of haematological parameters in Italian Large White pigs

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The determination of haematological parameters in livestock could become a routine practise, considering also the decreasing costs for these analyses. The possibility to use genetic associations between the individual genetic variability of these parameters and those of traits of direct economic interests could be useful to identify predictors of animal performances improving selection efficiency. In this study, we evaluated the genetic aspects of 33 haematological parameters in Italian Large White pigs. Blood samples were collected during slaughtering from 919 performance tested pigs (304 castrated males and 615 females), included in the sib testing program of the National Pig Breeders Association (ANAS). An extensive haematocrit and biochemical profile (including 15 and 18 parameters, respectively) of these samples was obtained using standard analytical procedures. Transformations were applied to normally distribute each variable. Pearson phenotypic correlation coefficients among all these parameters and the phenotypic measure of back fat thickness (BFT) obtained on the carcasses were calculated. Thirty-three bivariate mixed linear animal models, one for each haematological parameter, were used to estimate the genetic parameters for these traits and BFT. The fixed effects of sex (2 levels), the date of slaughtering (26 levels) and the second order covariate effect for the carcass weight were considered in the BLUP Animal Model. Variances were estimated with a REML algorithm using software VCE6. The pedigree file included 2371 pigs. Heritability values of the haematocrit parameters varied from 0.07±0.07 (eosinophils) to 0.54±0.09 (of both mean platelet volume and mean corpuscular volume) and for the biochemical parameters varied from 0.04±0.06 (glucose) to 0.93±0.13 (alkaline phosphatase), showing consistent genetic components. Finally, the genetic correlations with BFT ranged from -0.81±0.46 (eosinophils) to 0.55±0.13 (urea). Haematological parameters may represent important sources of information that could complement the classical production traits in pig selection programs. Other studies will be needed to evaluate if they could provide additional genetic information and if their inclusion as routine parameters in pig breeding programs could be economically sustainable.

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P-068

Use of microsatellite markers for genetic traceability of Girgentana dairy products

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Genetic traceability is based on the identification of both animals and their products through the study of DNA. With the goal of developing a genetic traceability system for dairy products, the aim of this study was to identify specific microsatellite markers able to discriminate among the most important Sicilian dairy goat breeds, in order to detect possible adulteration in Girgentana dairy products. A total of 20 microsatellite markers were analyzed on a total of 338 individual samples from Girgentana (GIR), Maltese (MAL) and Derivate di Siria (DdS) goat breeds. The first step was to identify breed specific microsatellite markers that can be used for the traceability of dairy products. Analysis of genetic diversity indexes showed that microsatellites panel was highly informative. Presence of private alleles was evidenced in each breed. We focused our attention mainly on alleles present at the same time in MAL and DdS and absent in GIR. Only eight microsatellite markers showed these alleles. To test these eight microsatellites, we first analyzed each of them on pools of DNA of single breed constituted by mixing an increasing number of individuals with known genotypes. From the visual analysis of electropherograms of DNA pools, it was possible to detect that only three of these eight markers (FCB20, SRCRSP5, and TGLA122) showed alleles useful for traceability purpose. In fact, these three microsatellites presented the smaller or the greater allele in MAL and DdS breeds and, therefore, it was not possible to confuse them with "stutter" (fragments smaller/greater than the real allele) when unknown alleles are present within the samples. We analyzed FCB20, SRCRSP5, and TGLA122 markers in DNA samples extracted from cheeses of GIR breed and we obtained very good amplifications. We compared electropherograms with those obtained from DNA pools prepared mixing DNA from GIR, MAL, and DdS in which private alleles of MAL and DdS were visible. From the analysis of electropherograms of DNA extracted from cheeses we did not detect specific alleles of these two breeds. To the best of our knowledge, this work was the first to extend the use of microsatellites for traceability purpose on dairy products. Considering our results, these microsatellite markers could be applied as potential tool in a genetic traceability system of GIR dairy products in order to detect adulteration due to MAL and DdS goat breeds.

P-069

Analysis of acetyl-CoA carboxylase gene polymorphisms in Valle del Belice dairy breeds

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Acetyl-CoA carboxylase is the flux-determining enzyme in the regulation of fatty acid synthesis within animal tissues. The expression of the mammary gland isoform of this enzyme, during lactation, is regulated by the acetyl-CoA carboxylase-α (ACACA) gene. The ovine ACACA gene, with 51 coding exons, is responsible for phenotypic variability observed in milk fat content and milk fatty acids (FAs) composition. However, before attempting association analyses between this enzyme and phenotypic traits of interest, a study on the genetic variability within this locus is required. The aim of this work was to sequence the encoding region of ACACA gene in Valle del Belice dairy ewes in order to identify polymorphic sites. Individual milk samples from 113 Valle del Belice ewes were collected. After fatty acid methyl esters (FAMEs) analysis of all samples, a selective genotyping approach was used. As criteria, the mean values of each FA±2 standard deviations were used to identify the highest and lowest phenotypic values. Two sets of 8 animals each were genotyped in a SNP discovery study of the ACACA gene. A preliminary analysis was performed with SAS using the PROC ALLELE procedures to estimate allele frequencies and Hardy-Weinberg Equilibrium (HWE) of identified polymorphic sites. The exact position of the SNPs was detected according to NCBI mRNA Reference Sequence (Acc. No NM_001009256). Sequencing analysis and alignment of the obtained sequences showed the presence of 14 polymorphic sites in the ACACA exons. In particular: C1441T in exon 11; 2 SNPs in exon 13 (T1783G and T1834C); T2347C in exon 17; A2575G in exon 19; 2 SNPs in exon 37 (C4534T and G4579A); and C5383T in exon 42. The most polymorphic was exon 53 which showed the presence of 6 SNPs (T6928C, C6991T, C6995T, G7005A, C7082G, and T7095C). All SNPs were in HWE. Analysis of SNPs frequencies showed that major allele was always at frequency >50% in all cases. The characterization of ACACA gene reported herein laid the basis for further association analyses needed to evaluate the potential use of these SNPs as genetic markers for fat content and FAs composition in sheep milk.

P-070

Combined principal component analysis and random forest approaches identified population informative SNPs in several Italian pig breeds

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Genetic variability among individuals from different populations or breeds is particularly relevant in many fields, including the investigation of evolutionary aspects and breed allocation with potential application in product authentication. Taking advantage from the availability of a high density single nucleotide polymorphism (SNP) genotyping platform in pig (Illumina PorcineSNP60 BeadChip array), we used this tool to identify a small subset of breed informative markers using an innovative statistical approach. On the whole, 2430 pigs (90 Apulo-Calabrese, 96 Casertana, 38 Cinta Senese, 144 Italian Duroc, 46 Italian Landrace, 1968 Italian Large White and 48 Nero Siciliano) were genotyped. A Principal Component Analysis (PCA) was carried out using chromosome by chromosome mapped SNPs, reducing the dimension of the dataset. Then the most informative SNPs based on the first PC were selected for subsequent analyses. A Random Forest (RF) approach was applied on the pre-selected SNPs, weighting analyses based on the breed-unbalanced sample size. The best 96 SNPs were selected using the Mean Decrease in the Gini Index and the Mean Accuracy Decrease that were evaluated using the Out Of Bag (OOB) statistics. Some of the selected SNPs were close to regions affecting production or other phenotypic traits that are different among the investigated breeds. This approach identified a set of markers useful for breed allocation that can be applied for authentication of mono-breed products in pigs.

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P-071

Estimation of genetic parameters of milk coagulation properties in Sarda dairy sheep

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Technological properties of milk are of great interest for the dairy sheep industry due to their influence on the cheese manufacturing. In particular, Milk Coagulation Properties (MCP) as rennet coagulation time (RCT, min), curd-firming time (k20, min), curd firmness (a30, mm) and individual cheese yield (ILCY) are currently evaluated as possible traits for selection programs. Aims of this study were to estimate heritabilities of MCP and ILCY and their genetic correlations with milk yield (MY) in Sarda dairy sheep. Individual milk samples were collected from 1018 Sarda ewes distributed on 48 herds located in Sardinia. Milk samples were provided by APA (Associazione Provinciale Allevatori, Sardinia, Italy) during the milk recording season 2014. RCT, a30, K20 were measured mechanically by using the Formagraph instrument (Foss Electric), ILCY were determined by individual cheese micro-manufacturing experiments. MY, RCT, k20, A30 and ILCY were analyzed by fitting both a single-trait and multiple trait animal model (ST_AM and MT_AM): the fixed effects of flock, parity, lambing month, days in milk (the last two included as covariate) and the random animal effect were included in the model. Pedigree file, provided by ASSONAPA, consisted in more than 1,8 million of records, however, we traced back to the last fourth generations from the investigated animals. A total of 5671 known ancestors were included in the numerator relationship matrix. Genetic parameters for MCP were obtained by REML estimation of (co)variance components using MTDFREML software. Estimates of heritability using a ST_AM for MY, RCT, a30, k20 and ILCY were 0.23 (±0.11), 0.31 (±0.02), 0.41 (±0.13), 0.28 (±0.12) and 0.27 (±0.12), respectively. Estimates of heritability using a MT_AM were 0.24, 0.15 and 0.21 for MY, CY and RCT, respectively. Genetic correlations were -0.88 (MY-ILCY), -0.43 (MY-RCT) and -0.05 (CY-RCT).

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P-072

Genome-wide association study for BRD susceptibility using a selective DNA pooling approach

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Bovine respiratory disease (BRD) is a major disease with a great economic impact on feedlot cattle industries, occurring in response to environmental exposure to a combination of viral and/or bacterial pathogens. The identification of the genes involved in the phenotypic variation for BRD resistance is the basis of the genomic selection for this disease. A selective DNA pooling (SDP) approach was applied to identify QTL for BRD susceptibility in Israeli Holstein male calves. The Kosher slaughtering was adopted, involving close and detailed examination of the lungs for adhesions. The control group included cattle presenting completely clear lungs, indicative of no BRD event, that were classified as “Glatt” kosher (GK). The case group included those presenting severe adhesions, indicative of BRD episodes in their life, which were classified as “non-kosher” (NK). Bovine DNA was extracted from whole blood samples collected in EDTA tubes using a commercial kit. DNA samples were quantified and diluted to 50 ng/uL for the pool constitution. DNA pools were constructed by taking equivalent amounts of DNA from each sample to obtain a final concentration of 200 ng/uL. Five GK and two NK pools were made of 21-31 calves each. Each pool was genotyped in duplicates using the Illumina BovineHD BeadChip according to the Infinium protocol. Statistical analyses were performed according to the SDP approach, and the proportion of false positive (PFP) method was used to obtain genome wise significance level. Annotation analysis of significant SNPs was carried out using online databases. A total of 142 significant SNPs distributed over 26 autosomes were associated to BRD susceptibility /resistance. Among the significant markers, some SNPs were annotated within or near (<1 Mb) genes involved in immune responses. This study brings evidence of significant associations between susceptibility/resistance to BRD and SNP markers on several chromosomes in known and newly disclosed QTL regions, which can be further characterized and used for genomic selection.

P-073

Bovine serum amyloid A3: analysis of the genomic region

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Serum Amyloid A belongs to a family of apolipoproteins considered one of the major vertebrate acute phase reactants. They are synthesized in response to infection, injury and inflammation. In mammals four genes, SAA1, SAA2, SAA3 and SAA4, have been described; in particular, in bovine mammary gland different products, proteins and mRNA, have been identified all deriving from SAA family genes, but the true identity of the involved genes is not yet clear. The bovine Hap Map Consortium stated that a duplication on BTA29 resulted in two genes, SAA3 and M-SAA3.2, which are located about 80 kb apart. They encode protein isoforms with 96% amino acid sequence identity. The aim of this study is to analyse the structure of the bovine SAA3 and M-SAA3.2 in order to distinguish their paralogous regions and to identify useful markers. To identify the position of the SAA3 and M-SAA3.2 coding sequences, the bovine genome assembly version Bos taurus UMD 3.1.1 was consulted, accessible through NCBI data base. First, the obtained sequences were aligned to highlight differences and to calculate degree of homology using MEGA6 and BLAST. The sequences of the two genes show some differences: M-SAA3.2 presents two insertions of 210 and 1303 bp. The first maps in intron 2, whereas the latter disrupts the sequence of the minimal promoter identified by Larson et al. (2006). To confirm the results of the in silico survey, we performed an *in vitro* analysis of the two genes using specific sets of primers. In a second step, a region of 20kb on BTA29, containing M-SAA3.2, was identified. Using the DesignStudio web-based tool (Illumina), a project was designed for resequencing this region with MiSeq NGS approach. The DNA of 95 bulls was extracted, amplified with the custom assay and sequenced. 446 SNPs were identified. The SNPs with minor allele frequency >0.05 were 144: 109 mapped upstream from the promoter, 8 into the promoter, 20 in the ORF region, and 7 downstream.

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P-074

Analysis of single nucleotide polymorphisms in Alpine ibex using the GoatSNP50 BeadChip

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The Gran Paradiso National Park is the oldest protected area in the Italian Alps and the Alpine ibex (*Capra ibex ibex*) is its symbol. This species, threatened with extinction at the end of the 19th century, survived only in the park. From this population, ibex was reintroduced into the European Alps. Single nucleotide polymorphisms (SNPs) are now the markers of choice for exploring genetic diversity in several species. Recently, SNP chips from livestock species have been used to identify SNPs in closely related wild species. In this study the goat 50K SNP chip was used to analyse Alpine ibex from Gran Paradiso National Park. DNA was extracted from blood samples of 8 animals sampled in the Park. Among all goat markers tested 45,966 were monomorphic and 1,329 polymorphic in the Alpine ibex. Minor Allele Frequency (MAF) distribution of polymorphic markers showed that the class that includes the highest number of markers (520) is the $0.4<\text{MAF}\leq 0.5$ class, and that overall more than 50% of markers (860) were very informative ($\text{MAF}\geq 0.2$). Based on their genomic position in domestic goat, polymorphic loci appear distributed on all autosomes and the X chromosome. A preliminary analysis of genetic distances with domestic goat breeds reared in Northern Italy indicates that Reynold's genetic distances between these breeds and the Alpine ibex ranged from 0.57 to 0.69, with the highest values for Orobica (0.69) and Valdostana (0.64). The high distance observed with Valdostana in our sample disagrees with the supposed introgression of Alpine Ibex in this goat breed, which is reared in the same area of the Gran Paradiso National Park. While waiting for the sequencing of C. ibex genome, this panel of 1,329 goat SNPs, is the richest panel of molecular markers nowadays available for the investigation of the genetic diversity of this wild species.

Acknowledgements

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P-075

Development of multiplex-PCR protocol to amplify 12S and 16S rRNA genes of mitochondrial DNA for traceability of Sicilian mono-species dairy products

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Species identification in dairy sector is important not only to safeguard public health but also to verify compliance with the Production Regulations of many typical dairy products (PDO/PGI). The most common fraud in dairy sector is represented by the mixture of milks from different species, resulting in mislabeling of protected designation of origin (PDO) products. For a rapid, specific and sensitive identification of cattle's, sheep's and goat's milk in mono-species Sicilian dairy products, species-specific multiplex-PCR protocol was developed. DNA from blood and experimental cheeses of Sicilian autochthonous breeds was used to amplify the 12S and 16S rRNA genes of the mitochondrial DNA. Modicana and Cinisara cattle breeds; Comisana, Pinzirita, and Valle del Belice sheep breeds; Girgentana, Maltese and Derivata di Siria goat breeds were the sampled autochthonous breeds. The use of species-specific primers for bovine, caprine and ovine species, allowed amplifying of fragments with different lengths, 256 bp, 326 bp, and, 172 bp, respectively. In the next step, amplification by multiplex-PCR of pools containing mixtures of DNA from two species at different percentage, showed the following sensitive thresholds: 0.1% for bovine/caprine and ovine/caprine DNA mixtures and 0.5% for bovine/ovine and ovine/bovine DNA mixtures. Finally, multiplex-PCR assay was applied to bovine/ovine and ovine/bovine experimental cheeses to detect the minimum thresholds for both species. The results showed the sensitive thresholds of 0.1% for bovine/ovine cheeses and 0.5% for ovine/bovine ones. The proposed assay represents a rapid and straightforward method for the detections of adulteration in Sicilian mono-species dairy products. Therefore, the ability to detect low levels of contaminating milk could be interesting to safeguard not only mono-species dairy products protected by European labels but also allergic or intolerant subjects.

P-076

Evidence of introgression of domesticated genes in several European wild boar populations

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Conservation programs of wild animal species that can breed with the domestic counterparts should monitor the level of introgression of domesticated genes into wild populations. One critical example in this field is the quite common hybridization between wild boars and domestic pigs that provides a directional flux of genetic material from domestic breeds into wild boar populations. This introgression has contributed to modify phenotypically wild boar populations. In this study we investigated this phenomenon analysing polymorphisms in two nuclear genes that are known to affect relevant and phenotypic traits that have been modified during the domestication process and that in the wild might have adaptive values: melanocortin 1 receptor (MC1R), affecting coat colour phenotypes; and nuclear receptor subfamily 6, group A, member 1 (NR6A1), affecting the number of vertebrae in the Sus scrofa species. A total of 232 wild boars from two different European regions, 146 specimens from Italy (26 from Sardinia and 120 from Emilia Romagna) and 86 from East European countries (15 from Serbia, 14 from Macedonia, 8 from Bosnia, 8 from Herzegovina, 16 from Montenegro, 10 from Croatia and 15 from Slovenia) were collected and genotyped for markers in these two genes. The most frequent allele of the MC1R gene was the wild type form in all populations. This allele is the original allele of the European wild boar populations. However, all other described domesticated alleles at this locus have been identified. For the other gene, the most frequent allele was that that is associated with a lower number of vertebrae. However, the domesticated allele (associated with a larger number of vertebrae) was observed in several populations even if at a lower frequency. Our results confirm the presence of introgression in European wild boars and the need of actions to prevent uncontrolled genetic flow from domesticated pigs into wild boars that might have important implications in modifying phenotypic traits of these populations.

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P-077

Identification of the causative mutation of the albinism determining the white coat colour of the Asinara donkey breed

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Asinara donkey (Asino dell'Asinara) breed is quite unique as it is characterized by a completely white coat colour that might be due to albinism. This breed is from the Asinara island (Sardinia, Italy) and is protected by the Asinara Island National Park in which about one hundred of these donkeys live. Apart from the coat colour, the other phenotypic features of this breed are similar to those of the Sardo donkey (Asino Sardo) population. In this study, we analysed the donkey tyrosinase (TYR) gene as a strong candidate for the albinism in the Asinara donkeys. TYR is a key melanocyte enzyme involved in the melanin production pathways. Disrupting mutations in the TYR gene cause different forms of albinism in many other mammals. A total of 40 donkeys (17 white Asinara donkey and 23 coloured donkeys of other breeds) were sampled and genomic DNA was extracted from hair roots. All five exons and parts of the intronic and flanking regions of the TYR gene were amplified from 10 donkeys (7 white Asinara and 3 coloured animals) using primers designed on the corresponding horse gene sequence retrieved from the EquCab2.0 genome version. Amplicons were sequenced using Sanger sequencing technique. A few missense mutations were identified in addition to several nucleotide differences between the two species, Equus asinus and Equus caballus. In particular, a missense mutation that occured in exon 1 was homozygous in all white donkeys whereas the alternative allele was always present in the coloured animals. The amino acid substitution involves a highly conserved amino acid position in all vertebrates that is in the binding site for the copper in the first catalytic domain (CuA) of the enzyme. A PCR-RFLP protocol was set up to genotype all donkeys sampled confirming the sequencing results: only white donkeys were homozygous for the identified missense mutation. These results confirm that the white coat colour of the Asinara donkeys is determined by a recesive albinism caused by a mutation in the TYR gene, opening new possibilities to establish conservation plans of the endangered Asinara donkey breed.

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P-078

Effect of β-lactoglobulin (LGB) and k-casein (CSN3) alleles on some characteristics of milk produced by Cinisara cows

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It is well known that β-lactoglobulin (LGB) and k-casein (CSN3) alleles affect milk cheese making properties. The purpose of this study was to analyse the A and B allele distribution at the LGB and CSN3 loci in Cinisara breed and their influence on milk and cheese making production traits. The Cinisara is a Sicilian breed which, according to DAD-IS database (FAO), consists of about 5,000 individuals and is reared mainly for milk production which is usually transformed into Caciocavallo Palermitano, a typical stretched-curd cheese. We typed 314 lactating cows at the LGB and CSN3 loci by means of RFLP-PCR analyses. The frequencies of LGB A and B alleles were 0.22 and 0.78, respectively; the frequencies of CSN3 A and B alleles were 0.46 and 0.54, respectively. Individual milk samples were analysed for total nitrogen (TN) and non-casein nitrogen (NCN) according to FIL-IDF standard procedure. The coagulation properties were measured using the Formagraph. Data were analysed using ANOVA procedure in which the fixed effect of CSN3 genotype or the fixed effect of LGB genotype was evaluate. The results of this study show that also in Cinisara the LGB A and B alleles are associated with significantly different effects on NCN (lower for BB genotype, $P<0.001$), and casein index (higher value for BB genotype, $P<0.0001$). As a consequence, the LGB alleles are associated also with significantly different effects on coagulation properties such as coagulation time (r) and curd firming time (k20) (lower value for BB genotype, $P<0.011$, $P<0.050$, respectively); while they are not associated with different effects on curd firmness (a30 and a2r). CSN3 A and B alleles are not associated with different effects on NCN, and casein index. On the contrary, they are associated with significantly different effects on coagulation properties: BB genotype shows lower value for coagulation time (r) ($P<0.0001$) and curd firming time (k20) ($P<0.0001$); higher value for curd firmness (a30) ($P<0.0001$) and curd firmness (a2r) ($P<0.0001$) than AA genotype. Our results confirm again that LGB and CSN3 genotypes affect cheese making properties and show that Cinisara cows, due to higher frequencies of alleles with positive effect on cheese making properties, produce a high quality milk for cheese processing.

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P-079

Variability of the IGF2 locus in the *Nero Lucano* pig population and its effect on quality and colourimetric characteristics of meat

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According to literature data, the IGF2 (Insulin-like Growth Factor 2) gene plays a role in regulating proliferation, differentiation and apoptosis of cells in many different tissues. Furthermore, polymorphisms located in intron 3 of this gene have been associated with meat quality traits such as intramuscular fat content, marbling and backfat thickness. The aim of this study was to analyze the genetic structure of two known IGF2 SNPs (-225C>G and -182T>C), located in the promoter region P2 of IGF2 gene, in the *Nero Lucano* pig and their effects on quality and colourimetric characteristics of meat. Till 10 years ago, the *Nero Lucano* pig was a breed in danger of extinction. Today, thanks to Basilicata Regional Institutions, such as ARA, Alsia, Comunità Montana del Medio Basento, and University of Basilicata, the *Nero Lucano* pig population shows an increasing consistency. We typed 153 pigs at IGF2 SNPs -225C>G and -182T>C by means of RFLP-PCR analyses using Eco0109I and DraIII restriction enzymes, respectively. The frequencies of -225 C and G alleles are identical to those of -182 T and C alleles, that is 0.13 and 0.87, respectively. According to the genotype distribution it appears that only two haplotypes are present in this population: -225G -182C, and -225C -182T. For each individual, the meat from two representative muscles (Longissimus dorsi, and Psoas) was collected. Colourimetric characteristics (CIELab lightness, L*; redness, a*; yellowness, b*; CIE, 1976), dry matter, total protein, fat content, ash (ASPA,1996) Fe heme (Hornsey 1956), oxymyoglobin (MbO2), deoxymyoglobin (deoxyMb), and metamyoglobin (metMb) content (Krzywicki 1979) where evaluated. Analysis of variance was performed on all the variables measured using the GLM procedure (SAS, 1996). The results of this study show that in *Nero Lucano* pig these two IGF2 haplotypes are associated with significantly different effects on chemical composition and colourimetric characteristics of meat. In particular, meat from pigs homozygous for the -225G -182C haplotype show higher values of fat, Fe heme, metMb, and lower values of protein, deoxyMb, MbO2, L* and b* parameters ($P<0.05$). No differences between the two haplotypes were observed for dry matter, ash and a* parameter. According to these results, the polymorphism at the IGF2 locus can be used for selection purposes in this autochtonous breed.

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P-080

Analysis of patterns of peaks in somatic cells count of Italian Holstein Friesian cows

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Mastitis is one of the major diseases in dairy herds. It induces economic costs for breeders mainly due to worsening of milk quality and increase of health care cost. Somatic cells count (SCC) are indicators of both resistance and susceptibility of cows to intramammary infections. Lactation-mean SCC or test-day SCC are generally used as mastitis indicators. Recently, other traits that are derived from SCC have been suggested as alternatives to improve genetic evaluations for mastitis resistance, such as maximum SCC, standard deviation of SCC, and patterns of SCC peaks. Aim of this research was to investigate patterns of peaks of SCC, for the definition of a new trait for breeding value estimation of udder health based on SCC in Italian Holstein Friesian cows. Patterns of SCC were used to distinguish lactations with short or longer periods of increased SCC, and also lactations with and without recovery from the increase in SCC. Data on SCC and production traits were extracted from the milk recording scheme (76,879,502 test-day records) of Italian Holstein Breeders Association (ANAFI). A subset was created with 9,041 Italian Holstein cows from 5,442 herds and they were edited to include records from the first 3 lactations and test-day records of SCC between 5 and 600 DIM. Healthy and recovered cows were assumed to have less than 200,000 somatic cells/ml, and cows with intramammary infections were assumed to have more than 400,000 cells/mL. Therefore, test-day records of SCC were categorised as low when the uncorrected SCC measured was < 200,000 cells/mL, and when the uncorrected SCC was > 400,000 cells/ml, the test-day record of SCC was categorised as high. An intermediate category was defined for SCC between 200,000 and 400,000 cells/mL. Preliminary analysis showed that a distribution of patterns of peak was 78.3% for the low SCC category, 13.7% for the intermediate SCC and 8% for the high SCC category. In particular the frequency of the low SCC category is larger in the first parity than in the other parities and the intermediate and high category were larger in the second and third parity. Within each parity the low SCC category was the largest while the high SCC was the smallest. In conclusion, the udder health can be improved through selection, and this information is partly already included in the PFT Italian index but, in the future, this could be changed with the use of the SCC patterns, or used next to the current index.

P-081

Canine hip dysplasia - Understanding the genetic basis to apply Genomic Selection – A Master Thesis in Veterinary Medicine

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Canine hip dysplasia (CHD) is the most prevalent orthopaedic disease in large breeds of dogs such as the German Shepherd Dog, Golden and Labrador Retriever, Bernese Mountain Dog. It is characterized by an abnormal formation of the hip joints leading different degrees of laxity and subluxation with subsequent formation of progressive osteoarthritis. Nowadays the CHD diagnosis is carried out by physical examination, including the evaluation of hip pain, and the degree of hip laxity with the Ortolani test in young dogs, while in mature dogs the range of motion and pain of hip joint are evaluated. Moreover, the X-rays are essential to confirm the diagnosis and to establish the severity of the disease. Hip scoring (classes from A to E, according to increasing severity in the FCI classification) is a procedure used to determine the degree of hip dysplasia in dogs and it is the sum of the points awarded for each of the radiographic features of hip joints. The results of several studies reported in literature highlight that selective breeding programs, based on genetic and genomic information, are valid alternative to reduce the incidence of CHD in dogs. CHD is a complex trait, which expression is influenced by genetic, nutritional, environmental and hormonal factors. Several quantitative trait loci (QTL) on different chromosomes have been found to contribute to this trait expression in various dog breeds, including Labrador retrievers. The aim of this Master Thesis in Veterinary Medicine is to target resequence the genome of a sample Labrador retrievers dogs in a case/control (dogs classified as A/D-E) study of CHD under a selective DNA pooling approach (SDP). Blood samples will be collected during the routine CHD diagnosis practices and genomic DNA will be extracted with a commercial kit. The chromosome regions that will be investigated will be chosen on the bases of those reported in literature and associated with CHD. The resulting sequencing data will help to find new genomic/genetic variations which may be involved in the disease occurrence.

P-081 bis

Effect of genes related to meat quality traits on progeny of selected bulls of Italian Limousine breed

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The aim of this study was to analyze polymorphisms in candidate genes for meat quality traits in the Italian Limousine beef breed. Chemical (proximate analysis and fatty acid profile) and physical (sample cut, colour, pH, cooking loss, shear force and free water) traits from steaks of 97 steers progeny of 9 selected bulls were recorded. DNA was extracted from the same animals and analysed at 10 SNP mutations in 7 different genes: myostatin (MSTN), dyacilglycerol-O-acyltransferase (DGAT), calpain (CAPN), calpastatin (CAST), leptin (LEP), thyroglobulin (TG), fatty acid binding protein (FABP). Allele and genotype frequencies, heterozygosity, and Hardy-Weinberg equilibrium for each SNP were estimated. Haplotype reconstruction through Phase software was also performed for SNPs located on the same chromosome. The association among SNPs or haplotypes and the recorded traits has been performed through a linear mixed model incorporating the effect of the pedigree with the pedigreemm package in R. Two SNPs at myostatin gene (F94L and Q204X) and one located in the thyroglobulin gene (C422T) were fixed whereas the other SNPs presented a MAF 0.05. Fixation of the first two SNPs might be either the result of the selection program developed so far or the bias due to the small sample size. No significant departures from Hardy-Weinberg equilibrium were identified for the genotypes, except for the CAST282 located in the calpastatin gene where a moderate excess of heterozygous genotypes was observed. Accounting for haplotypes did not change the association results in any of the traits analyzed. Two out of three SNPs of the CAPN gene (CAPN316 and CAPN530) and one at CAST gene (CAST282) were associated with meat tenderness, free water content and fatty acid profile. Thus the combined effect of favourable alleles for CAPN and CAST markers resulted in improved meat quality traits providing further evidence that selection based on these markers might be feasible. There are also evidence that SNPs at FABP, DGAT and CAST genes were associated with fat traits confirming the important role of these genes in lipid metabolism of intramuscular fat. No significant association between LEP gene and meat quality traits has been found. Due to the moderate size of the sample is not possible to draw final conclusions but the result suggests to deepen the studies at the final aim of incorporating markers information in the selection criterion of Italian Limousine breed.

pagepress

P-082

The investigation on Sakiz ewes (Chios) from different age groups in Aegean Region, Turkey

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The present study was carried out to analyze relationships among traits and the effect of body condition score on body weight and some body measurements of 109 Sakiz ewes raised at a private farm in Aegean Region, Turkey. The animals were enrolled in four age groups as Group 1 (06-12 months, n: 42), Group 2 (13-18 months, n: 18), Group 3 (19-24 months, n: 25) and Group 4 (25 months and above, n: 24). The animals involved in the present study composed of cattle grazing at 9.00 and 16.30 at pasturage. Potable water was available as *ad libitum*. Body Length (BL), Wither Height (WH), Chest Depth (CD), Chest Girth (CG) and Shinbone Circumference (SC) were considered as some body measurements of ewes in study. WH and CD were measured by a measuring cane. BL, CG and SC were measured by using a measuring tape. Individual body weights were determined by a weighing scale in wee hours of the morning before grazing. In this study, assessment of BCS of sheep was performed within five-rank system by Russel, 1984. Sheep were enrolled in 4 groups (2.0, 2.5, 3.0, 3.5≤) according to BCS. Overall means of body weight (BW) 37.02 kg, body length (BL) 85.01 cm, wither height (WH) 69.27 cm, chest depth (CD) 52.11 cm, chest girth (CG) 93.44 cm and Shinbone Circumference (SC) 9.16 cm were found for Sakiz ewes. The means of BW, BL, WH, CD, CG and SC were increased as BCS. The highest means of BW, BL, WH, CD, CG and SC were determined by the group with score of 3.5≤ and the lowest means were found by the group with score of 2. The effects of age on BW, BL, WH, CG and SC were found statistically significant ($P<0.01$), while the effect of age on CD was found non-significant ($P>0.05$). The effect of BCS on BW, BL, WH, CD, CG and SC were found significant ($P<0.01$). The positive and significant correlations were among WH, BL and BW (respectively, 0.659 and 0.741) ($P<0.01$). Besides, the correlations among CG and WH, SC and WH, CD and BL were deemed statistically significant ($P<0.01$). These correlations showed that BW could be estimated relatively rightly from WH and BL than other body measurements in Sakiz ewes. The results of this study showed that it should be improved necessary of techniques as BCS due to the important effects of BCS on body development and growth of ewes.

P-083

Research of relationships between some udder traits and milk yield of Maltiz x Saanen

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In this study, the relations between some udder traits and milk yield in Maltiz x Saanen crossbreed goat were researched. The material of study is constituted of 58 head Maltiz x Saanen crossbreed goat that same age and lactation number at raised at a private farm in Aydin. Udder depth (UD), Udder bottom height (UBH), Udder Upper Height (UUH), Udder Circumference (UC), Distance between Teats (DBT) was measured by a measuring cane. Teat Length (TL) and Teat Diameter (TD) were measured by caliper. The shape of udder was determined with method of Mavrogenis. The relations between milk yield and udder traits were investigated by Pearson Correlation Test. In study, lactation length (LL), daily milk yield (DMY) and lactation milk yield (LMY) were 233.6 days, 1.71 kg and 399.8 kg, respectively. The mean of udder depth (UD), udder bottom height (UBH), udder upper height (UUH), udder circumference (UC), distance between teats (DBT), teat length (TL), teat diameter (TD) were 12.69, 31.41, 45.26, 41.06, 11.47, 3.99 and 1.89 cm, respectively. The significant correlations were found between LMY-UUH and LMY-UC (0.270 and 0.261, respectively) ($P<0.05$). The significant correlations were found between UD-UBH (0.793) and UUH-UBH (0.813) ($P<0.01$). As the conclusion, investigation of genetic associations between udder traits and milk yield is necessary and udder traits will be more affect associated with genetic selection studies for milk yield in cross goat breeding in future.

P-084

Effect of simulated hail damage on yield and quality in forage maize

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The Po Valley experiences the greatest incidence of hail events in the world due to its particular terrain configuration and defoliation represents the main consequence of hail storm on maize. The effects of hail damage on maize largely focused on grain yield but not on maize for forage. In recent years, biogas production from maize silage has been rapidly increased. We aimed to verify the effects of hail damage simulated by artificial defoliation on yield, chemical and nutritional feature as well as on BMP of maize grown in the Po Valley. The trial was carried out in 2012 at the experimental farm Vittorio Tadini Podenzano, Piacenza, Italy according to a randomized block design with three repetitions. Four level of defoliation (0, 33, 66 and 100%) at three different grow stages (V12, R1,R3) were applied and plants were harvested when the undefoliated maize was at waxy stage (37.0 % DM). Chemical composition, aNDFom digestibility (aNDFD) and Biochemical Methane Potential (BMP) was measured on dried samples. The nutritive value was calculated using the Milk2006 spreadsheet. Increasing the degree of defoliation progressively reduced forage yield ($P<0.01$) up, whereas delaying the leaf removal reduced the impact on dry matter yield ($P<0.01$). Both time and severity of defoliation affected forage features, with the largest effects for total defoliation at V12 stage. In particular, leaf removal reduced dry matter (DM) content at harvest from 37.0 to 30.9 % ($P<0.01$), increased aNDFom (from 44.9 to 56.3 % DM; $P<0.01$) and reduced starch contents (from 23.9 to 14.1 % DM; $P<0.01$). The effects on aNDFD were more variable but total defoliation increased aNDFD by 6% *vs.* the undefoliated maize, in particular at V12 and R1 stages. Anticipating the defoliation decreased the nutritive value, that was also markedly decreased by total leaf removal ($P<0.01$). BMP was not affected by time of defoliation ($P>0.05$) but complete leaf removal reduced it by 6.3% ($P<0.01$) compared to the control. On the whole, maize defoliation affected most DM yield but also forage features. Further researches are necessary to better study the effects of stage of defoliation on maize grown under the Po Valley conditions.

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P-085

Effect of birth weight on growth of piglets of Sarda breed during suckling time

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Sarda breed pigs are raised on small farms and the most relevant product are suckling piglets, slaughtered at about 8-10 kilograms of body weight, used for the preparation of traditional dishes. Aim of this study was to investigate the effect of birth weight on growth performance of suckling piglets of purebred Sarda reared in smallholder farms. A total of 84 piglets from 10 litters were assigned to three classes of body weight at birth (Light (L), Intermediate (M), and Heavy (H)) Individual weight of piglets were recorded weekly until weaning and average daily gain (ADG) was calculated. Effects of birth weight litter size, sex and time on individual ADG were tested with an ANOVA model. The averages of BW at birth for L, M and H classes were 1142, 1392 and 1678 g, respectively. The ADG during suckling time was positively affected ($P<0.01$) by the birth weight (114, 122 and 134 g for L, M and H respectively). As expected, piglets from medium-small litters (6 to 8 pigs born alive) had an ADG higher than medium-large (9 to 12 piglets) ($P<0.01$). In all classes of birth weight the ADG decreased in the early weeks reaching the minimum in the fourth week. Then it showed a slight increase towards a plateau for the next four weeks. This result suggests that sow's milk wasn't able to meet the nutritional needs of potential growth in pre-weaning piglets. This nutritional deficiency seemed to disappear when piglets consumed creep feed. Further observations, perhaps with more animals, are needed to evaluate the nutritional requirements of piglets of Sarda breed during suckling time.

P-086

Environmental performances of the main Italian beef production systems

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Italian agriculture, included animal breeding, contributes for 7.0% to national greenhouse gases (GHG) emissions and also accounts for 95% of national ammonia emissions. Beef and dairy cattle production systems are considered the first contributor to the environmental impact of livestock sector mainly due to enteric fermentation emissions but also because of the lowest feed conversion rates compared to monogastric species. This study aimed to estimate, using an LCA approach, the environmental impact of the main Italian beef production systems that are: fattening of young bulls and cow-calf system. The former includes the French – Italian young bulls (FI-YB) and Piedmontese young bulls (P-YB); the latter, autochthonous young bulls (A-YB), autochthonous Piedmontese young bulls (AP-YB) and French young bulls (F-YB). A sample of twenty-two Italian representative beef specialized farms, were identified thanks to breeders associations and beef chain actors. Each beef production system was analyzed from cradle to the farm exit gate, including both calf-to-weanlings and finishing stage, farm inputs, animal transport. Kilogram of live weight (LW) has been adopted as functional unit (FU). Biophysical allocation has been applied to split impacts between the co-products of the target systems. Environmental performances are summarized in the following categories: carbon footprint (CF), acidification potential (AP), eutrophication potential (EP), energy use, land occupation. Calf-to-weanlings stage represents the major source of emissions independently from production type. FI-YB resulted to be the best environmentally-friendly together with the other intensive system (P-YB) due to the higher birth percentage per suckler cow and cattle for replacement rate too collected respectively in the French case study (91%, 21%) and in the Piedmontese one producing 5-months weaners (89%, 6%). An high variability was observed among production systems because of farm management, feeding system and land use. Results are in the range of those collected by other authors for the main French beef systems and Italia report about young bulls chain. (4) A beef production system based on specialized calf-to-weanlings plus finishing farm seems to be more sustainable than a cow-calf one.

P-087

The rearing of *Venerupis decussata* (Linnaeus, 1758) in a Sardinian coastal lagoon (Italy)

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The high rate of the grooved carpet shell clam Venerupis decussata (Linnaeus, 1758) consumption in Mediterranean countries has determined the over exploitation with a reduction of wild stocks. Recently, also in Sardinia, due to decline of the natural clam stocks, a great interest for farming this species has been increasing. The aim of the present work was to test the performance of V. decussata juveniles coming from a Sardinian bivalve pilot-scale hatchery in order to evaluate their growth rate under natural conditions. The experimental breeding was carried out in Tortolì coastal lagoon (eastern Sardinia ; Italy) from January 2014 to January 2015. Two groups of Venerupis decussata supplied by the hatchery were used: the group A, born in March 2013 and group B born in May 2013. In January 2014, clams of group A showing mean shell lengths of 22.22±1.99 mm and a total weight of 1.67±0.43 and individuals of group B having mean shell lengths of 15.68±2.14 mm and a total weight of 0.64±0.28 g, were sown on a natural substrate of Tortolì lagoon in two different areas of 100 m^2 each with a seeding density of 100 clams m^{-2}. Every two months samples of Venerupis decussata (30 specimens per group) were collected and their shell lengths and total weight were measured. Hydrological variables (temperature, salinity, pH, dissolved oxygen and chlorophyll a) were monitored by multiparameter probe. After one year, specimens of group A showed a mean shell length of 33.33±2.13 mm and a total weight of 5.98±0,50 g, while the values detected in the group B were 29.56±2.86 mm and 4.30±1.49 g, respectively. The increase in average monthly for shell length and total weight was of 0.93 mm and of 0.36 g in group A and of 1.16 mm and 0.31 g in group B. Both groups showed a growth peak during May and September 2014 and a stationary phase during July. The trial is still ongoing in order to reach the commercial mean length of 35 mm, according to the Sardinian law.

P-088

Life cycle assessment of dairy sheep production system in Appenino Lucano val d'Agri-Lagonegrese National Park

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Sheep milk production is a significant sector for the European Mediterranean area including the regions of southern Italy. The main objectives of this study were to investigate the major environmental impact categories generated from dairy sheep farming in the Appenino Lucano val d'Agri-Lagonegrese National Park by using a Life Cycle Assessment (LCA) approach. The environmental impact of eight dairy sheep farms within the national park was evaluated. In all farms the sheep were Italian Merinos breed. The functional unit (FU) was 1 kg of Fat and Protein Corrected Milk (FPCM) with a reference milk fat and protein content of 8.0 and 5.3%, respectively. Average herd size was 350 heads and the average FPCM per lactating sheep was 89.20 kg/year. The average farm size was 62 ha. In addition to milk, all farms produced meat and wool. Therefore, all inputs and outputs were partitioned between milk and the other co-products, on the basis of their economic value. The following internal farm activities were taken into account: energy consumption, manure management, transports, enteric fermentation, on-farm feed productions and management of sheds. Enteric methane emissions were quantified using the national emission factor (8 kg CH4/head/year). Impact categories investigated were global warming (GW), acidification (AC), eutrophication (EU) and non-renewable energy use (NRE). The LCA was carried out with the assistance of a commercial software package: SimaPro 8.01, and the EPD 1.04 (2008) module of this package was used in the evaluation of impacts categories. The average environmental impacts associated with 1 kg of FPCM were: GW 3.16 kg CO_2-eq, AC 39.5 g SO_2-eq, EU 10.4 g PO_4(-3)-eq and NRE 38.3 MJ-eq. For the GW, the process showing the highest impact was enteric fermentation (72.9%) followed by feeding management (22.9%)

with biogenic CH4 as the prevalent chemical compound. The environmental impact due to AC and EU was split into feeding management and emissions from manure inside the shed and direct on the pasture. For the AC and EC the main polluting compounds were NH_3 in air followed by N_2O in air and PO_4 in water. The component with the highest environmental impact in terms of NRE was crude oil followed by energy produced from natural gas. We could conclude that dairy sheep farming should be systematically monitored to minimize the impact of its activity on environmentally sensitive areas (*e.g.* national parks) without impairing its competitiveness.

P-089

Environmental impact of insect rearing for food and feed: state of the art and perspectives

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The demand for feed and animal food products is expected to rise sharply until 2050. In addition, livestock production plays a role in the overall anthropogenic impact. Thus, valuable protein alternatives such as edible insects are welcome. Edible insects have an excellent protein value, low feed conversion ratio (FCR) and can be massively reared on low-value substrates. Based on the available literature, this paper is aimed at reviewing the environmental performances of insect rearing and drawing the perspectives for future investigations. To date, few papers focused on the environmental impact of insects farming and used the Life Cycle Assessment (LCA) as standardized methodology. Impacts such as Global Warming Potential (GWP), energy use (EU) and land use (LU) have been estimated for the mealworm meal (MM) production. If compared to some animal food products, the GWP of MM ranges from about a half (pork) to less than a tenth (beef) (Tab. 1), mainly due to the weak climate-relevant emissions of this insect. In addition, the low LU of MM production sounds promising and may be partially explained by the low FCR (2.2) observed for this species. With the exception of beef and pork, the EU of MM is higher than the literature values for the other animal food products due to the rearing facility climate-conditioning. For feed production, housefly larvae grown on organic wastes (including poultry manure) have been studied. The data were obtained from a commercial-exploited testing site. If compared to literature data on fishmeal (FM), housefly larvae meal (HLM) exhibits a very low impact on climate changes, but is comparable to soybean meal (SBM) production (Table 1). Producing HLM requires less agricultural land than SBM as no dedicated crops are required. The energy demand of HLM production is more than twice the EU for SBM meal, but it is lower than for FM. Even if comparisons between these studies are not easy due to several methodological aspects, edible insects appear more sustainable than other animal products or feedstuffs. However, other environmental indicators (*e.g.*, acidification potential) should be investigated to get a complete understanding of the environmental performance of edible insects as food and feed. The different rearing strategies adopted have a significant effect on the sustainability of the mealworm and housefly rearing cycles. Studies comparing different insect species and/or different rearing strategies should be undertaken.

P-090

The effects of slaughter age and restricted feeding on growth, carcass and meat quality traits of dairy breed lambs

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This experiment aimed to investigate the possibility to raise the carcass weight of lambs of dairy breed and produce low-fat meat by increasing the slaughter age and applying strategies to reduce feeding level. At 35d of age, 70 weaned lambs of Valle del Belice breed were divided into 2 groups and adapted to housing in multiple boxes and experimental diets over a 10-d period. The groups received *ad libitum* pelleted alfalfa hay and concentrates differing in the 20% inclusion of durum wheat bran (0WB, 20WB) used to reduce cost and energy level. After 45d of experiment, both groups were divided into 3 subgroups; 2 of them with 15 lambs were slaughtered at 90d of age (90L), whereas the other 4 subgroups received the same concentrates *ad libitum* (120L) or restricted at 75% of *ad libitum* intake (120R) for 30d until slaughter at 120d of age. Feed intake and live weight of lambs were regularly measured. At slaughter, carcass traits and tissue components of hind leg were recorded. Longissimus dorsi (LD) meat was evaluated for pH, colour, thawing and cooking losses, WB shear force and sensory properties in triangle tests. In both phases, 45-90d and 90-120d of age, the diet did not influence feed intake and growth of lambs fed *ad libitum* (90L and 120L), whereas under feed restriction the lambs fed 20WB showed a reduction in weight gain than 20R lambs fed 0WB (105 *vs.* 170 g/d, P,â§0.05). In all production systems, the diet did not affect the carcass weight (12.6 *vs.* 12.4; 14.7 *vs.* 13.7; 15.6 *vs.* 14.9 kg for 0WB *vs.* 20WB in 90L, 120R and 120L), whereas the 20WB diet reduced carcass yield of 120R lambs (52 *vs.* 56 %, P,â§0.01). The simultaneous feed and energy restriction for 120R lambs fed 20WB diet resulted in the lowest performance. The fat deposition, as perirenal and pelvic fat and adipose tissue of hing leg, increased from 90L to 120R, and to 120L lambs, regardless of diet. The LD traits were affected by the production system, since 90L lambs showed higher water losses, and lower tenderness and red colour than older lambs. At triangle tests, the panellists perceived significantly the differences due to diet for 90L and 120R meat, and the effect of feeding level. Thus, increasing the slaughter age of dairy breed lambs from 90 to 120 d of age lead to the production of heavier carcasses with improvements in meat quality in terms of tenderness and reduced water losses, especially due to a higher but moderate fat content, also when lambs were exposed to a 75% restricted feeding.

P-091

Variation of casein and fatty acids milk contents in Italian Browns Swiss cows

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The interest in functional foods has considerably enlarged and the development of products with particular features for the food market has been encouraged.

The aim of this study was to evaluate the variations of protein, casein, saturated (SFA), unsaturated (UFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids contents in the milk of two groups of Italian Brown Swiss cows, either conventionally reared in indoor period of housing or consuming pasture during summer. Milk components were obtained from samples collected during the national routine (conventionally reared) and 'extraordinary' (mountain pasture period) milk recording scheme in 2008 and 2013 in herds located near Sondrio. Milk samples were processed with the MilkoScanTM FT6000 (Foss Electric, Hillerød, Denmark) for the identification of milk casein, SFA, UFA, MUFA and PUFA composition. The groups were analyzed separately per year and the environmental factors (pasture/indoor, parity, data of sampling, days in milk, days from collection to analysis) affecting milk protein, casein, and fatty acids contents were included in the GLM and MIXED procedures of SAS 9.3. A total of 778 milk samples were available, including 234 records from indoor and 544 observations from pasture feeding. Pasture intake affected the content of casein (%) and the proportion of fat in milk (g/100g) through its direct effect on energy and protein intake, enhancing milk casein levels (from 2.90 to 3) and reducing the concentration of milk SFA in milk from grazing cows (from 2.32 to 1.88). This study has confirmed in a field study conducted in different locations and time, that in a low input grazing system the total production exhibits a higher true casein content and a minor amount of SFA in milk: pasture intake affects the proportions of fat and casein fractions in milk through their direct effect on energy intake. Additionally, as milk was transformed in cheese, the cheese yield was calculated as 'kg of cheese per 100 kg of milk' resulting to be 10.4 from cows reared indoor resulted 10.4, while this value increased to 12 from milk of pasture based diet cows. The farmers and breeders producing Bitto, "Similar Bitto", and "Latteria" cheeses may disclose to consumer the better nutritional properties of pasture milk (i.e. less SFA) respect to indoor milk to raise awareness of the quality of marketed productions. Acknowledgments: This study was funded by Regione Lombardia projects n705 (BruCa) n. 1328 (LattOmega).

P-092

Food waste valorization: mango peels enhance *in vitro* rumen microbial fermentation

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Mango (*Mangifera indica* L., Anacardiaceae) by-products derived from fruit processing still contain bioactive phytochemicals that could be recovered to develop new functional feed additives or ingredient in ruminant nutrition. This research evaluated the effects of mango peels on rumen microbial fermentation (microbial growth, pH, NH3-N and volatile fatty acid concentration) by an *in vitro* rumen batch incubator system. Mango peels were tested as raw material and their water, ethanol and heptane extracts and fermented products, at three different concentrations. The *in vitro* batch culture incubation system was carried out with rumen fluid withdrawn from three rumen-fistulated not-lactating dairy cows. The rumen fluid was added in anaerobic condition to a mineral salt buffer, mixed in a bottle warmed at 39°C and standardized at pH 6.8±0.1. One hundred millilitres of solution were placed in glass bottles supplied whit a substrate for microbial growth (0.8 g/100 mL alfalfa hay and 0.2 g/100 ml corn meal). The bottles were purged with anaerobic grade N_2/CO_2 (80/20, v/v), closed, and incubated in a water shaking bath at 39°C for 24 h. Each test was evaluated in duplicate. The pH was determined at 0, 4, 9, 24h of incubation. At 0 and 9h of incubation the production of VFA and NH3-N concentration was determined. Total bacterial count (TBC) was evaluated at 0h and after 24h of incubation. Statistical analysis was performed using PROC MIXED of SAS. Mango peel increased total VFA concentration compared to the control ($P<0.001$). Mango peels water extract and mango peels raw material decreased the NH3-N concentration compared to the control ($P<0.05$ and $P<0.001$ respectively). Mango peels stimulated the rumen microbial activity, thus improving the efficiency of fermentation. Therefore, the present study highlights that the use of mango peels contribute to food waste minimization and its valorization as beneficial feed supplement can contribute to mitigate the environmental impact of livestock.

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P-093

Food waste valorization: the effectiveness of mango peels in mid-lactating dairy cows

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The by-products of industrial mango (*Mangifera indica* L., Anacardiaceae) processing may amount to 35–60% of the total fruit weight, and are still rich in biologically active compounds. Therefore, considering the results obtained from *in vitro* rumen trial, we investigated the effect of mango peels supplementation on mid-lactating dairy cows. Twenty-four multiparous Italian Friesian dairy cows were assigned to 300 g/day of mango peels or to a control diet. The treatment with mango peels lasted for 14 days. Body condition score, milk production, milk quality parameters, blood biochemical parameters, milk and plasma immunoglobulins IgG and IgG1 and serum reactive oxygen metabolites (ROM) were evaluated. Furthermore, the bacterial culture of quarter milk samples for mastitis pathogens (Staphylococcus aureus, Coagulase-negative-staphylococci, Environmental streptococci) was performed according to standards of the National mastitis Council (NMC, 1999). Statistical analysis was done using the MIXED procedure of SAS. Milk yield tended to improve in treated animals. Milk parameters were unaffected by treatment. The blood biochemical parameters were within the reference intervals of healthy cows, both in control and in treated animals, although mango peels supplementation decreased triglyceride level ($P<0.05$). Plasma and milk IgG and IgG1 were not affected by treatment. Regarding milk microbiological test, milk obtained from dairy cows treated with mango peels showed a lower Staphylococcus aureus mammary infection among the quarter samples. This effect observed in mango treated animals could be related to polyphenolic mangiferin. The present study highlights that the use of mango peels contribute to food waste minimization and to its valorization as beneficial feed supplement.

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This study was a prosecution of the SAFEWASTES project. The researches on mango peels were coordinated by Prof. Reinhold Carle (Universität Hohenheim, Stuttgart, Germany) which also provided mango peels.

P-094

Environmental assessment of different livestock systems in the peri-urban areas: a farm level approach

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The present work introduces a method for an environmental assessment at farm scale. A new diagnostic tool for farmers and policymakers that allows an easy evaluation based on a set of 18 indicators aggregated in 5 main sustainability sub-pillars: diversity, spaces management, farming practices, natural resources, energy (see Table). We sampled 30 livestock farms (cattle, swine, poultry) located within the South Milan Agricultural Park, a local institution that includes the city of Milan and the municipalities adjacent to it, and we collected their data by interviews and database. These areas are characterized by relevant fragmentation and important constraints imposed by the Nitrates Directive which often classifies them as Nitrate Vulnerable Zones. Therefore, the difficulties are definitely more, especially because here, more than elsewhere, local institutions impose stringent environmental and architectural regulations. Following these considerations, we created a method able to provide synthetic and easily interpretable indexes and to perform a ranking of their environmental sustainability, exploring the weaknesses and investigating new ways of management. It is able to highlight the peculiarities that distinguish the environmental impact of the strategic decisions of each farm, such as the production differentiation, the related activities (*e.g.* agritourism), the bioenergy production and the short supply chain. The output of the method consist of a radar diagram representing the performance of the farm. The results indicate predictable differences among farms of different type of livestock and production method (organic or not) which, however, are often not completely decisive for the achievement of high values of sustainability. In fact, other aspects, such as higher level of multifunctionality and greater farmland, were often key factors in reaching better performances. These results underline that the method is able to highlight the factors that affect the environmental management of the territory. These are often in agreement to the new EU regulations in terms of CAP (*e.g.* Greening and the RDP) and the European and local normative, still towards a greater involvement of farmers in the protection of the environment and the rural landscape. On the other hand, they follow the modern concepts of the peri-urban agriculture, increasingly linked to the multifunctional aspects associated with the social, environmental and productive use of the rural world.

P-095

Chemical and nutritional characterization of natural pasture in an Apulian Trojan oak wood and effects of Podolian cattle grazing on grass consumption

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The trial was carried out in a farm located in an Apulian Trojan oak wood in Mottola (TA). Three non grazed testing areas (A, B, C) of 10 ha each, representative of the local wooded pasture, were selected. In each area, 3 square (25 m^2) surfaces were randomly demarcated. The grass was cut in order to simulate animal grazing in each square surface and mixed altogether in a single pool which was promptly weighed and analyzed. An average production of grass of 1 kg/m^2 (25 kg/area), corresponding to a production of about 10 tons/ha, was found for the 3 areas. The chemical composition, fatty acid profile and the nutritional value of grass, estimated by IVGP, were assessed. The effect of animal grazing on fire prevention was evaluated by using a different stocking rate of adult Podolian cattle fed exclusively on pasture. 0.5, 1 and 2 animals/ha were respectively introduced in the areas A, B and C. The trial lasted from February to May 2014, with animals having free access to grazing on the testing areas. At the end of the trial, new 25 m^2 square samples were identified in order to cut and analyze grass residuals. Grass consumption in the 3 testing areas was inversely proportional to the cattle stocking rate, with estimated daily ingestion equal to 54.05, 51.35 and 45.64 kg/head/day, respectively recorded for an animal load of 0.5, 1 and 2 animals/ha. This consumption of grass is suitable for the feed intake of adult Podolian cattle. The gradual decrease of grass ingestion by animals following the increase of the stocking rate is presumably due to mechanisms of competition between animals that occur with a higher animal load. The grass showed a significantly ($P<0.01$) higher protein (19.84 *vs.* 10.30 %DM) and fat content (3.01 *vs.* 0.89 %DM) at the beginning of the trial in comparison to the end. In February grass showed better digestibility since significantly ($P<0.01$) lower crude fiber content (20.55 *vs.* 34.55 %DM) as well as lower NDF (52.75 *vs.* 71.33 %DM) and ADF (27.00 *vs.* 40.54 %DM) values were recorded. In May grass showed the highest amount of linoleic (C18:2ω6cis) and linolenic (C18:3ω3) acids that were significantly ($P<0.01$) higher in comparison with February. Exploitation of natural pasture in an Apulian Trojan oak wood contributes to the maintenance of plant and animal biodiversity. Identification of an adequate animal stocking rate may ensure their feeding requirements and potentially prevent fires in wood ecosystems by reducing dry and flammable vegetation.

P-096

Index of welfare, housing systems and hair cortisol level in dairy cows

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There is increasing interest in animal welfare topics and the question on how to measure it is still under debate. Dairy cattle are often challenged with stressful practices but housing conditions are among the most important. Determination of hypothalamus-pituitary-adrenal axis activity evaluates stress conditions in farm animals, however, blood cortisol concentrations vary due to several factors and the release of cortisol is generally irregular and difficult to interpret. The use of hair as a suitable biological matrix to assess chronic stress in animals has been recently proposed by many authors. To establish dairy cow welfare an index method, named TOS-BBL, was developed taking into account parameters mainly related to farm characteristics and housing systems; among these, free-stall and tie-stall housing represent a discriminant factor influencing animal welfare. The aim of this study was to verify the correspondence of the TOS-BBL index with the hair cortisol concentration (HCC) and the influence of the housing system on the same parameters in dairy cows. Eighty hair samples were carefully obtained using clippers from the tail switch of ten pluriparous dairy cows reared in eight farms located in Mugello area (Florence). HCC analysis was performed according to the method described elsewhere. Animal welfare level was performed by TOS-BBL index method that defines increasing welfare levels with a 1-5 scale. Spearman's correlation between TOS-BBL and HCC was calculated; HCC was related with housing system by ANOVA test; differences between welfare index and housing system was determined by Pearson Chi square test. Results showed a significant negative correlation between welfare index and HCC ($r=0.345$, $P<0.0017$) suggesting a correspondence between the two measures. HCC resulted influenced by the housing system ($P<0.0001$) with higher level of this stress indicator in tie-stall (2.76 pg/mg, SE 0.632) than in free-stall (1.63 pg/mg, SE 0.221). Mean welfare score was 1.7±0.48 and 4.2±0.40 respectively in the tie-stall and free-stall systems ($P<0.001$). However HCC resulted lower than those found in Italian-Friesian cows by other Authors. These first results indicate the correspondence between TOS-BBL index and HCC. In the inspected farms animals seem not affected by chronic stress, although free-stall system assure more suitable living condition.

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P-097

Mineral supplementation and bone characteristics in beef calves

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Mineral supplementation in animal feed show not only physiological, but also environmental and economic aspects that could suggest its reduction. Minerals, above all calcium and phosphorus, are stocked mainly in bones and as sources of such minerals are feeds, the effect of diet mineral supplementation on bones characteristics of 32 fattening bulls slaughtered at 18 (Y) or 24 (O) months of age was studied on two breeds with a different growing rate (Friesian, F; and Limousine, L). During the last rearing period (106-268 d), feed rations were designed to meet the needs of animals for an increase of 1 kg/d, by adding (HM) or not (LM) a supplement of dicalcium phosphate (1.5% on feed). Initial and final animal live weights, so as carcass weight, were recorded to calculate the productive indices (average daily gain, ADG; and carcass yield, CY), and at slaughter the left fore-shin of each animal was collected to study metacarpus characteristics (weight, length, middle circumference, wall thickness, weight:length ratio, middle cross areas – whole bone, medullary cavity, ring bone wall –, ashes content). Data were studied by ANOVA according to treatment, breed and slaughtering age. No negative effects of diet were observed on health and productive parameters: ADG was, as planned, around 1.00 kg/d in all groups, showing only interaction diets-breeds and diet-age ($P<0.000$), while CY was higher, as expected, in L than F (58.75 *vs.* 51.42 %, $P<0.000$) and in O than Y (56.97 *vs.* 53.85 %, $P<0.000$), showing interactions diets-breeds and diet-age ($P<0.05$). Metacarpus was heavier in O than Y (520 *vs.* 421 g, $P<0.01$), longer in F than L (246 *vs.* 223 mm, $P<0.01$) as well as in O than Y (242 *vs.* 224 mm, $P<0.05$), showing interaction breed-age ($P<0.05$), with a longer circumference in L than F (120 *vs.* 115 mm, $P<0.05$) and in O than Y (122 *vs.* 113 mm, $P<0.000$), and a thicker wall in O than Y (7.3 *vs.* 5.3 mm, $P<0.000$), showing interaction breed-age ($P<0.001$). The weight:length ratio was higher in O than Y (2.14 *vs.* 1.88, $P<0.01$). Bone area was larger in O than Y (1100 *vs.* 921 mm^2, $P<0.000$), so as wall area (823 *vs.* 768 mm^2, $P<0.000$), while no differences were found in the medullary cavity area (about 266 mm^2). Only the ashes content in bones was slight higher in HM than in LM (69.9 *vs.* 69.4% on DM, $P<0.01$). Therefore, it could be concluded that none real benefit due to mineral supplementation in the diet of the last rearing period of beef calves has been highlighted.

P-098

Effect of different rotational stocking management of Brachiaria brizantha on productivity of system

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Pasture management can affect plant growth and thus interfere with animal production. Marandu palisade grass (Brachiaria brizantha, Hochst. ex A. Rich.) swards subjected to year-round grazing by crossbred heifers were evaluated under the conditions of Central Brazil. The following three stocking systems were tested: 1) a grazing cycle of 28 days (T28d); 2) when the sward reached a pre-grazing height of 30 cm (T0.3m), and 3) when the sward reached a pre-grazing height of 30 cm under irrigation (T-irrig0.3m). The heifers were submitted to a rotational grazing system receiving only mineral supplement, except for the winter period when the animals from treatments T28d and T0.3m were supplemented with dry matter at 1% of body weight. The following variables were analyzed: heifer weight gain (WG), stocking rate, and productivity. The 2- or 3-day grazing cycles were evaluated using a completely randomized block split-plot design consisting of three repetitions per stocking system and four paddocks per system. There was an effect of the interaction between grazing system and season for WG, stocking rate, and productivity. During autumn, winter and spring, plant height was less than 30 cm for all treatments and never reached the 30-cm pre-grazing height target in T0.3m or T-irrig0.3m. There was an effect of stocking management (P=0.0055), season (P<0.001) and interaction (P<0.0001) on WG, stocking rate and productivity. The lowest WG was obtained in winter for the T-irrig0.3m treatment. The mean WG was 0.45, 0.49 and 0.43 kg day-1 for T28d, T0.3m and T-irrig0.3m, respectively. The stocking rate was higher in summer and autumn and productivity increased. The mean stocking rate was 4.7, 6.2 and 6.8 UA ha-1 for T28d, T0.3m and T-irrig0.3m, respectively. Lower productivity (P=0.0155) was observed for T28d when compared to T0.3m and T-irrig0.3m. The productivity rates were 662.2, 741.7 and 758.3 kg WG ha-1 per season for T28d, T0.3m and T-irrig0.3m, respectively. Stocking strategy affects heifer weight gain, stocking rates, and productivity. The management of palisade grass using a pre-grazing plant height of 30 cm was associated with a better stocking rate and productivity than a fixed cycle of 28 days for regrowth. However, the implementation of this management strategy is difficult because it requires different numbers of paddocks in each season.

P-099

Effect of feeding systems on growth performances and meat traits of suckling Messinese kids

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The aim of this study was to evaluate the effect of the feeding system of the dams to compare kid growth performances and meat quality in relation to different kinds of milk fed and live weight at slaughter. The study was carried out on 102 suckling Messinese kids, divided into two groups called: Group SE represented by fifty kids (30 males, 20 females) reared with their dams bred in a semi-extensive system, and Group E represented by fifty-two kids (39 males, 23 females) reared with their dams bred in an extensive system. Dams of the Group SE were fed exclusively spontaneous pasture; in the evening the dams were kept in the stable. Dams of the Group E were kept exclusively outdoors and their pasture areas were characterized by the presence of Quercus suber; therefore, the animals were fed also acorn *ad libitum*. Each kid was weighed every 10 days, from birth to weaning (average 50 days); at the slaughter, the weight of the hot carcass to determine the relative yield was calculated. Then, carcasses were chilled at 4°C for 24h. Meat quality measurements on the Longissimus dorsi muscle of each carcass were measured. Data were subjected to ANOVA. The trend of Body weight (initial body weight SE 3.22kg; E 3.57kg) showed better growth performance in the kids of Group E than that of Group SE, as testified by the final body weight (E 10.53kg *vs.* SE 9.40kg.; P<0.001). The yield at slaughter did not show any significant difference between the two groups (E 57.31% *vs.* SE 58.42%; P= 0.32). The final pH showed significant lower values in the muscle of the kids of the Group E (E 5.63 *vs.* SE 5.96; P<.001); this could be due to the different energy content of the breast milk, fed by the kids, thus determining a different accumulation of muscle glycogen and a relative glycolytic activity. As regards the color parameters, significant differences were observed for the Hue with lower values in the meat of Group E than that of the Group SE (E 46.24 *vs.* SE 62.64; P<0.005). The cooking loss (E 25.53% *vs.* SE 19.60%; P<0.05) and the tenderness (E 5.04 kg/cm^2 *vs.* SE 3.63 kg/cm^2; P<0.05) were significantly higher in the meat of the Group E that that of the Group SE. The chemical composition showed similar values between the groups. Results could represent a starting point, in a system of food chain traceability, to identify the relation between the typical feeding system of goats in the Nebrodi area and the performances growth and the meat traits of suckling kids.

P-100

Potentiality of five tanniferous plants for reducing methane production in the rumen

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It has been shown that tannins in trees and shrub foliage can reduce methane production in the rumen. So, the objective of the present study was to evaluate the potential of five species of native plants existing in the temperate climate region of Mexico to reduce methane production in the rumen. The *in vitro* gas production technique was used to evaluate Amaranthus spinosus L., Cosmos bipinnatus, Commelina coelestis Willd., Eupatorium glabratum kunth and Galinsoga parviflora cav.,Willd, at three levels (10, 20 and 30%) of substitution of ryegrass (Lolium perenne var L) from a basal forage diet. The experimental diet consisted of 64.6% ryegrass, 6.3% corn stover, 20.8% corn and 8.3% canola meal. The gas production was recorded at 1, 2, 3, 4, 5, 6, 7, 8, 12, 16, 20 and 24 h of incubation. After 24 hrs the incubations were stopped and a gas samples were taken to measure methane production (CH4). A complete randomized experimental design was used and the results were analyzed by analysis of variance. The results showed a significant decrease (P<0.05) in methane production with all plants, but specially with Amaranthus spinosus L. and Commelina coelestis Willd at levels of 20% and 30% respectively in comparison with the control diet. The digestibility of the NDF (DNDF) after 24 hrs of incubation was not affected (P>0.05) by the inclusion of these experimental plants. A highly significant interaction (P<0.01) between plant species and the replacement levels on methane production at 24 hrs post-incubation was observed. All plants reduced the methane production at level 2 (10% of inclusion), but at level 3 (20% of replacement) four plants (Amaranthus spinosus, Cosmos bipinnatus, Eupatorium glabratum and Galinsoga parviflora) kept CH4 production low, possible due to their tannins content. However, C. coelestis increases methane production at level 3, a response possibly associated to its high content of fibre. Results also shows that A. spinosus increased the CH4 production at level 4 (30%) possibly due to both, its high content of crude protein (18%) and because it replaced 30% of the grass in the diet, which has less protein. Results suggest that Amaranthus spinosus L. and Commelina coelestis Willd at levels of 20% and 30% respectively have potential to reduce methane emissions by cattle and should be evaluated *in vivo*.

P-101

Methane production in dairy goats fed flax or hemp seeds supplemented diets

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Aim of the study was to evaluate the effects of diets enriched with polyunsaturated fatty acids (PUFA) using flax (F) or hemp (H) seeds supplementation, in comparison with a control diet (C), on lactation performance, *in vivo* total-tract DM and NDF digestibility, and methane (CH4) emissions in lactating dairy goats. Twelve Alpine multiparous dairy goats were divided in 4 groups, each similar for milk production during early lactation; the goats of each group were then randomly assigned to the three dietary treatments after the lactation peak yield. In the supplemented diets, the flax or hemp seeds substituted the same amount (6.4% of DM) of a mix composed of corn meal (60%) and soybean meal (40%), in order to have isonitrogenous diets (CP: 12.2% on DM). The chemical composition (% on DM), in terms of ether extract (EE) and starch, was as follows, for C, F and H diet, respectively: 2.23, 4.78 and 4.08% of EE; 14.9, 13.0 and 12.8% of starch. n6/n3 ratio was 6.73, 0.66 and 4.83, for C, F and H. The goats, milked once daily, after an adaptation period of three weeks to the experimental diets and the metabolic cages, were housed inside open-circuit respiration chambers to allow the measurement of CH4 emission and the collection of faeces. The average value of DIM during data collection was 86. No differences among treatments were observed for DM intake (1.92 kg/d, on average) and diet DM (63.0%) and NDF (50.3%) digestibility. Milk production, in terms of yield (2.34 kg/d, on average), crude protein, lactose and urea, was not influenced by the seeds supplementation, while milk fat increased using the seeds (3.43, 4.26 and 4.23%, for C, F and H, respectively, P=0.007). CH4 emissions (39.9 g/d, on average) were not different among diets, nor if CH4 is expressed in relation to DM intake (20.7 g CH4/kg of DMI) or NDF intake (47.4 g CH4/kg of NDF intake) or in relation to the intake of digested NDF (94.3 g CH4/kg of digested NDF). PUFA supplementation using flax or hemp whole crude seeds, positively affects milk fat concentration, without interfering with the digestion of the fibre and DM. On the contrary, these seeds, at the concentration tested in the present trial, were not effective in reducing CH4 emissions, probably due to the lack of a negative effect on digestibility. The evaluation of bio-hydrogenation of the polyunsaturated fatty acids in the rumen is needed to provide a more comprehensive interpretation of the results. The research was funded by FilAgro.

P-102

Preliminary study on morphologic characteristics of the Pezzata Mochena goat breed

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The Pezzata Mochena goat breed is one of the forty-three autochthonous Italian local goat breeds with a Herdbook managed by ASSONAPA; the origin is from the Province of Trento (north-eastern Italy) and the Herdbook was established in 2005. Due to the lack of recent information about the morphological characteristics of the breed, a research was carried out to assess biometry, with the aim to evaluate if a selection on body measures took place in the last years. The database was represented by 60 Pezzata Mochena adult goats (53 females, 7 males) measured between 15 and 102 months of age (mean value 44 months), at eight farms from two Alpine valleys (Valle dei Mocheni and Altopiano di Pinè); the animals were individually measured once with a Lidtyn stick or with a metric tape. The measurements were taken for height at withers (HW), chest circumference (CC), cannon bone circumference (CBC) and body length (BL). The data were submitted to analysis of variance (fixed effects of sex, valley, herd nested within valley and, as a covariate, age at the measurement). The results showed that the sexual dimorphism was never significant (P>0.05) (HW: 69.4 and 71.8 cm; BL: 75.7 and 78.4 cm; CC 86.1 and 89.2 cm; CBC: 9.7 and 10.0 cm for females and males, respectively), while for all the variables, the age at measurement was always highly significant (P<0.001). Also the valley was not a significant factor (P>0.05) (HW: 71.0 and 70.3 cm; BL: 77.4 and 76.9 cm; CC 88.0 and 87.4 cm; CBC: 9.9 and 9.8 cm for Altopiano di Pinè and Valle dei Mocheni, respectively). The effect of the herd factor nested within valley was significant (P<0.05) for HW and BL and highly significant (P<0.001) for CC and CBC. The results of our study are in line with those reported in the literature for this breed, and show that i) the Pezzata Mochena can be defined as a large sized goat breed, with a good potential for meat production; ii) the breed has homogeneous body measures regardless of the sex and of the breeding area, iii) the breed has not been subjected to selection in the last years for body dimensions, because the need is actually to consolidate and expand the effective number of the population. The Pezzata Mochena goat represents an interesting opportunity to combine the need for a robust type of animal with the productive conditions of an extensive breeding in the mountain area of northern Italy.

P-103

Cortisol measurement in milk but not in hair is related to dairy cow health

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The aim of the trial was to assess the relationship between cortisol concentration (in milk - MC and hair - HC) and dairy cows health. Cows were sampled in 6 Italian Simmental (IS) and 4 Italian Holstein (IH) commercial farms, were clinical healthy and between 50 and 270 Days In Milk (DIM). Cows were housed in free stalls with cubicles and milking parlour, had free access to *ad libitum* total mixed ration (TMR) based on corn silage and their management was similar across farms. Formulated was offered twice a day, after the morning and the afternoon milking. The day of official milk recording, 100 ml of milk samples were collected from each cow at the morning milking and hair was sampled from the tail switch for cortisol analysis. Blood was sampled before the morning meal. Plasma samples were analyzed for Zn, total protein (TP), albumin, ceruloplasmin (CuCp), haptoglobin (Hp) and paraoxonase (PON): all indices related to the inflammatory response. For statistical analysis, animals were classified for parity with ordinal value of 1 for first calving, 2 for second calving and 3 for cows with more than 2 calvings. A K-mean cluster procedure was used to cluster the animals on the base of haematic parameters in 3 classes representing different animal health levels (C1 = better state; C2 = mild state; C3 = worse state). The effect of animal health class on cortisol concentrations was assessed with an univariate model including also the fixed effects of breed, parity, the interaction of class per breed and the linear relationship with DIM. Cows in C3 showed higher (P<0.01) values of MC than cows in C2 and C1. No significant effects were observed for the breed factor both in MC and HC, whilst a significant effect of parity was evidenced for HC concentrations, with higher values in cows with higher parity number (P=0.000). HC was significantly higher in C1 cows, even though a significant effect of the interaction breed x class was also found. These data suggest that milk cortisol is related to the acute phase response to stress and can be used as a biomarker of short term activation of Hypotalamus Pituitary Adrenal axis (HPA) and as an indicator of lactating cows health condition. Instead, HC is probably an indicator of long-term stimulation of HPA in relation to the environment. These results offer a new perspective for the definition of animal welfare by means of endocrine biomarkers.

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P-104

Who pays for animal welfare?

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Animal welfare standards prescribed by law are also statutory management requirements that must be respected by farmers who receive direct payments. Animal welfare is a value for the society as a whole, thus the relevant costs should be shared. Member States have the possibility to implement measures for improving animal welfare through EU rural development funding. For the period 2007-2013, measure 215 ”Animal Welfare Payments “ was included in Rural Development Programmes in 9 countries; in Italy, it was implemented only by 8 regional administrations. To apply for Measure 215, farmers must implement farming techniques that improve animal welfare more than the usual good animal husbandry practices (statutory requirements). Animal welfare at farm level and cross compliance were assed at two dairy cattle farms; structural and management actions to improve the animal welfare were identified and the costs of such actions were estimated; the economic impact of the potential support under measure 215 in the rural development plan was also simulated. At the time of assessment, no severe break of compliance was detected at the two farms; however, some weaknesses were identified and improvement of housing and management were proposed in order to maintain the current animal welfare status and avoid future failures. The profitability of investments was evaluated by calculating their net present value (*i.e.* of the present value of the sum of the future cash flows produced by the investment). Investments to improve animal welfare resulted partly self-funded in the mid and long term due to the higher milk yield and the better animal health that were expected; the table shows how costs change. However, in the short term, large part of expenses resulted fully born by farmers if not supported by a public grant or higher market prices. When potential income from measure 215 was considered, net present value resulted positive for both farms(21,700 € and 23,000 € over a period of 5 years) indicating that the future earnings from the investments exceed the cost of the investments and any further cost. In conclusion, measure 215 resulted effective in rewarding farmers who would adopt standards of animal husbandry which go beyond mandatory standards. Hopefully, in future, higher market prices will compensate costs for improving animal welfare; to achieve this, voluntary schemes effective in creating a consumer confidence must be set.

P-105

How much does it cost to improve comfort in dairy barns? A case of rubber flooring

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There is some evidence that covering concrete slatted floors of dairy cows barn with slatted rubber mats has positive effect on locomotion comfort. An economic analysis was done to evaluate the suitability of the investment when purchasing rubber for concrete slatted floors. The analysis was based on the results provided by two experimental trials comparing the use of rubber and concrete or two different type of rubber for flooring feeding area. Rubber floor, when compared with concrete, showed a positive effect on locomotion score and hoof health whereas the two rubber floors didn’t affect differently animal welfare; the two rubbers were equal for thickness and weight, but different for the anti-slip top surface, the structure of the lower surface, the original raw material (one was obtained from recycled material the other from virgin rubber) and price. The economic analysis took into account the costs of the investment and the effects on hoof health measured as results of the experimental trials; the analysis used a model validated and already adopted in previous research projects by CRPA. The model provides estimates of costs for: - depreciation for each type of rubber floor taken into consideration; -labour required for the operations for isolating, containing and caring for sick cows; -veterinary care, assessed in proportion to the number and severity of injuries detected; - drugs and health material used prescribed by the veterinarian to treat animals; - loss of revenue resulting from the decreased milk production due to health problems; - estimated worsening of the reproductive performance due to hoof health problems. In conclusion, the economic analysis estimated that, under conditions in which the experiment took place (revenues and expenses for the years 2012 and 2013), for a stable of 100 cows, the benefit obtained with the use of rubber is about 1800 €/year in the case of production of fresh milk and about 2000 €/year in the case of milk for processing into Grana Padano and *Parmigiano Reggiano*.

P-106

Loss in milk yield and related composition changes resulting from different levels of somatic cell count in Valle del Belice dairy sheep

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Mastitis is the most common and costly disease in the dairy industry and is responsible for several production effects. Mammary infections cause high somatic cell count (SCC) and severe damage to udder tissue and result in important losses of milk yield and quality, curd and cheese yields in dairy ewes. The aim of this study was to determine the effect of different levels of SCC on milk yield and composition in Valle del Belice dairy sheep. The original data set used for this study included 92,261 records of 6,763 ewes. Data were collected between 1994 and 2006 in 15 flocks. Test-day records of milk yield (MY), fat % (F%), protein % (P%), and SCC were collected following an A4 recording scheme. Records with incomplete information were deleted. Moreover, records with less than three test-day measurements within lactation for milk traits were discarded. After editing, the dataset consisted of 17,060 observations on 2,418 ewes. To evaluate loss in test day MY, F% and P%, five different classes of SCC were arbitrarily defined (1: SCC ≤500,000; 2: 500,000< SCC ≤ 1,000,000; 3: 1,000,000< SCC ≤1,500,000; 4: 1,500,000< SCC ≤ 2,000,000; 5: SCC >2,000,000). A linear model as factorial design of fixed effects was used with the GLM procedure of S.A.S. statistical package. The following model was used: y= FK+OP+AOP+STG+SCC+DIM+e, where y is the response variable measured (MY, F% or P%), FK is the effect of flock (15 levels), OP is the effect of parity class (4 levels), the covariate AOP is the age of lambing, STG is the season of lambing (3 levels), SCC is the fixed effect of somatic cells count class (5 levels), DIM is the class of days in milk (5 levels) and e is the random error. All fixed effects included in the model were significant (P<0.001). The estimated losses in MY according to the level of SCC used were approximately 20%, (903g for SCC ≤500,000 and 726g for SCC > 2,000,000). However we had an increase of 4.9% and 2.7% on F% and P% respectively. In particular for F%: 5.75% for SCC ≤500,000 and 6.05% for SCC >2,000,000; for P%: 7.43% for SCC ≤500,000 and 7.64% for SCC >2,000,000. Results suggest the need for the establishment of prevention and control programs of mastitis based on SCC routine monitoring in dairy sheep herds. These values could be suitable for use as basic input in calculations of economic losses due to mastitis.

P-107

Modelling the spatial distribution of Theileria parva (Theiler 1904), causative agent of East Coast Fever disease in cattle

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Theileria parva is a protozoan emo-parasite, which affects Bos taurus and Bos indicus cattle populations causing East Coast Fever disease, one of the most relevant cattle plagues in sub-Saharan Africa causing the death of $\sim 1.1\times10^6$ animals per year and an annual loss of $\sim 168\times10^6$ USD. T. parva occurrence on the territory is bound to three conditions: i) the presence of susceptible bovine host populations; ii) the presence of its main tick vector Rhipicephalus appendiculatus; iii) suitable ecological conditions for the survival of both the vector and the parasite in all their developmental stages. While the environmental drivers affecting the vector occurrence have been extensively investigated, studies focusing solely on the conditions determining the presence of the parasite are still lacking. The present study aims therefore at investigating the ecological conditions needed to maintain the parasite-vector-host biological system. In the course of the EU-funded project Nextgen, 590 cattle blood samples from 204 georeferenced locations covering the whole Ugandan country have been tested for the presence/absence of T. parva DNA. The values of 19 bioclimatic variables and topographic data (altitude, aspect and slope) for each sampling site were derived from WorldClim (Global Climate Data) and Shuttle Radar Topography Mission (SRTM) databases. A classification tree model approach was used to test bioclimatic and topographic variables together with geographical coordinates. This analysis revealed latitude as the main geographical driver for T. parva occurrence in Uganda, with potential interactions among temperature seasonality, temperature annual range and precipitations of the wettest month in the southern regions (latitude≤−0.15). For central-northern regions, instead, mean diurnal range, territory aspect and slope were the variables influencing most the presence of the parasite. This preliminary work represents a first step for the development of a full probabilistic model for T. parva occurrence in sub-Saharan Africa.

P-108

Influence of mycorrhizal fungi on sorghum and maize crops: preliminary results

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Aim of this study was to evaluate the effects of vesicular-arbuscular mycorrhizal fungi Glomus spp. on sorghum and maize crops. The maize-trial site was an irrigated buffalo farm in the Volturno plain (10 m above sea level), the sorghum-trial site was a dryland dairy farm in the Fortore area (680 m above sea level). Seeds of Zea mais (cv Indaco class FAO 600, Limagrain®) and Sorghum sudanese (cv Hermes, Hi-Gest technology, Alforex®) were inoculated with a commercial product (Aegis, Italpollina ®) based on Glomus intraradices (700 spores g^{-1}) and Glomus mossae (700 spores g^{-1}) at the dose of 20 g inoculum/kg seed. The experimental thesis were the following: MC (not inoculated maize seeds, fertilization: N 240 kg ha^{-1}, P 120 kg ha^{-1}1), MM (inoculated maize seeds, fertilization: N 120 kg ha^{-1}), SC (not inoculated sorghum seeds, fertilization: N 120 kg ha^{-1}, P 90 kg ha^{-1}), SM (inoculated sorghum seeds, fertilization: N 120 kg ha^{-1}). For each crop, inoculated and not inoculated seeds were sown in two contiguous areas of about 3 ha. Percentages of colonization were measured 30 d after seeding using the grid-line intersect method. Yield components were estimated on 4 lines 10 m long per treatment. Additional 4 samples, consisting of 3 representative plants each, were collected in order to evaluate plant traits and, only for maize, leaf area. Data of each crop were analysed using ANOVA with treatment as factor. Percentage of infection was always greater in mycorrhizal roots. Compared to MC, mycorrhizal maize showed significant higher values or a tendency toward higher values for almost all variables examined. Similar results were observed for mycorrhizal sorghum (SM). Biomass and DM yield were improved in both mycorrhizal cultures. Results showed that mycorrhizal treatment improved the maize and sorghum crops in presence of reduced P/N fertilization.

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P-109

Meat texture of Piemontese young bulls measured by two physical methods

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Warner-Bratzler (WBsf) and Texture Profile Analysis (TPA) are the most widely used mechanical methods to measure meat texture. The WBsf measures the maximum force (PF) required to shear round cores obtained from cooked meat. The TPA, based on the imitation of the chewing process and performed with a double-compression cycle on raw meat, gives a wide range of texture properties, such as hardness, cohesiveness, gumminess, springiness, adhesiveness and chewiness. To obtain more information from WBsf and TPA, some modifications to the original protocols were suggested: 1) to perform WBsf on raw meat strips in order to identify the initial yield (IY), as a measure of the strength of the myofibrillar structure and to calculate the difference between PF and IY (PF-IY) as an index of connective tissue toughness; 2) to perform TPA using a modified square compression cell that avoids transversal elongation of the sample; the stress at 20% and 80% of the maximum compression would assess the myofibrillar and connective tissue strength, respectively. The aim of this study was to compare the modified WBsf and TPA methods on beef during ageing and to assess correlations. At 2 and 10 days post mortem two steaks were taken from LTL muscle of 20 Piemontese bulls. Raw meat strips, 1 cm2 in cross section, were sheared perpendicular to the fiber direction with a WBsf device or compressed perpendicular to the fiber axis with a square compression probe (1 cm2) at 20% and 80% of sample thickness. Data were analysed by the GLM procedure. The lower IY and the higher difference PF-IY at d10 in comparison with d2 certify the tenderizing effect of ageing on myofibrillar tissue. No significant differences for PF were found, because the measurements were taken on raw meat and therefore the tenderizing effect of cooking on connective tissue was lacking. A significantly lower force was detected at d10 for hardness at 20%, which reflects the tenderizing effect of the proteolytic enzymes on the myofibers. On the contrary, as ageing does not influence the mechanical resistance of connective tissue, no significant differences were detected for hardness at 80%. Both methods are suitable to evaluate meat texture, but the poor correlations between the WBsf and TPA parameters (r ranged from -0.2 to 0.4 and four out of thirty were significant) indicate that these tests are complementary and measure different aspects of meat texture.

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P-110

Efficiency of AI in adult buffaloes synchronized by Ovsynch and progesterone-TAI protocols

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The aim of this study was to evaluate the efficiency of two synchronization protocols for artificial insemination (AI) in adult buffaloes, analysing data recorded in two farms over a period of 4 years. Despite of the seasonality of the species (that is a short-day breeder), AI were carried out from February to October in each year, to meet milk production with market requirements for mozzarella cheese production. All animals underwent two synchronization protocols: the Ovsynch-TAI Program (OV) and the Progesterone Releasing Intravaginal Device Program (P4). Buffaloes treated by OV received a GnRH agonist (buserelin acetate, 12 mg) on Day 0, a PGF2α analogue (luprostiol, 15 mg) on Day 7, and a further GnRH (12 mg) on Day 9, with AI performed on Day 10 (16 hours after the last GnRH). Animals treated by P4 received a progesterone releasing intravaginal device on Day 0, an administration of equine Chorionic Gonadotrophin (eCG 750 IU) and PGF2α analogue on Day 10 (at the time of progesterone device withdrawal) and a GnRH injection (12 mg) on Day 12 with AI performed on Day 13 (16 hours after the last GnRH). Pregnancy rate (PR) was assessed by ultrasound on Day 45. Statistical analysis of the data was carried out by chi-square test, according to the period of the year in which AI were performed and parity. A total of 2,881 AI were analyzed. The overall PR was 46.7% and no differences were present between OV (47.1%) and P4 (46.0%). Primiparous buffaloes showed higher ($P<0.01$) PR than pluriparous counterparts (50.0 *vs.* 44.8%, in primiparous and pluriparous animals, respectively) throughout the year and, particularly, in May (62.4 *vs.* 39.3%, in primiparous and pluriparous animals, respectively; $P<0.01$). P4 treatment resulted in higher ($P<0.01$) PR in June in primiparous buffaloes (54.2 *vs.* 31.1%, in P4 and OV group, respectively), and in March in pluriparous counterparts (50.4 *vs.* 30.1%, in P4 and OV group, respectively), while no differences were assessed in other months. On the contrary, a higher PR was recorded by OV treatment only in pluriparous subjects in May (43.0 *vs.* 29.7%, in OV and PR group, respectively; $P<0.05$), June (63.6 *vs.* 33.6%, in OV and P4 group, respectively; $P<0.01$) and July (52.3 *vs.* 26.1%, in

OV and P4 group, respectively; $P<0.01$). In conclusion, synchronization protocols would be utilized on the basis of both animal order and season to improve the efficiency of AI in buffalo species.Work supported by Rural Development Funds (FEASR 2007-2013)-PSR Campania Region-Measure 124. Project FORAGE: Optimization of water utilization in forage agricultural management

P-111

Effects of mycorrhized maize grain on milk and on rumen environment of Italian Holstein dairy cows

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The aim of the study was to evaluate the effect of the mycorrhized maize grain, both on rumen microbiology and milk production of dairy cows. Animals were divided into two groups (7 animals each), homogeneous for the main production and physiological parameters. Trial lasted 100 days, after three adaptation weeks. Six cannulated cows were also used for rumen microbial analyses in a cross-over design. Farm ration for lactating cows included 5 kg maize grain, mycorrhized (E) and not mycorrhized (C) administered as unifeed (0.91 Milk FU/kg Dry matter (DM), 155 g/kg DM crude protein) and *ad libitum* basis. Residues were weighed weekly. Dry matter intake (DMI) and average daily gain (ADG) were calculated. Chemical characteristics of feedstuffs and diets were determined (AOAC, 1995; Goering and Van Soest 1970). Milk FU were calculated according to INRA (1988). Milk sampling and recording were carried on every two weeks and fat, protein, urea, somatic cells were determined (ASPA, 1995). Rumen content samples were collected before feeding and analyzed for pH and microbial populations (protozoa and fungi, direct counts). qPCR quantifications of total bacterial were performed according to Huws *et al.* (2010). Differences were tested by GLM procedure (SAS, 2001) using the mono-factorial model with diet as fixed effect. No meaningful differences came out in maize grain proximate composition. Milk yield was the same in the two groups, but E group showed an increased milk protein conten. This is likely due to a higher DMI (22.35 *vs.* 21.11 kg/d, $P=0.015$) for E group, which also showed a tendency to a higher ADG (272.21 *vs.* 124.72 g/d). Further investigation are needed in order to clarify the degradability of the E diet. In the rumen, protozoa counts were significantly higher in E diet (+15.6%, $P<0.05$) and total bacterial behaved accordingly (6.91 *vs.* 6.19 log10 pg g (-1) DM, $P<0.01$). No differences were observed either on rumen pH or fungal density. Mycorrhized maize as feed seems to improve milk protein content and animals' overall conditions as confirmed by a higher DMI and ADG.

P-112

Influence of animal profile performance parameters of INRAPORC® on simulation errors

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IINRAPORC® is a system of models that evaluate effects feeding strategies on nutrient utilization of pigs 15 to 150 kg BW, through mathematical modeling. Informations of the animal profile are required for calibration, such as: initial BW, precocity, protein deposition, feed intake at 50 kg BW (Y50) and maintenance adjustment factor (MANT). These parameters may be optionally provided by values observed (expert calibration procedure) or estimated by the system (standard calibration). Different calibration procedures, characterized by the combination of the estimated and measured animal profile parameters, were evaluated to determine the effect on the performance estimations for pigs finished from 78 to 145 kg BW under a typical nutritional and genetic south brazilian conditions. The initial weight of 6 castred males under restricted feeding management was 78.5±4 kg each contributed 13 data BW and backfat thickness (BT), collected once a week. BW was obtained by weighing and BT by ultrasound between the last thoracic vertebra and the first lumbar, distancing 65 mm lumbar back line (P2) maintaining the fixed distance throughout the data collection period. However the method of obtaining BT used by INRAPORC the distance of the lumbar back line increased with the growth of the animal. Average values observed of BW and BT were compared with simulated data using the calibration models of Statgraphics Centurion 15.2.11.0. Hypothesis tests were performed to evaluate the intercept and the angular coefficient ($b0=0$ and $b1=1$). When the value of MANT was set at 1.00 and Y50 information imput (expert calibration procedure), a better estimate of BW was observed, with no significant intercept of 4.48 kg ($P=0.088$) and the angular coefficient 0.93 ($P=0.004$), but the standard calibration showed the significant coefficients: $b0 = 7.25$ kg ($P=0.004$) and $b1 = 0.90$ ($P\leq0.001$). BT was better estimated by standard calibration system that presented significant intercept less -1.94 mm ($P=0.009$) than the expert calibration -2.37mm ($P=0.003$), both methods resulting linear regression coefficients not different of the unity ($P\geq0.475$). The simulations showed results overestimated for BW and BT, in the weight range tested. The best estimate of BW was obtained by expert calibration procedure. The standard calibration proved to be better to estimate the BT, this may occur because it does not consider the BT observed, once your collection methodology is different from that used in INRAPORC.

P-113

Standard error increment percentage influenced by INRAPORC® simulations to extrapolate their results as means of a known population

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INRAPORC® is a deterministic system, so their estimates represent the values of an animal with average potential within a population and do not consider the variation of population. Thus the system can provide errors in results because the individual development kinetic can behave differently of the mean of population development kinetic. The aim of this study was to evaluate how much INRAPORC® simulations can influence the standard error of a known population, in order to verify the level of system error contribution, for a population variation when extrapolating the average data system output as the average of a known population. With the calibrated INRAPORC® were simulated the average of BW and backfat thickness (BT) at slaughter, for 2 populations of castrated males (n=76+116) that have similars BW initial (BWi) and populational deviation (P>0.49), bloodline and diet. BWi was 80.7±8kg and the slaughter was spread in 4 phases to 100kg (n=22+28), 115kg (n=18+29), 130 kg (n=18+31) and 145kg (n=18+28) of BW, were used 4 observations of BW and BT, of each population. The animals were weighed on the farm, 6 hours before slaughter. The BT was collected in the slaughterhouse with a pachymeter in P2, as brazilian system for carcass classification. To perform the analysis, it was compared the standard error values resulting from the real population mean and standard error values resulting simulation, as average population, and calculated the percentage difference between them. This procedure was performed in each slaughter phase for BW and BT. By expert calibration performed before this study showed significant systematic error of 2.37mm, overestimating the BT. Thus, this value was reduced to estimated data in the present study. Statgraphics Centurion® was used to analyze the Quadratic-Chi distribution of these percentage differences of standard errors for any slaughter weight, considering the level of 68.3% probability density, ie by 1 standard deviation unit (σ), to quantify the upper limit of the percentage increase of standard error. As a result, the influence of the system to the percentage increase of standard error at level of 1 σ was 15.9% (n=8) for BW and 18.2% (n=8) for BT. By extrapolating the average estimates of the system to the average data of the population is interesting to consider larger deviations, with percentage increase in the standard errors of the characteristics, especially to stipulate performance targets values and nutritients levels.

P-114

Validating a calibration of INRAPORC® based on a small population applying it to greater business population

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IINRAPORC® is a system of models that can be used to evaluate effects of different feeding strategies on performance of pig 15 to 150 kg BW, through mathematical modeling. The program is calibrated with data obtained from animals fed *ad libitum* and it estimates the performance for animals under feed restriction program. The aim of this study was to evaluate whether the system calibration, performed from a small group of animals (experimental conditions), could be applied to a larger group (commercial pig farms) to estimate the BW and backfat thickness (BT). The system calibration was based on data from 6 males fed *ad libitum* and validated through control group of 6 males under feed restriction (2.72 kg/d), from 13 observations for BW and BT. Data of two commercial pig farms of 76 (CPF1) and 116 (CPF2) animals under feed restriction and slaughter weight of 100kg (n=22 e 28), 115kg (n=18 e 29), 130kg (n=18 e 31) and 145kg (n=18 e 28), were used totalizing 5 observations of BW and 4 for BT, in each farm. The initial BW and feed intake at the CPF1 was 85.02±7.09kg – 2.73kg/d and CPF2 was 77.83±7.27 kg – 2.36kg/d, respectively. All animals groups were the same bloodline genetic and received the same diet. The observed data of BW and BT were compared with simulated by the system, using the Statgraphics Centurion 15.2.11.0. Hypothesis tests were performed to verify the null of intercept and the proximity of the value of 1.00 to the angular coefficients. Intercepts and linear regression coefficients were not significant (P≥0.52) for BW in both pig farms and control group. Similary, the intercepts and linear regression coefficients were not significant (P≥0.07) for BT at the CPF1 (+2.81mm and +0.75) and CPF2 (-2.52mm and +1.01). The values of intercepts and linear regression coeffi-

cients for BT at the CPF2 were consistent with those observed in the control group. For these animals by expert calibration, the intercept was significant -2.37mm ($P \leq 0.01$) and the linear regression coefficient was not significant +1.01 ($P=0.73$). The feed intake observed at the CPF2 (2.73 kg/d) was close to observed in the control group (2.72 kg/d) and it may explain the BT results between these groups. The Inraporc's calibration based on a small group of animals can be used to estimate BW and BT accurately. However, the level of feed intake and the number of observations can alter the coefficients and intercepts, as well as their statistical significance in hypothesis tests.

P-115

Two-dimensional difference in gel electrophoresis (2D-DIGE) for milk proteins characterization in Girgentana goat breed

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Milk proteomics has rapidly become an eligible approach, since the protein fraction constitutes the most biologically relevant component in milk. Proteomic applications can vary greatly from straightforward protein identification to complex characterization of post-translational modifications and protein-protein interactions. The aim of this work was to compare protein profile of Girgentana whole milk samples between lactation periods and geographical areas by two dimensional difference in gel electrophoresis (2D-DIGE). Milk samples were collected in two flocks located in two different areas of Sicily at three different time points from calving: 30, 55, and 120 days, respectively. Protein pools were labelled with CyDye fluorophores Cy3, Cy5, and the internal standard was labelled with Cy2. Electrophoresis was carried out using pH range 3-10 in the first dimension and 12.5% polyacrylamide gel to separate proteins in the second dimension. Image and statistical analyses were performed using Image Master 2D Platinum software. Several spots on the gels appeared as strings, demonstrating that isoforms of differing charge, resulting from post-translational modifications, are present in milk. Protein spots showing more than 1.5 fold change in spot volume (increased for up-regulation or decreased for down-regulation), with a statistically significant ANOVA value ($P \leq 0.05$), were considered differentially abundant. Among these, a set of ten spots were excised from preparative gels and identified by liquid chromatography tandem mass spectrometry. To assign protein identity, data were filtered following the application of identification criteria based on number of unique peptides ≥2. Milk fat globule EGF factor 8 protein variants, β-lactoglobulin, β-casein and serum albumin were successfully identified. 2D-DIGE allowed giving a general picture of milk protein distributions over 3-10 pH range. These preliminary results in addition to the identification of others interesting spots will be used to generate a reference proteomics map for the Girgentana goat breed.

P-116

Combined effect of dimethylacetamide and non-permeating cryoprotectants on quality of frozen/thawed sperm

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The aim of this study was to assess the effect of different cryoprotectants (permeating and non-permeating compounds) on the quality of post-thawing chicken semen. Twenty-seven adult (30 weeks of age) Lohmann male chicken breeders were used. All birds were housed in individual cages at 20°C, exposed to a 14L:10D photoperiod and fed a commercial standard chicken breeder diet. Males were divided in three different groups (9 birds/group). All the ejaculates collected within one group were pooled into one semen sample, then splitted into 4 aliquots, each one assigned to one treatment. Semen collection was repeated on four days to process 48 pooled semen samples in total (12 replicates/treatment). Ejaculates were collected into graduated tubes; volume and concentration of pooled semen samples were recorded. Semen aliquots were diluted to a concentration of 1.5 $\times 10^9$ sperm/mL using 4 different diluents: Lake pre-freezing (LPF, control treatment), LPF added with 0.1M trehalose (LPF-T treatment), LPF added with 0.1M sucrose (LPF-S treatment) and LPF added with 0.1M trehalose + 0.1M sucrose (LPF-TS treatment). The diluted semen was immediately cooled at 4°C for 30 minutes and transferred to the laboratory. Dimethylacetamide was added into semen aliquots to finally obtain 6% DMA concentration and 1×10^9 cells/mL sperm working concentration. Semen was packaged in straws (0.25 mL), frozen for 10 minutes over a nitrogen bath at 3 cm of height and thawed in water bath at 38°C for 30 sec after at least 7 days. Sperm viability (SYBR14/PI staining) and motility (SCA system) were assessed in fresh and thawed samples. The recovery rates (%) of viable and motile sperm after freezing/thawing were also calculated. Analysis of variance was performed considering time and treatments as sources of variation. The mean proportion of viable and motile sperm measured after thawing were 46 and 38% respectively and not significant differences were found between treatments. Progressive motility and the recovery of progressive motile sperm were significantly improved in semen samples diluted in LPF-T (3.8% and 21.4% respectively) compared to LPF-S (2.7% and 15.3%), LPF-TS (3.0% and 16.9%) and control (2.9% and 15.9%) treatments. The present results show a significant positive synergic action of DMA and trehalose on motile function of cryopreserved chicken spermatozoa.

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P-117

Artificial insemination in buffalo heifers

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Reproductive performances in buffalo heifers are mainly affected by nutrition, rather than photoperiod compared to adult counterparts. Therefore, artificial insemination (AI) is usually carried out from March to September with high pregnancy rate (PR), in order to meet milk market requirements for mozzarella cheese yield. However, no information are available on the utilization of different synchronization protocols in different periods of the year. The aim of this study was to compare the efficiency of Ovsynch (OV) and progesterone (P4) based treatments in buffalo heifers throughout the mating period. The trial was carried out on 1521 heifers bred in two farms over a period of 4 years. The animals were shared into two homogeneous groups, according to age and body condition score: Group OV (n=724) was treated by the Ovsynch-TAI Program, which consists in a GnRH agonist injection (buserelin acetate, 12 mg) on Day 0, a PGF2alfa analogue (luprostiol, 15 mg) on day 7, and a further GnRH (12 mg) on day 9, with AI performed on Day 10 (16 hours after the last GnRH). Buffaloes in Group P4 (n=797) received a progesterone releasing intravaginal device on Day 0, an administration of equine Chorionic Gonadotrophin (eCG 750 IU) and PGF2alfa analogue on Day 10 (at the time of progesterone device withdrawal) and a GnRH injection (12 mg) on Day 12 with AI performed on Day 13 (16 hours after the last GnRH). Pregnancy rate (PR) was assessed by ultrasound on Day 45. Statistical analysis of the data was carried out by chi-square test, according to the period of the year in which AI were performed. The overall PR recorded in Group P4 was higher (P<0.05) than that recorded in Group OV (58.2 *vs.* 51.9%, in P4 and OV, respectively). In particular, P4 treatment resulted in higher pregnancy rate in March (64.7 *vs.* 46.6%, in P4 and OV, respectively; P<0.01) and June (60.4 *vs.* 49.1%, in P4 and OV, respectively; P<0.05), while no differences were recorded in other months. These results suggest that also in buffalo heifers a seasonal trend may be present. Therefore, synchronization treatments would be applied according to the season in which AI are performed. Work supported by Rural Development Funds (FEASR 2007-2013)-PSR Campania Region-Measure 124. Project FORAGE: Optimization of water utilization in forage agricultural management

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P-118

Analysis of small RNAs in bovine blastocysts using deep sequencing

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MicroRNAs are short non-coding RNAs and are important in controlling, amongst other things, reproductive functions, such as oocyte maturation, early embryonic development and implantation. During pregnancy, specific microRNAs synthesized by the embryo or endometrial cells are present in plasma in a stable form and are transmitted to specific tissues. Profiles of microRNAs are altered in sub-fertility pathologies, such as polycystic ovary syndrome and male factor infertility. This prompted the examination of microRNAs as biomarkers for fertility. We developed a deep sequencing approach to analyze all microRNAs present in bovine blastocysts in order to characterize the early stages of bovine embryo development. Total RNA samples were extracted using three different procedures from pools of 30 blastocysts. Libraries were made using the Illumina TruSeq Small RNA kit and were sequenced on an Illumina HiSeq2000 with a 50 single-read module. Raw reads were trimmed using ‘Trimmomatic’ and trimmed sequences were used for novel small-RNA discovery using ‘miRDeep2’. Known bovine and human small-RNAs (mature and precursors) were used for comparison in order to help the discovery process. Preliminary results showed that the number of trimmed reads ranged from 4.4 M to 18.5 M. The method of RNA extraction seems to influence the expression of identified microRNAs which ranged from 424 species to 832. The best method allowed the identification of

474 known and 316 novel microRNAs. Gene ontology analysis will be carried out in order to classify microRNAs which characterize the embryo at the blastocyst stage. This work significantly improved the methodology for the analysis of microRNAs in bovine blastocysts and is being used to identify microRNAs associated with differences in reproductive success which may be used as biomarkers in cattle reproduction.

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P-119

Hepatoprotective substances in sow's feeding

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In sows, the transition period from late gestation to lactation has received sparse attention, even though substantial physiological changes occur at that time. The transition period is crucial for the sow and offspring because nutrients are reallocated to the mammary tissue, massive mammary growth occurs and milk production is being initiated. The target organ is liver in which an accumulation of non esterified fatty acids (NEFA) and biosynthesis and lipoprotein release occur. Artichoke extract (AE) has choleretic (improves biliar production through hepatic function), cholagogue (promotes the discharge of bile from the system), detoxifying and diuretic properties. The hepatoprotective activity helps the animals facing nutritional stress situations. Therefore the objective of this study was to determine the effect of lactation diets supplemented with artichoke extract (Bedgen 40TM, Bedson, Spain) on the performance of nursing sows and their litters. Two trials with a total of 85 sows (Large White) were performed. Animals were assigned to two treatment groups balanced for parity: control diet (CON) and diet supplemented with 150 g/t of AE. Dietary treatments started at the farrowing (day 1) and ended at 19 days of lactation, the duration of the trial was chosen to standardize the average duration of lactation since a fractional weaning was practiced. Backfat at the P2 position and body weight were registered on entry to farrowing house and weaning. Blood samples were collected from 40 sows (20 per treatment) at day 1 and day 19, for the assessment of the total antiradical activity by mean KRL test, lipid profile and activity of hepatic enzymes. Piglet data were collected. Results showed that blood total protein and ALT were lower ($P<0.05$) in sows treated with AE. No other differences were found on blood analysis. Dietary treatment did not influence any productive parameter The average body weight at weaning of litter piglets was 45.61 and 46.69 in CON and AE group respectively. Parity affected number of piglets born alive, birth weight, birth after fostering, number of weaned piglets resulting higher ($P<0.005$) in sows belonging to (2-3-4) parity class than (0-1) and (,â•5) parity class. Changes in backfat thickness were significantly ($P< 0.05$) lower in AE than in CON group (3.54 *vs.* 1.93 mm). The experimental dietary supplement had a positive effect on the condition of sows that with an equal feed intake reported a lower reduction of thickness and on some parameters of hepatic metabolism

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P-120

Effect of season on the ovum pick-up efficiency in Calabrian Podolic cattle

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The use of advanced reproductive technologies is fundamental to preserve biodiversity and limit genetic erosion of species and breeds. The aim of the study was to evaluate the effect of season on the efficiency of *in vitro* embryo production (IVEP) in Calabrian Podolic cattle. OPU was carried out every month on 19 pluriparous Podolic cows, during summer and autumn, for a total of 8 replicates. Animals underwent OPU with (400 UI PMSG i.m. 3 days before OPU) or without treatment in each season. Following follicle puncture, cumulus-oocyte complexes (COCs) were selected and processed according to standard procedures for *in vitro* embryo production (IVEP). The following parameters were recorded: total number of antral follicles (TF), large (LF), medium (MF) and small follicles (SF); total COCs; recovery rate (% COCs/TF); cleavage and blastocyst rates. Data were analyzed by Student T test. Overall, 390 COCs were recovered and 104 transferable blastocysts were produced. No differences were found between summer and autumn in the number of TF (5.1±0.5 *vs.* 5.2±0.4), COCs (2.4±0.3 *vs.* 3.4±0.5), cleavage (52.8±10.5 *vs.* 55.2±6.9) and blastocyst rates (29.1±7.1 *vs.* 31.1±5.0). However, the PMSG treatment increased ($P<0.01$) the number of TF (5.9±0.3 *vs.* 4.6±0.3; $P<0.01$) and MF (1.6±0.2 *vs.* 1.1±0.1; $P< 0.05$), while no differences were found for LF and SF (0.5±0.1 *vs.* 0.7±0.1 and 3.8±0.3 *vs.* 2.9±0.3, respectively). However, the recovery rate was lower in donors treated with PMSG (45.6±7.3 *vs.* 70.3±6.2; $P<0.05$, respectively), leading to a lower number of total COCs recovered (2.4±0.3 *vs.* 3.4±0.4,

$P<0.05$, respectively). Interestingly, cleavage and blastocyst rates increased when animals were treated with PMSG (68.2±4.9 *vs.* 46.3±5.1; $P<0.01$ and 38.2±5.4 *vs.* 21.3±4.4; $P<0.05$, respectively). The improved developmental competence observed after PMSG may be due to the increased number of MF, as this parameter is known to be related to follicular size. In conclusion, season did not affect OPU-IVEP efficiency in Calabrian Podolic cattle and it was demonstrated the feasibility of IVEP for the preservation of the breed. Furthermore, PMSG treatment increased follicular population, as well as oocyte developmental competence. Nevertheless, the decreased recovery rate resulted in similar number of embryos produced, suggesting that PMSG priming is not worth to increase the overall embryo production in the field.

P-121

Management of reproduction in cattle breeding farms in the Greater Cheliff Algerian

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In Algeria the control of reproductive performance is one of the important positions and difficult to manage in cattle. Ideally, calving and calving interval should be a year, but the evolution of this parameter in the case of cattle farming in the region shows great Cheliff frank degradation of this criterion, with QT prolongation especially for improved breeds of cattle. This deterioration of reproductive performance is also observed for the other criteria for measuring fertility, and inevitably leads to increased farm costs: economic costs extra inseminations, lost time of insemination failures, or low-performance reform of animals. These criteria should be easily identifiable in each operation, and must be based on acts or events reliable and as far as possible, available to all animals in the herd. In order to determine the economic performance of meat cattle farms, a study was conducted in 2011 in the Greater Cheliff northern Algeria. The sample consists of 102 farms and farmers. The data collected were subjected to statistical analysis using the SPAD 5.5 software. This multi varied statistical treatment was necessary in order to differentiate, clarify and order the animal production practicing reproductive face of strong heterogeneity seen existing cattle farms. Through the data from our survey, more than half of the farmers, 55% of all farmers practicing natural mating, while the practice of artificial insemination is only 33% of farms. However the practice of both artificial insemination and natural mating is 10%, this shows the reluctance of farmers to use artificial insemination. Examination of these reproductive parameters reveals driving control problems, which results in a loss to the farmers. Mismanagement of reproduction is causing poor performance, due to poor reform strategy, to breeding, gestation control and heat detection. For this purpose, the reproduction in cattle is far from mastered aitre

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P-122

Body weight gain comparison between suckling piglets and piglets on a transition feeder

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Hyperprolific sows have the capacity to produce litters with 15 or more piglets. Large litters can be economically positive but increase the number of piglets with low birth weight (less than 1.0 kg). These small piglets have reduced survival rates and poor weight gains because larger piglets tend to suckle more and to gain more weight than their litter mates. The transfer of piglets from large litters to other litters in the first days of life is common practice to increase the chance of small piglets to survive but it may be insufficient in hyperprolific farms. An alternative is to transfer small piglets for a Transition Feeder, a pen for approximately 45 piglets with an automatic feeder that warms water and mixes it with a meal. Mother's feeding pattern is mimicked by dispensing warm feed once an hour. This experiment evaluated the body weight gain for litters from 66 sows from January 6 to February 23, 2014 in a commercial pig farm located in Santa Catarina, Brazil. Piglets were randomly selected and grouped according to their body weight at 10 days of age: 105 piglets with 4.90 kg average live weight were allocated to the Treatment Group (TG), 69 piglets with 4.95 kg average live weight formed the Control Group (CG), and 64 piglets with 5.85 kg average live weight were considered the "Natural" Group (NG). TG piglets were transferred to the Transition Feeder at 10 days of age where they received liquid diet, mixing of water with high digestibility ingredients and high levels of lactose (21%) to adequately replace the milk. The feeder and feed formula are tailored for newly weaned pigs, from age 10 days. CG and NG piglets remained in their litters until weaning at 21 days old. Analysis of variance was done using SAS 9.0. The statistical model included the effects of farrowing room, parity order, piglet gender, class of number of piglets born, class of piglets birth weight, treatment and residuals. The SNK test at 5% was used to compare weight gain from day 10 to day 21 of age. TG piglets gained significantly more body weight gain (2.26 kg) then CG piglets that remained with the sow (2.04 kg) and did not significantly differ from NG piglets (2.47 kg). It was concluded that the Transition Feeder is a viable alternative to recover body weight of small piglets from 10 days of age.

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P-123

Constitution of *Nero Siciliano* pig breed genetic reserve: flow cytometry evaluation of epididymal sperm cells

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In the framework of the research program "Integrate knowledge for sustainability and innovation of the agro-food Made in Italy (CISIA)" the genetic reserve of the Nero Siciliano local pig breed was created. This breed is farmed in marginal areas and few males are available for semen collection. In this context epididymal sperm extraction was the only option to collect gametes. Semen quality evaluation and determination of its fertilizing ability are essential aspects in the practice of assisted reproduction in domestic species, especially in local breeds, where few chances to store semen are available. With flow cytometry (FCM) technique an objective and quick assessment of semen potential fertility is obtainable. The aim of this study was to evaluate different quality parameters of boar frozen-thawed epididymal sperm by using advanced technologies as FCM and computer assisted sperm analysis (CASA). Testicles were obtained from 12 boars after surgical castration performed directly on farm. Epididymal sperm were extracted from cauda epididymis by using the retrograde flushing technique. At freezing-thawing, the following sperm parameters were evaluated by FCM: viability (SYBR-14/propidium iodide (PI) staining), acrosomal status (FITC-PNA/PI staining), membrane lipid disorder (Merocyanine 540 hydrophobic dye), mitochondrial membrane potential (JC-1 potentiometric probe) and sperm DNA fragmentation (acridine orange dye). Sperm motility and kinetic parameters were assessed by CASA system. A total of 1401 semen doses of Nero Siciliano local pig breed were stored with suitable sperm quality. The mean values of measured parameters, standard deviations, minimum and maximum values, on frozen-thawed samples, were: viable sperm: 41.3±9.2 % (31.0-61.8); live acrosome intact sperm: 34.6±9.7 % (18.9-52.9); live sperm with low lipid disorder: 26.6±9.5% (15.6-48.3); sperm with high mitopotential: 38.7±10.6% (22.1-61.2); sperm DNA fragmentation index (% DFI): 5.6±2.7 (2.6-11.6); total motile sperm: 44.0 ± 12.0% (21.2-68.0); curvilinear velocity: 89.9±9.2 μm/s (77.3-103.8); average path velocity: 39.1±7.7 μm/s (25.0-47.8); straight-line velocity: 8.4±2.8 μm/s (5.5-14.0); linearity index: 9.2±2.7% (6.5-14.2). The current study is a concrete example of the application of advanced techniques, like FCM or CASA, for the validation of frozen-thawed epididymal boar sperm as gametes source in animal genetic resources cryobanks.

P-124

Effects of dietary phosphorous supplementation during the rearing phase of Italian Friesian dairy heifers

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Current dietary P requirements for growing dairy cattle are based on old trials and are formulated to achieve a target average daily gain (ADG) independently from P use efficiency. The aim of this study was to evaluate the effect of a reduced dietary P supplementation for dairy heifers on growth performance and P excretion to reduce the environmental impact of the diet. Thirty-sixth heifers received daily a basal mixed diet, calculated according to the mean body weight (BW) and the planned ADG (0.8 kg/d) of the group, to avoid constant refusals above 5% of the distributed amount. The same commercial concentrate, but with two different mineral P supplementations (avoiding changes in the Ca content), was individually fed to 18 heifers for each P level group by an automated feeder from 6 to 12 mo of age, increasing the amount from 1.0 to 1.5 kg/head/d in the 180 d period. On average, diet P resulted to be 0.30 and 0.35% (on DM) for the low (P1) and high (P2) level group respectively. Wither height, hip height, body length, heart girth, cannon bone circumference, and BW were monthly recorded for each heifer. Individual feces were sampled at 12 mo of age, from the rectum, to assess fecal dry matter and P content, before the end of the dietary treatment. At the same time, an individual urine sample was collected by spontaneous emission or just after a mild vulvar stimulation. Urine was analyzed to assess concentration of creatinine, Ca, P, Mg, Na, K, and Cl. Individual data on body size measurements were processed to estimate individual growth curve by the Laird's function; the obtained individual curve parameters, as well as data from feces and urine, were analyzed by ANOVA to test the effect of P supplementation. Dietary P supplementation did not affect the growth curve parameters for BW and the considered skeletal variables. None of the urinary variables was affected by dietary treatment. On the contrary, a significant difference in fecal P concentration was found between diets, with 4177 and 6406 mg/kg DM in P1 and P2 respectively (max SE = 340; P=0.0003). These results confirm that feeding heifers a concentrate with lower inorganic P supplement during the rearing phase determines a proportional decrease of fecal P excretion, without worsening of growth performance.

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P-125

Relationships between rumination time with metabolic status and inflammatory conditions around calving in dairy cows

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Rumination has long been associated with health in dairy cattle and, more recently, changes in rumination time (RT) have been related to stress factors and diseases. The aim was to study the relationship between RT and inflammation conditions around calving in order to early detect animals with health problems. The trial involved 23 Italian Friesian dairy cows kept in an experimental free stall barn. From 20 d before calving till to 40 days in milk on all the cows the RT was continuously measured by using a rumination monitoring system (HR-Tag), summarizing the RT in 2-h intervals. Blood samples from the jugular vein were collected before the feed distribution at -14, -7, 5, 10, 20 and 30 d (±1 d) from calving. The samples were analyzed to estimate the parameters of the energy, protein, and mineral metabolism. Albumin, cholesterol and bilirubin data collected in the peripartutient period were used to calculate the liver functionality index (LFI). On all the cows the daily milk yield and all health-related problems were recorded. The mean value of daily RT before calving (from –20 to –2 d) averaged 479 min/d (range 264-599 min/d among cows). A reduction of daily RT of 70% (on average) of the value observed during the dry period was observed on the day of calving. After calving the RT increased and reached almost stable values after 15 DIM (from 15 to 40 DIM the mean RT was 452 min/d). A negative partial correlation between total bilirubin and RT in lactating cows ($r=-0.35$; $P < 0.01$) was observed. A positive correlation between paraoxonase measured at 10 DIM and RT (recorded between 3 and 6 DIM) was also observed. A positive relationship between RT, recorded between 2 and 10 DIM, and the liver fertility index (LFI) was observed ($r=0.75$; $P<0.001$). The LFI, accounting the variation of some negative acute phase proteins or related parameters, measures the consequence of inflammation occurring around calving. The synthesis of these negative acute phase proteins are reduced in case of inflammation, and LFI is lower, and LFI below 0 is considered poor. Cows with an acceptable LFI value ($LFI > -0.26$) have ruminate more than 450 minute/day, as well as healthy cows. These results indicate that inflammatory conditions are associated with a slower increase of RT during the first ten days of lactation. These results seem to suggest that a very early detection of health dis-

orders around calving can be performed through the monitoring of rumination in real time.

P-126

Effect of different dietary aromatic essences on fatty acid profile and oxidative status of rabbit meat

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Increasing consumer knowledge of the relationship that links diet and health had promoted a notable interest for functional food ingredients. Rabbit meat due to highly digestible, tasty, low calories, high nutritional value and low fat content could represent an excellent base to produce particularly appreciated functional food. The objective of the present study was to evaluate the impact of a dietary supplementation with plant acqueous extracts on the fatty acid profile of raw rabbit meat (Longissimus dorsi). Five groups of twenty New Zealand White (NZW) weaned rabbits were submitted to the following isoenergetic and isonitrogenous dietary treatments: Standard diet (S); Standard diet + 150 ppm vitamin E (E); Standard diet + 0.2% oregano extract (O); Standard diet + 0.2% rosemary extract (R); Standard diet + 0.1% oregano extract + 0.1% rosemary extract (OR). The extracts were were obtained with an enzyme aided extraction of leaves using water as solvent (Phenbiox srl). Each diet contained the following supplementation: 50 ppm vitamin E, CLA 0.5% (from soy oil), 3% Omega Lin® (extruded linseed, Mignini & Petrini) and 0.5% vitamin-mineral premix. The rabbit were slaughtered at 80 d of age. Total fatty acid profile and antioxidant status (TBARs) were measured on fresh meat (after 24 hours of storage at + 4°C). Data were analyzed using the GLM procedures of the statistical software STATA. The addition of oregano and rosmary extract, as well as the vitamin E supplementation, had no effects on the fatty acid profile. In all samples, linoleic acid (C18:2n-6) was the most abundant fatty acid, ranging from 25.5%±0.4 to 28.1%±0.6, followed by oleic (C18:1n-9) and palmitic (C16:0) acids, both reaching 20% of total fatty acids. Furtermore, the fatty acid classes increased in the following order: monounsaturated (MUFA) < saturated (SFA) < polyunsaturated (PUFA) fatty acids. Interestingly, all the samples analysed were characterized by a PUFA/SFA ratio ranging form 1.1 to 1.3 (recommended value >0.7). The PUFA n-6/n-3 ratio ranged from 3.1 to 3.6 (recommended value <5). The lowest meat TBARs concentration was recorded in the E and O groups. However, all dietary treatments improved the oxidative stability compared to the S group. In conclusion, the plant acqueous extracts evaluated in this experiment showed no effects on the fatty acid profile but beneficial effects on the antioxidant status of rabbit meat.

P-127

Effect of diet supplementation with linseed as linolenic acid source on native immunity and oxidative traits of dairy cattle

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In a field trial the effect of long term diet supplementation with different levels of extruded linseed and energy levels on plasma concentration of haemolytic complement assay (HCA), Reactive Oxygen Metabolites (ROM), antioxidant power of plasma (AP), serum albumin and total protein were evaluated. in early lactation dairy cows. One hundred and two Holstein Friesian cows, reared in Parmigiano Reggiano cheese area, homogeneous for lactation number, days in milking (DIM) (41+/-12d), BCS (3.17+/-0.14) and productive level were allotted into three experimental groups: control group (C); 280g/d Omega-Lin®-Mignini&Petrini (O280); 450 g/d Omega Lin®-Mignini&Petrini (O450) and fed isonitrogenous TMR diets. Omega Lin® contained 70% of extruded linseed. Blood samples were taken by coccygeal vein and blood parameters evaluated. The fixed effect of different classes of DIM (class 1: 10-19 days; class 2: 20-39 days; class 3: 40-59 days: class 4: 60-90 days) and their interactions were evaluated. Diet treatment significantly affected HCA, total protein and AP levels showing the highest (55.52CH50/150 μ, 70.56 g/L and 453.30 μM HCLO) levels in O450 cows ($P<0.0001$), respectively. In particular γ-globulin are abundant in O450 ($P<0.0001$). Diet x DIM interaction showed a significant statistical effect ($P<0.0047$) for ROM and serum albumin ($P<0.001$). Serum albumin showed the highest level in O450 group within 20 DIM while ROM showed the lowest levels in the same group from 20 to 60 DIM ($P<0.0001$) respectively. The maintenance of an efficient immune system is essential for animals to satisfactory performance and integration in the diet of substances with immunostimulant action is extremely useful. In conclusion HCA values explain the better innate immunity status in O450, confirmed to high value of AP too; and so the serum albumin and total protein concentrations were to ascribe to better body condition in these dairies mainly in peripartum period. In conclusion, a dietary flaxseed extruded lactating cows has been shown to have a positive effect on the immune system without going to overburden the oxidative status of animals.

P-128

Ketosis risk and milk loss in commercial dairy farms

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The aim of the present study was to evaluate the effects of subclinical ketosis (SK) on milk yield and quality in relation to the level of milk beta-hydroxybutyrate (BHB) in commercial dairy farms. Individual milk samples (about 360,000 observations collected in 465 dairy farms of the Po plain from March 2013 until January 2014) were analysed for chemical composition (protein, fat, lactose, urea) and BHB level by infrared spectrometry (Milkoscan FT, Foss, DK). Since SK affects mainly cows in early lactation, only cows with less than 20 DIM were included in the study. The concentration of 0.12 mmol/l of milk BHB was used as threshold value to divide cows into 2 groups: healthy (H; BHB<0.12) and cows at risk of SK and clinical ketosis (SK; BHB≥0.12). Data were analysed by GLM SAS procedure taking in account also farm and month effect. The risk of prevalence of SK cows was affected by DIM: for example prevalence was 29.2% for cows with less than 6 DIM (n=1,934) and 18.7 % for cows with less than 20 DIM (n=15,859). Average BHB levels were 0.047 and 0.158 mmol/l for H and SK cows, respectively. The number of parities was on average 2.1 without difference between the two groups. SK affected milk production: 36.4 *vs.* 35.2 kg/d respectively for H and SK group (P<0.001). The average lactation length was 336 and 323 DIM, respectively for cows of SK and H group. Milk fat (%) was higher for SK (4.73) than H (4.11) cows (P<0.001), hence milk fat protein ratio was different between groups (1.45 *vs.* 1.25, respectively for SK and H groups, P<0.001). Lactose content was higher for H group (4.74%) than SK group (4.63%) (P<0.001). In conclusion, a rather high risk of prevalence of subclinical ketosis was registered in commercial dairy farms, particularly in cows at the beginning of lactation (<20 DIM), often not diagnosed. It is confirmed that subclinical ketosis hampers milk production, and milk BHB proves to be a valuable predictor of this disease.

P-129

Chronic toxicity and bioaccumulation of Selenomethionine in juvenile mullet (*Liza aurata*)

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Because of its bioaccumulation in the aquatic food chain, Selenium (Se) pollution, resulting from various anthropologic activities (mainly agriculture and industry) can become a primary environmental emergency in some coastal areas and their surrounding lagoons. These are very sensible ecosystems, highly exposed to the pollution released by the drainage of agricultural water. At the same time these humid areas are the elective habitat of several fishes, including mullets and some aspects of the Se polluting effects on these species are still not well known. An 8-week growth trial was conducted to determine the sensitivity of juvenile (60 g) mullets (Liza aurata) to the toxicological effects of elevated dietary selenium (Se). The fishes were fed with three purified diets supplemented with Se in the form of l-selenomethionine (SeMet), resulting in dietary concentrations of 0 (control), 30 and 60 mg kg/diet SeMet on a dry matter basis in three Effects of dietary SeMet on survival, growth, whole-body proximate composition, tissue Se concentrations, and histopathology were determined. Selenium concentration of tissues and whole fish were determinate with spectrophotometer Atomic Absorbtion (Shimadzu AA 6800) after samples homogenization and mineralization. No mortality or differences in hepatosomatic index in all dietary treatment groups were observed. A significant decrease in growth rate (SGR), feed efficiency (FE) and body weight increase (BWI) was observed in mullet fed at or above 20 µg Se/g diet. No significant differences have been detected in moisture, crude protein and lipid content. Dietary SeMet concentration were positively correlated with selenium accumulated in the muscle, liver, gill, gastrointestinal tract (GIT) and whole body in a dose-dependent manner. Histopathological alterations and lesions observed in mullets fed above 30µg SeMet/g diet showed vacuolar degeneration and moderate glycogen depletion in the liver and hydropic degeneration and tubular cell death in the kidney, likely a result of Se-induced oxidative stress. Results show that even at the lowest SeMet added concentration tested of 30 µg SeMet/g diet, a level that can be reached in the chain-food of polluted lagoon, SeMet is toxic to juvenile mullets.

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P-130

Colostrum, n-3 FA status and immune response of newborn kids as influenced by maternal lipid supplementation

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Long-chain n-3 polyunsaturated fatty acids (LC n-3 PUFA), mainly EPA (C20:5 n-3) and DHA (C22:6 n-3) play a crucial role during pregnancy and peri-natal growth. In particular, for DHA, a specific role during fetal and neonatal development has been recognized, but DHA status of the mother and the new-born may be sub-optimal if maternal intake is insufficient. The aim of the study was to evaluate the effect of supplementing saturated fatty acids (FA) or LC n-3 PUFA to the diets of late gestating dairy goats on FA profile of colostrum and plasma of newborn suckling kids and on their immune response. The saturated FA supplement (calcium stearate, ST) was rich in 16:0 (26 %) and 18:0 (69.4 %) and the LC n-3 PUFA supplement (fish oil, FO) was rich in EPA (10.4 %) and DHA (7.8 %). Starting from the last wk of gestation until 3 wk after kidding, 23 multiparous Alpine dairy goats were divided into 3 groups: C (control diet), FO and ST. FO and ST diets were formulated to administer 30 and 50 g/head/d of FA during pre- and post- kidding. Newborn kids were fed colostrum from their own dam within 2 h of birth. Individual colostrum was sampled within the first 24 h postpartum for FA determination. Neonatal blood samples were collected weekly from birth until 21 d of life for evaluation of FA profile and immune response. The source of maternal lipid supplement did change the FA profile of colostrum and the n-3 FA status of newborn kids. Maternal FO supplementation enhanced total n-3 PUFA in colostrum (1.78 *vs.* 0.72 and 0.76 g/100g FA, P<0.01) and in plasma of suckling kids during the first 3 wk postnatally (5.27 *vs.* 2.32 and 3.27 g/100g FA, P<0.01, in FO, C and ST respectively). IgA at 2 d after birth was higher in the plasma of kids born from ST dams (0.54 *vs.* 0.20 and 0.14 mg/ml, P≤0.01) and also the overall mean was higher (0.18 *vs.* 0.07 and 0.07 mg/ml, P≤0.01 in ST, FO and C respectively). Circulating concentrations of IgG were higher for the whole experimental period in plasma of kids born from ST *vs.* FO (17.14 *vs.* 9.80 mg/ml, P<0.01) and C dams (11.43 mg/ml, P<0.05). In conclusion, the n-3 FA status of newborn kids can be improved by supplementing the maternal diet with n-3 PUFA and is associated to the increased supply of EPA and DHA from maternal circulation during fetal development and from colostrum during early neonatal period. Unexpectedly, in the present trial, kids from mother fed ST showed the most significant variations in immune response

P-131

Arterial blood gas analysis on acute lactic ruminal acidosis induced experimentally on Santa Inês sheep (*Ovis aries*)

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One objective of the production model in Brazil is to obtain high weight gain in shorter time, and this fact sometimes results on mistakes in feeding sheep, causing disorders such as acute lactic ruminal acidosis (ALRA), which can lead to organic disfunctions and metabolic acidosis. To show what happens on the arterial blood parameters, this study aimed to relate the changes on the values of hemogasometry. It was used 10 healthy ewes maintained on basal diet of Tifton hay *ad libitum*, with free access to water and mineral mixture during the period of one month. For the induction of ALRA, the animals received 15 grams of saccharose/kg of body weight, orally, before morning feeding. After the induction of ALRA the arterial blood for hemogasometry was collected from carotid artery on the following moments: 2, 4, 6, 8, 10, 12, 16, 20, 24, 28, 32, 36, 48, 72, 96, 120, 144 hours and on the second, third and fourth week; using syringes containing lithium heparin anticoagulant, and processed in Cobas b 121 System. The parameters obtained from the hemogasometry were: pH, partial pressure of oxygen–PO_2 and carbon dioxide–PCO_2 (mmHg), oxygen saturation–SO_2 (%), bicarbonate concentrations–HCO_3, Na+-sodium ion, K+ potassium ion, iCa–ionized calcium, Cl--chloride ion and excess/base deficit-EB (mmol/L). Associations between the variables and time by linear, quadratic and cubic regression was assessed using statistical software (SAS 9.1, SAS Institute Inc., USA), with statistical significance set at P<0,05. All blood gas variables showed significant differences, except SO_2 (P>0,05). The results show marked changes on the parameters analyzed from 2 hrs after the induction of ALRA, such as initial occurrence of metabolic alcalosis evidenced by the average pH values in 7,50 after 06 hrs of induction and reaching its lowest average of 7,27 with 16 hrs, values that characteristic metabolic acidosis. Another important parameter altered was the values of base deficit averaging 6,46 mmol/L in 04 hrs, reaching values of -12,40 mmol/L after 20 hrs. The values

of ions have little changed with respect to baseline values, with changes mainly in the ranges from 2 hrs to 72 hrs post induction. Seventy two hours after induction the hemogasometry values were normal for the specie. This study indicates that the hemogasometry test is an important tool of evaluating animals with ALRA and indicating which parameters need to be corrected, thus avoiding the death of the affected animals.

P-132

Effects of replacing flaked corn with sorghum meal as the main energy source in lactating dairy cows diets

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The purpose of this experiment was to compare flaked corn and sorghum meal on lactating cows performance and health status. Steam-flaked corn has a rate of ruminal starch degradability of 25%/hour compared to a 7 %/hour for sorghum meal, with an intestinal digestibility of 90% and 75%, respectively. Diets were formulated using 38% grass hay, 4% straw, 21% protein and mineral supplement and 38% of either corn flakes (C diet) or sorghum meal (S diet). Eight lactating Holstein dairy cows, averaging 204 days in milk and producing 31 kg milk/day were divided in two homogenous groups and fed the two experimental diets in a cross over design with a 8 days adaptation and 5 days collection (13 days for each period). Dry matter and water intake, milk production and quality, and rumination time were recorded daily. Ruminal pH was measured continuously at 10 min intervals. Fecal samples were collected twice daily (at 8 am and 8 pm) for pH and dry matter content. Ruminal fluid and blood samples were collected at the end of every period. Data were analyzed with GLM – ANOVA for repeated measures using the software Statistica v.10. Dry matter intake was not different between treatments whereas water intake was higher in S compared with C diet (155 *vs.* 138 liters/day; $P \leq 0.05$). Milk production did not differed between treatments, whereas milk fat was higher for S diet (3.78% *vs.* 3.54% for S and C diet, respectively; $P \leq 0.05$). Milk urea was lower in C diet compared with S ($P \leq 0.05$). No difference was found for rumination time and ruminal pH. VFA profile showed higher content of acetic ($P \leq 0.05$), isobutiric ($P \leq 0.01$) and isovaleric acids ($P \leq 0.01$) for S diet, while propionic acid was higher in C diet ($P \leq 0.01$). In C diet the C2 to C3 ratio was 2.2 compared to 3.5 in S diet ($P \leq 0.01$). No differences were found in fecal pH and dry matter. Blood parameters showed a lower plasma urea ($P \leq 0.01$) and phosphorus ($P \leq 0.05$) content for C diet. In conclusion, replacing flaked corn with the same amount of sorghum meal did not affect productive performance although a better fat content in milk was achieved, as sustained by an higher ruminal acetic acid production. On the other side, the flaked corn-based diet improved nitrogen utilization, due to highly digestible carbohydrate available for ruminal fermentation, resulting in a decreased urea content both in the rumen and in the blood.

P-133

Oxidative stress in horses with experimental small colon intraluminal obstruction – preliminary results

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Initially, believed that the treatment of ischemic bowel diseases was only restore circulation through surgical correction. However, later noted that some intestinal section evolved to necrosis. The advance of pathophysiology knowledge found that the tissue reperfusion, although essential for the preservation of cells, also exacerbates pre-existing lesions, called reperfusion injury. Ischemia is defined as reduction or interruption of blood flow and is considered a major cause of tissue damage. One reason is the increased production of reactive oxygen species (ROS) in the post-ischemic tissue. These ROS are normally produced by mitochondria, being removed by the cellular defense system. The balance between removal and production of ROS is of fundamental importance. If there is instability, the ROS initiate the oxidative stress, producing tissue changes by lipid peroxidation in cytoplasm and membrane cell, protein oxidation and nucleic acids fragmentation. Therefore, the aim of this study was to evaluate the occurrence of oxidative stress by the instability between oxidative and antioxidants indicators in horses with experimental intraluminal obstruction. For this purpose, eight healthy adult mixed-breed horses were subjected to small colon distension using a surgically implanted latex ball in the lumen (Ethical approval: CEBEA- Protocol no: 007568-09). Blood samples were obtained before intestinal distension (M0), after 4 hours of distension (M4- when the ball was deflated and removed) and at 12-hour intervals until 72 hour after latex ball removal (M12, M24, M48, M60, M72). Data were submitted to a normality test and then analysis between moments was performed by using One-Way ANOVA and Tukey's post hoc test. This model induced alterations in glutathione (GSH) and vitamin E (Vit E) after tissue reperfusion. Nevertheless the malondialdehyde (MDA) showed no significant difference. GSH concentration (μmol/gPT) (mean+-standard deviation) at M24, M36 and M48 was significantly higher when compared to M0 (M0=7.37+-0.61; M24=9.07+-0.67; M36=9.77+-1.05; M48=9.33+-0.72) whereas Vit E (μmol/L) showed higher means at M48, M60 and M72 (M0=8.69+-4.15; M48=15.22+-7.18; M60=14.94+-7.66; M72=14.93+-8.70). Thus, it was concluded that reperfusion after latex ball removal is associated with increased antioxidant substances (GSH, Vit E) which can prevent the formation of catabolites (MDA) associated to oxidative stress.

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P-134

Physiological growth performances in new born puppies of Labrador Retriever breed. Preliminary results

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The study aimed to define the standard growth curves in Labrador Retriever breed during the first 4 weeks of life, with the aim of optimizing the specific assistance postpartum. The definition of the physiological growth parameters of puppies in relation to the breed, such as weight at birth and average daily gain, represents the tool to monitor the daily development and to ensure neonatal survival in dogs . In our study we selected weight values of 147 healthy full-term puppies relative to 22 parturitions from Labrador Retriever breed bitches. Puppies were fed only with mother milk, no other food or liquids were added. A length of 63±1 days was considered a full-term gestation from ovulation day (65±1 days from LH peack). The pregnant bitches were fed under standard conditions with a dry maintenance feed 50% increased dose, referring to the normal daily requirement from the 28th day of pregnancy. The diet was gradually replaced from the 55th day of pregnancy with dry puppies feed until weaning. No pharmacological treatments were used to promote milk production. The puppies dead during the first two weeks were not included in the study. The puppies were weighted immediately after birth and every 24 hours for the following 28 days using an electronic scales. Statistical analysis of body weight was performed using a one-way analysis of covariance (male *vs.* female), with the age of bitches as the covariate, by the GLM procedure of SAS. The bitches in our sample completed 22 pregnancies with an average of 6.7 weaned puppies. Males were 50% and females 50%. The growing was linear during monitoring time and no weight loss during the first three days after birth was registered. The average body weight at birth was 440.80±81.62g, nearly doubled in 9 days (954.75±188.81g), tripled in 17 days (1,369.67±390.41 g) and quadrupled in 21 days (1.794,75±445.04 g) after birth. The average body weight at initial weaning period (Day 28) was 2,682.31±494.82g. No significant effect related to sex was recorded.

P-135

Metabolomics information provides potential predictive internal phenotypes for production and performance traits in Italian Duroc pigs

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Over the past decades, selection programs in pig breeding have been based on final phenotypes, like growth, feed efficiency and carcass traits that are the results of complex biological mechanisms and their interactions with environmental factors. These complex biological mechanisms can be dissected into fine molecular phenotypes using metabolomics information. The metabolome of a biological system can provide a readout of the integrate response of the cellular processes to genetic and environmental factors. This reductionist approach can also be used to predict and model the outcome of end phenotypes based on internal phenotypes, represented by metabolomics data. In this study, we investigated the relationships between metabolomics data and production traits using a population of 389 performance tested Italian Duroc pigs. These pigs were tested within the Sib testing program at the Central Test Station of the National Pig Breeder Association (ANAS). After slaughtering in a commercial abattoir, blood samples were collected on these animals and plasma concentration of about 200 metabolites was determined by mass spectrometry analysis. Estimated Breeding Values (EBVs) for several traits (average daily gain, feed:gain ratio, back fat thickness and lean meat content), and numerous other carcass traits, measured after slaughtering, were calculated and provided by ANAS. Those EB*vs.* and carcass traits were used to identify correlated metabolites. After filtering of metabolomics data, 116 metabolites were selected for further analyses. Identification of the most important metabolites associated with carcass and performance traits in the investigated pigs was obtained using PLS methods, in addition to a new statistical validation approach based on permutations. Selected metabolites from the groups Acylcarnitines, Amino Acids, Biogenic Amines, Glycerophospholipids, Sphingolipids and Sugars, might represent new potential molecular markers useful to predict end phenotypes. The molecular dissection of complex traits will help to understand the fine biological pathways affecting complex traits.

P-136

Milk and plasma IgG levels in primiparous and multiparous cows, during the first week of lactation

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Bovines are born agammaglobulinemic and achieve immune protection by transport of colostral immunoglobulins into the bloodstream through intestinal epithelium. This transfer depends on initial permeability of intestinal mucosa and transcytotic mechanisms mediated by receptors. Despite immunoglobulin transfer ceases within 48 hours after birth, receptors persist into adulthood. In bovine colostrum/milk the predominant immunoglobulin is IgG (subclasses IgG1 + IgG2), which mainly derives from maternal blood. During the first week of age, interaction between ingested IgGs and intestinal receptors stimulates intestinal mucosa thickness and villi length of the newborn. During the first week of lactation, colostrum/milk and blood IgGs were analysed in 7 primiparous and 7 multiparous Fresian dairy cows, in order to identify factors affecting their level. All cows were fed a total mixed ration *ad libitum*. On day 0, 1, 3 and 7 of lactation, milk yield and composition were determined and colostrum/milk and blood samples were tested for IgG1 and IgG2 by ELISA. Data were analysed by the PROC MIXED RM method of SAS. Colostrum/milk yield ranged between 11.5 and 34.7 L/d, without differences between primiparous and multiparous cows, throughout the study period, but protein and casein were higher ($P<0.05$) in colostrum/milk of multiparous ones, from day 1 to 7. By contrast, on parturition day, fat (95.4 *vs.* 58.8 g/L, SE=9.35, $P<0.01$) and urea (0.81 *vs.* 0.65 g/L, SE=0.04, $P<0.05$) were higher in colostrum of primiparous cows. In both groups, maximum levels of IgGs were observed at parturition. Total IgG was twice higher in colostrum of primiparous cows (2.47 *vs.* 1.26 g/L, SE=0.30, $P<0.05$), but this difference was entirely due to IgG2 (1.20 *vs.* 0.37 g/L, SE=0.15, $P<0.01$), whereas colostrum IgG1 did not differ between groups.. In both groups, colostrum/milk IgG1 correlated ($P<0.05$) negatively with milk yield and positively with milk protein. In primiparous cows, colostrum/milk IgG2 correlated ($P<0.01$) negatively with milk yield and positively with milk urea. Maximum levels of blood IgGs were observed at parturition, without differences between groups. Results suggest that milk yield, protein and urea, parity and distance from parturition are all factors affecting the level of IgG in colostrum and milk. Results suggest that IgG1 and IgG2 are transferred from maternal blood into milk through different processes and the efficiency of IgG2 transfer seems to decrease with parity.

P-137

Effect of cocoa husks diet on lipid metabolism and liver composition in fattening pigs during the hot season

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Cocoa husks are byproducts from chocolate production, characterized by high contents of proteins, lipids, dietary fibre and antioxidants, polyphenols in particular. The present study evaluated the effect of cocoa husks feeding on lipid metabolism and liver composition in finishing pigs. Eight castrated male Duroc x Large White pigs (135 ± 4.06 kg, mean ± SE) were randomly assigned to one of two groups: CTRL, fed a conventional pelleted diet based on cereals, and COCOA, fed a diet obtained by substitution of 10% of the conventional diet with coarsely-ground cocoa husks. The experimental diets were isoproteic and isoenergetic, but EE (4.53 *vs.* 3.60 % DM), NDF (14.2 *vs.* 12.8 % DM), ADF (7.19 *vs.* 5.03 % DM), ADL (2.22 *vs.* 1.21 % DM) and total polyphenols (15.4 *vs.* 8.18 g/kg DM) were higher in COCOA than CTRL diet. The trial was conducted during the hot season (T = 27.1 ± 2.49 °C; Ur = 65.0 ± 4.65 %) and lasted 6 weeks, after which blood samples were taken before the first meal of the day, and analyzed for lipid parameters. Pigs were then sent to the slaughter house, where body and liver weights were recorded and liver samples were collected and analyzed for composition. Obtained data were analyzed by one-way ANOVA. Consumption of cocoa husks for 6 weeks reduced ($P<0.01$) by 10 % DM and energy intake, but had no effect on body and carcass weights and hot dressing percentage. Cocoa husks diet increased plasmatic level of HDL cholesterol (38.5 *vs.* 25.9 mg/dL, SE=5.34, $P<0.05$), without affecting total and LDL cholesterol. In humans, increased HDL cholesterol is associated with reduced risk of cardiovascular diseases. The intake of polyphenols has been shown to increase plasma HDL cholesterol in both humans and animals, but the mechanism by which polyphenols stimulate HDL cholesterol synthesis by the liver remains unclear. Cocoa husks feeding did not affect liver cholesterol, but reduced liver weight (1634 *vs.* 1833 g, SE=88.5, $P<0.05$) and liver content of soluble protein (66.7 *vs.* 84.8 mg/g, SE=4.43, $P<0.01$), DNA (3.20 *vs.* 4.74 mg/g, SE=0.34, $P=0.01$) and glycogen (24.6 *vs.* 88.4 mg/g, SE=10.7, $P=0.01$). By contrast, cocoa husks increased liver ether extract (15.1 *vs.* 11.8 mg/g, SE=1.13, $P=0.05$). Previous studies showed that liver weight decreases in pigs exposed to high/discomforting temperatures, in order to reduce endogenous heat production. For the same purpose, animals decrease protein synthesis and store energy in the form of fats, because protein synthesis is energetically expensive and largely contributes to total heat production, whereas fat deposition is a more efficient and less thermogenic process. This leads to a shift toward the use of carbohydrates as energy substrate and a consequent reduction in glycogen storage in the liver. Results suggest that, during the hot season, cocoa husks diet may foster liver metabolism, in order to promote animal coping with environmental conditions. This hypothesis is supported by the fact that COCOA pigs needed less feeding to reach similar body and carcass weight of the CTRL ones.

P-138

Use of prolactin as an indicator of stress in purebred and crossbred cattle in a farm of Yucatán (Mexico)

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The aim of this study was to evaluate the adaptability to the tropical climate of purebred and crossbred cattle reared in a ranch near Tizimín, Yucatán, Mexico, through the prolactin (PRL) serum levels comparison. The PRL has a well-documented role in milk production, and plays a role in stress correction, immune system and thermo-regulation. The trial involved 11 calves: six females and five males, three Brahaman (B), three Chianina reared in America (CNA), three crossbred Chianina Italiana (CNI)xCNA and two crossbred Marchigiana (MRI)xCNA. Serum blood samples were collected twice a day at 6.00 am and at 1.00 pm, in the four Yucatán typical seasons: February (dry season start), May (dry season end), September (rainy season height), November (between rainy and dry season). In total, 22 samples for four seasons were collected. The hour collection choice was determined by relevant ambient temperature (AT) and relative humidity (RH) difference at 6.00 am (AT 21.9 °C; RH 81.5 %) and at 1.00 pm (AT 33.5 °C; 45.1 %). In February, at the trial start, the average age of these animals was 87.0 days, and at the end (November) 348 days. The PRL serum was determined in Italy by radioimmunoassay in the DIMEVET laboratory in Bologna. Data were analysed by a one-way general linear model considering, as factors, sex, breed, month, collection hour, and their interactions. Differences between means were tested with the Tukey test. Correlation between PRL, AT, RH and age were calculated. A PRL level significant difference between sexes ($P<0.0001$), breeds ($P<0.017$) and months ($P<0.002$) was found, but not between collection hours. Interactions sex x breed ($P<0.022$), sex x month ($P<0.009$) and breed x month ($P<0.028$) resulted significant. As expected, females showed a double PRL level than males (54.2 ng/mL *vs.* 20.5 ng/ml). Concerning breeds, CNA showed the highest PRL level (58.1 ng/ml) and MRI x CNA the lowest (16.7 ng/mL). The highest PRL level was found in May (99. 2 ng/mL) and the lowest in November (20.2 ng/mL). No significant correlations between PRL and the other parameters (AT, RH and age) were found. In conclusion, the highly stressing conditions of May, at the dry season end, determined a significant PRL increase in both sexes. No clear genotype influence was found in this trial, probably due to the low subject number. Higher serum PRL in cattle suggests its use as a stress indicator in tropical climate.

P-139

Administration of conjugated linoleic acid in late dry period and early lactation: effects on blood, rumination and productive performance of primiparous and pluriparous dairy cows

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The study investigated the effects of the conjugated linoleic acid (CLA) supplementation in dry period and early lactation on blood (metabolic and inflammatory markers), rumination time and productive performance of primiparous (PR) and multiparous (MU) transition dairy cows. Fifty nine Holstein cows (n=17 PR and n=42 MU) were assigned to control (CTR) or treated group (TRT). TRT cows (9 PR and 20 MU) received a daily supplement containing lipid-encapsulated CLA (Lutrell® pure, BASF, Ludwigshafen, Germany). The integration of CLA was of 20 g/d from -20 to 0 days from parturition (DFP) and 40 g/d from 1 to 7 DFP. The CTR cows (8 PR and 22 MU) received the same amount of placebo (hydrogenated vegetable fats). Blood samples were collected at -20, -3, 1, 3, 7 and 28 DFP for the analysis of the metabolic profile. The body condition score (BCS) was measured after each blood sampling, rumination time (SCR Europe, Podenzano, PC, Italy) and milk yield were recorded daily. Data were evaluated by ANOVA using the MIXED procedure (SAS Inst.). No effect of CLA supplementation were observed for milk yield and rumination time for both PR and MU, while in MU the drop of BCS from -3 to 28 DFP showed a lower value ($P<0.1$) in TRT. The reduced body lipid mobilization in MU-TRT group is confirmed by lower blood concentrations of β-hydroxybutyrate at 3 DFP ($P<0.1$) and non-esterified fatty acid at

7 DFP ($P<0.1$). At 1 and 3 DFP, creatinine concentrations (index of muscle tissue) showed a marked reduction in MU-TRT *vs.* CTR ($P<0.05$). The inflammatory condition showed inconsistencies between PR and MU cows. The CLA supplementation allay the inflammatory in MU, as confirmed by higher plasma zinc and lower bilirubin concentrations at 3 DFP ($P<0.1$), lower haptoglobin and higher paraoxonase concentrations at 7 DFP ($P<0.1$). However, PR-TRT *vs.* CTR cows showed contradictory blood changes: the higher concentrations of zinc at 1 DFP and paraoxonase at 3 and 28 DFP ($P<0.05$) suggest a better health status, contrariwise the higher ceruloplasmin, myeloperoxidase and ROMs values at 7 DFP ($P<0.1$), denote a more severe inflammation. Overall, the CLA supplementation showed greater effects in MU compared to PR cows. The week after calving, CLA modulates the utilization of body reserves and attenuates the typical inflammatory response. The less prolonged metabolic effects measured in the present study in comparison with previous experiments seem due to the shorter time of CLA treatment.

P-140

Hemogasometric variables of sheep (*Ovis aries*) induced to lactic ruminal acidosis by feeding high concentrate diet

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This study verified changes due to lactic ruminal acidosis, induced by high proportion of concentrated feed on daily diet, in hemogasometric values of Santa Inês sheep, a Brazilian rustic, without wool ovine breed used for meat production. Seven ewes were kept in pens, maintained at least for 60 days on basal diet of Tifton hay (Cynodon dactylon), drinking water and mineral mixture *ad libitum*. For induction, they received a diet, based on the average consumption of adaptation period (2 kg of organic matter/animal/day), in which was replaced, daily, 10% of roughage for concentrated feed (76.8% ground corn, 18.2% soybean, 5% mineral core) up to 80%, percentage maintained thereafter. Assessments were performed before (control), daily for 15 days after dietary change, every other day until the end of the first month and weekly in the rest of the five-month experiment. Samples were collected under physical restraint in hoof trimming crush, in dorsal decubitus, by auricular or carotid artery puncture, into syringes with lyophilized lithium heparin (BD Preset, BD Diagnostics, Brazil), avoiding air bubbles. After sealed and mixed, they were stored at -4ºC for up to 30 minutes. Measurements were performed through a benchtop analyzer (Cobas b 121 System, Roche Diagnostics GmbH, Germany). Associations among variables and time by linear, quadratic and cubic regression were assessed using statistical software (SAS 9.1, SAS Institute, USA), with $P\leq0.05$ significance level. Blood pH and oxygen saturation - SO_2 were not associated with time ($P>0.05$). Partial pression of oxygen - PO_2 ($P\leq0.05$) and anion gap - AG ($P<0.01$) tended to raise throughout the period. Partial pression of carbon dioxide (PCO_2) reduced in the first nine days, increased up to the eighth week, and then decreased ($P<0.05$). Bicarbonate - HCO_{3-}, carbon dioxide - $ctCO_2$ ($P<0.001$) and base excess - BE ($P<0.0001$) concentrations showed reduction before the 14th day, increased up to the 10th week and decreased again. Strong ion difference – SID curve fell until the 12th day, then tended to lift ($P<0.01$). Ingestion of great proportions of concentrated feed altered most variables, mainly in the first days, during adaptation of sheep to the new diet.

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P-141

Serum enzyme activities of sheep (*Ovis aries*) induced to lactic ruminal acidosis by feeding high concentrate diet

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This study verified changes due to lactic ruminal acidosis, induced by high proportion of concentrated feed on daily diet, in the serum enzymatic profile of Santa Inês sheep, a Brazilian rustic, without wool ovine breed used for meat production. Seven ewes were kept in pens at least for 60 days on basal diet of Tifton hay (Cynodon dactylon), drinking water and mineral mixture *ad libitum*. For induction, the animals received a diet, based on the average consumption of adaptation period (2 kg of organic matter/animal/day), in which was replaced, daily, 10% of roughage for concentrated feed (76.8% ground corn, 18.2% soybean, 5% mineral core), up to 80% of the diet, percentage maintained thereafter. Enzymatic assessments were performed before (control), daily for 15 days after dietary change, every other day until the end of the first month and weekly in the rest of the five-month experiment. The samples were collected under physical restraint in hoof trimming crush, in dorsal decubitus, by external jugular venipuncture into plastic tubes of 10 mL volume without anticoagulant. Serum fractions were separated by centrifugation as quickly as possible, and stored at -70ºC in plastic sterile microtubes. The enzyme activities (U/L) were measured, through semi-automatic spectrophotometer analyzer and commercial kits (LabQuest, LabTest, Brazil). Associations among variables and time by linear, quadratic and cubic regression were assessed using statistical software (SAS 9.1, SAS Institute, USA), with P≤0.05 significance level. Aspartate aminotransferase – AST levels increased before the ninth day, decreasing up to sixth week and raising again for the rest of the experiment (P≤0.05). Alkaline phosphatase - AP activity increased markedly until the 84th day, and then decreased (P<0.001). The association of lactate dehydrogenase - LDH and time was almost constant, with a slight reduction between the first and the seventh weeks (P≤0.05). Gamma-glutamyl transferase - GGT levels increased considerably in the first 11 days, with other marked rise from the 10th week to the end of the experiment (P<0.0001). Creatine kinase - CK concentration tended to reduce throughout the observational period (P<0.0001), probably due to the adaptation of animals to sampling.

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P-142

Serum electrolyte variables of sheep (*Ovis aries*) induced to lactic ruminal acidosis by feeding high concentrate diet

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This study verified changes due to lactic ruminal acidosis, induced by high proportion of concentrated feed on daily diet, in the serum electrolyte variables of Santa Inês sheep, a Brazilian rustic, without wool breed used for meat production. Seven ewes were kept in pens at least for 60 days on basal diet of Tifton hay (Cynodon dactylon), drinking water and mineral mixture *ad libitum*. For induction, they received a diet, based on the average consumption of adaptation period (2 kg of organic matter/animal/day), in which was replaced, daily, 10% of roughage for concentrated feed (76.8% ground corn, 18.2% soybean, 5% mineral core) up to 80%, maintained thereafter. Assessments were performed before (control), daily for 15 days after dietary change, every other day until the end of first month and weekly in the rest of the five-month experiment. Samples for total calcium - Ca, phosphorus - P, magnesium - Mg (through semi-automatic spectrophotometer - LabQuest, LabTest, Brazil) and chlorides - Cl (by electrolyte analyzer - Roche 9180, Roche, Brazil) measurements were collected from external jugular, into tubes without anticoagulant, and serum fractions were stored at -70ºC. Samples for sodium - Na, potassium - K and ionized calcium - iCa assessments, from auricular or carotid artery, were obtained into syringes with lyophilized lithium heparin (BD Preset, BD Diagnostics, Brazil), packed at -4ºC for up to 30 minutes, and evaluated by benchtop analyzer (Cobas b 121 System, Roche Diagnostics GmbH, Germany). Associations among variables and time by linear, quadratic and cubic regression were assessed by statistical software (SAS 9.1, SAS Institute, USA), with P≤0.05 significance level. Ca (P≤0.05) and P (P<0.01) levels reduced in the first eight days, increased up to the 13th week, and decreased for the rest of experiment. Oppositely, iCa have risen before the 10th day, fell up to the 12th week, then increased until the end (P≤0.05). K means tended to rise throughout the experiment (P<0.01). Mg increased until the ninth week, then decreased to baseline values at the end (P<0.01). Na concentration reduced before the ninth day, tending to lift thereafter (P<0.001). Ingestion of great proportions of concentrated feed altered all variables analyzed.

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P-143

Archaeal population in the horse gastrointestinal tract

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After the digestion in the stomach and small intestine by endogenous enzymes, the digesta goes through the large intestine. Here the symbiotic microbiome (bacteria, protozoa, fungi and archaea) of the caecum and colon in some species, like Equidae, largely contributes to the total energy supply, producing short chain fatty acids. This makes Equidae very effective hindgut fermentors. In spite of this, the knowledge on the composition and fermenting activity of horse caeco-colic microbiome is still rather limited in these animals. The latest research indicates a large number of uncultured microorganisms in the horse digestive tract and considerable differences among the equine caecal, colonic and rectal microbial populations. This work aims to evaluate the archaeal community of the horse digestive tract and to compare the methanogens between caecum and rectum. The total DNA was extracted from lyophilised samples of horse caecal and rectal content. The archaeal communities were analyzed by the amplification and sequencing of 16S rRNA gene, using a clone library approach. The phylogenetic analysis was carried out by MEGA 5.2 software program, using the maximum likelihood method. The analysis of the methanogens residing in the horse digestive tract revealed differences between the caecal and rectal samples. In the caecum 64% of the methanogen sequences have a low similarity (96%) with the uncultured archaeon and the cultivable Methanocorpusculum, 2 clones have a good similarity (99%) with the uncultured archaeon obtained from equidae faeces and 35% of the sequences represent a cluster of the unknown archaea. In the rectum 90% of the sequences have 96-99% similarity with the uncultured archaeons found in the equidae faeces and rumen content. 50% of the rectal methanogen sequences are phylogenetically related to the cultivable species Methanomicrococcus, Methanocorpusculum and Methanobrevibacter, the other 50% represent a cluster of unknown archaea. The horse methanogenic archaea profile differs between caecum and rectum. Most of the sequences (85%) are related to the unknown uncultured archaeons, which makes very difficult the evaluation of our results. The methanogenic archaea of the horse digestive tract represent new microorganisms, showing that this ecosystem is still rather unexplored. The research was carried with the contribution of the following programmes: Erasmus+; Visiting Professor and Master and Back 2012/2013 Regione Autonoma Sardegna.

P-144

The effect of the genetic type and handling immediately prior to stunning on pro- and macro- glycogen in two porcine muscles

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The muscular glycogen plays an important role on the extent of the pH decline after slaughter. Two glycogen fractions are described, pro- and macro-, which are metabolized with different priority during physical efforts. The aim of the present study was to evaluate the contents of pro-, macro-, total glycogen, lactate and pH in two muscles of pigs belonging to three genetic types submitted to a rough or gentle pre-slaughter handling. Twenty-eight pigs from Large White (LW), Duroc (DU) and Pietrain halothane-gene-free (P) breeds were slaughtered with the average final weight of 114.0, 123.0 and 115.2 kg. Half of each group, being 5LW, 5DU and 4P, was subjected to one of pre-slaughter handling. Measures of pH and samples were collected from Longissimus thoracis and Semimembranosus at 1 h post mortem. In the rough pre-slaughter handling group (RPH) the animals were submitted before stunning to a fast driving supported by the use of electric prods, accordingly to the EU regulations. In the gentle pre-slaughter handling group (GPH) the animals were driven slowly without electric prods. The data were processed by a model including the fixed effects of handling, genetic type and their interaction. In Longissimus thoracis, the effects of handling, genetic type or interaction in the contents of pro-, macro and total glycogen were not observed. The content of macroglycogen was lower in animals of the RPH group ($P<0.001$) in Semimembranosus: 9.7 *vs.* 16.7 µmol/g. The only interaction was observed between handling and genetic type in total glycogen of Semimembranosus ($P<0.05$), showing that LW pigs of GPH have higher levels than DU of RPH (106.3 *vs.* 54.4 µmol/g). The total glycogen of these two groups was similar to the others. The lactate content in Longissimus thoracis was higher in RPH than GPH ($P<0.01$) (50.9 *vs.* 37.0 µmol/g, respectively) while the genetic type did not show any effect. In Semimembranosus, the effect of both handling and genetic type or their interaction on the lactate contents was not observed. The pH in animals submitted to RPH was lower in both muscles ($P<0.05$) while the genetic type or the interaction between handling and genetic type did not show any effect. We conclude that the effects of handling and genetic type on the levels of pro-, macro- and total glycogen varies according to the measured muscle, being more evident in Semimembranosus and these contents tend to be lower in animals submitted to a rough pre-slaughter handling..

P-145

Effect of dietary inclusion of Spirulina on nutrient apparent digestibility in chinchillas

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Spirulina (*Arthrospira platensis*) is a blue-green microalgae characterized by a high level of protein as well as potential anti-obesity, antioxidant and hypolipidemic effects. The objective of the study was to evaluate the nutrient apparent digestibility of two isoprotein (15.7% crude protein), isoenergy (16.8 MJ/kg) and isofiber (17.8% crude fiber) pelleted diets for chinchillas, with or without Spirulina supplementation. With this aim, 20 growing chichillas (Chinchilla lanigera) of both sexes, with an average body weight (BW) of 268 g, were fed either a control diet (diet C) or an experimental diet with Spirulina at 2% inclusion level (diet S). The animals were individually allocated in digestibility cages for an adaptation period of 7 days followed by a 5 day-digestibility trial. During the digestibility trial, for each subject feed intake was calculated and faeces were collected and weighed. Diets and faeces were analysed for dry matter, organic matter, crude protein, ether extract, ash, starch, crude fiber, and fiber fractions content. The effect of the diet was tested by ANOVA using the results from the animals that completed the trial: 5 on diet C and 8 on diet S. The treatment showed no difference on feed intake (0.10 g DM/kg BW for C and S diets). The energy digestibility of the experimental diets (C: 41.3 *vs.* S: 49.1%), as well as the digestibility of dry matter (C: 38.0 *vs.* S: 44.9%) and organic matter (C: 40.7 *vs.* S: 47.3%) were similar although generally low. Contrarily to what was observed in rabbits and guinea pigs, enriching the diet of chichillas with Spirulina resulted in higher crude protein (C: 50.0 *vs.* S: 59.8%; $P<0.05$) and ether extract (C: 70.2 *vs.* S: 80.3%; $P<0.05$) digestibility as well as in a higher starch (C: 98.7 *vs.* S: 99.0%; $P=0.05$) digestibility. In chinchillas, the dietary inclusion of Spirulina has the potential of improving the nutritional value of the diet.

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P-146

Influence of early microbial association and dietary medium chain triglycerides on development of gastric function in pig

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Several environmental factors shape the gut microbiota in the early life, but it is not clear if early microbial colonization affects the gut functional development. The priming effect of oral association with microbiota of different complexity and of dietary medium chain fatty acids (MCT) was tested on the gastric transcriptome profile, in 24 pigs obtained by caesarean delivery (day 0), housed in two separate rooms, that received 45 mL pasteurized sow colostrum at 1 and 5 h after birth, and 2 mL of a starter microbiota (10^7 CFU of each Lactobacillus amylovorus, Clostridium glycolicum, and Parabacteroides spp.) on d 1, 2 and 3 of life. In addition, the pigs received either a complex microbiota in 2 mL of either a placebo inoculant (simple association, SA) or an inoculant consisting of diluted feces of an adult sow (complex association, CA) on d 3 and 4. Piglets were fed *ad libitum* a milk replacer diet till d 4 and then till d 21 a diet with either 7% coconut oil (MCT) or soybean oil (CTRL). Samples of oxyntic tissue were obtained of all pigs euthanized on d 21. Total RNA expression was analyzed by Affymetrix Porcine Gene 1.1ST array strips. Exploratory functional analysis of gene expression values was done by Gene Set Enrichment Analysis. SA phenotype enriched 292 gene sets compared to CA. Most of them were related to mitosis and cell cycle phases, and DNA and RNA replication. CA phenotype enriched 125 gene sets compared to SA. Provision of the MCT diet enriched 184 gene sets, compared with CTRL, including sets related to response to virus, chemokine activity and binding, response to stimuli, and neurite development. Provision of the CTRL diet enriched only 14 gene sets, compared with feeding the MCT, related to energy and lipid metabolism. Several nodes of genes related to anion channel activity were upregulated in CA-associated pigs fed MCT, compared to SA-associated pigs, fed the same diet. The results suggest that a. early association of newborn pigs with a complex microbiota favorably prevents the activation of pathways related to cell division in the gastric mucosa, compared to the association with a simple microbiota, presumably in relationship with previous observations that a short encounter of gastric tissue with a complex microbiota can be sufficient to influence the intestinal microbiota in the subsequent period and b. provision of an MCT diet has an effect on gastric transcriptome similar to the early association with a simple microbiota.

P-147

Microscopic differential cell counting and viability evaluation of milk somatic cells during lactation in goat

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Milk contains different cell populations commonly known as somatic cells, changing their distribution under the influence of several factors as physiological status and stage of lactation, and useful to assess the transcriptional activity of the mammary gland in livestock. The aim of this study was to compare the differential somatic cells counting (DCCs) and cell viability in goat milk samples collected at morning milking (MM) and 2 hours later (2H), in order to verify if some difference occurred between the two sampling times. This investigation was carried out within a project addressed to optimize studies on gene expression in goats mammary gland. In 11 pluriparous Alpine goats lactations were studied for 21 wks. Individual milk yield was recorded in three different time points (TP), at 38±5 days (TP0), 91±7 days (TP1) and 140±7 days (TP2) of lactation. Milk samples were collected twice a day: at MM and 2H, in the different TPs. For DCCs, 150 mL of milk were centrifuged and the pellet obtained was washed and resuspended in PBS. Slides were smeared and stained with May Grunwald-Giemsa stain. Two hundred cells were evaluated per slide under a light microscope, classified as neutrophils, lymphocytes, macrophages and epithelial cells, and expressed as a percentage of the total number of cells counted. Cell viability was estimated by trypan blue exclusion test. Two hundred cells were counted using a Neubauer's chamber, and discriminated as live or dead. A two-way analysis of variance (General Linear Model, PROC GLM, SAS Institute) was used to analyse the effects of TP and milking distance (MM and 2H) on milk somatic cells composition and viability. Treatment effects were declared significant at P≤0.05. Percentage of neutrophils increased significantly during lactation, whereas % of lymphocytes, macrophages and epithelial cells decreased. The percentage of viable cells in milk increased significantly from MM to 2H samples (63.97±2.35 and 77.01±2.25, respectively) but was not affected by lactation stage (TP0=70.0±3.4; TP1=69.5±2.4; TP2=72.0±2.4). Moreover the milking distance didn't affect somatic cells composition (neutrophils: MM=54.7±2.9, 2H=55.6±3.5; lymphocytes: MM=35.4±2.5, 2H=33.4±3.0; macrophages: MM=3.9±0.3, 2H=4.1±0.4; epithelial cells: MM=5.7±0.8, 2H=6.6±1.0). Results obtained in this study suggest that somatic cells are more viable and transcriptionally active in samples collected 2 hours after morning milking.

P-148

Estimation of dry matter intake in dairy cows fed TMR using different markers

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Knowledge of individual dry matter intake (DMI) is important to select cows for dairy efficiency, to reduce the livestock environmental impact and to improve profitability. The alkane technique has been widely used to estimate feed intake mainly in grazing ruminants but also in group fed dairy cows, but limited information is still available on dairy cows fed a total mixed ration (TMR). Estimations are more frequently carried out using the even-chain C32 as external marker and the odd-chain C31 or C33 as internal ones, and total faeces collection. A similar recovery for odd-chain, naturally occurring, and even-chain dosed alkanes in faeces is assumed, but this is not always the case. Acid insoluble ash (AIA) is alternatively used as internal marker. On commercial farms, total faeces collection is not possible but it is necessary to limit faeces collection to one or few times per day, though the daily excretion pattern of alkanes can affect accuracy of estimates. The aim of this study was to evaluate the reliability of the alkane ‚Äì AIA technique, based on faecal grab sampling, to predict feed intake in lactating cows fed a TMR diet. The trial was carried out on 6 lactating cows fed a TMR diet, housed in a free stall box and dosed for 14 days with 0.5 g/d of C32 n-alkane, administered in 100 g of corn meal, top fed once a day at feeding time. Daily feed intake was measured with Calan gates and ranged from 17.2 to 26.5 kg/h/d. During the last 7 days of dosing, faeces were collected around at feeding time that was left to vary +/- 0.5 hour to simulate a commercial farm situation. Average estimated DMI were 22.2, 19.72 and 20.37 kg/d based on AIA, C31 and C33 as internal marker, and did not differ ($P>0.05$) from measured DMI (21.3 kg/d). The coefficients of determination of the regression equations between observed and predicted DMI based on the AIA: C32, C31:C32 and C33:C32 pairs were 0.93, 0.93 and 0.89, respectively ($P<0.01$). The maximum absolute difference between measured and predicted was 2.0 kg/d. The alkanes technique allows to estimate DMI in group fed cows and housed in free stall barns with a fairly good accuracy.

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P-149

Fat and fiber in natural ingredient diets and purified diets for laboratory animals

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The present study was designed to evaluate nutritional composition and lipid quality in rodent diets. For this purpose seven natural ingredient diets (NIDs) and three purified diets (PDs), have been selected and analysed for dry matter (DM) crude protein (CP), ether extract (EE) crude fiber (CF) and neutral detergent fiber (NDF) and ash contents. Lipid peroxidation has been determined by peroxides values (PV) and Thiobarbituric acid value (TBA value). Recorded data were analysed for raw means, except for NDF and CF for witch correlation has been tested (PROC CORR, SAS 9.4). The mean chemical composition (expressed on DM) of the NIDs and PDs were repsectively: CP 20.4 and 15.24%, CF 4.4 and 2.23%, NDF 19.86 and 3.5%, EE 5.4 and 7.56 %, Ash 2.3 and 19.86%, PV 115.1 and 102.6 meq O_2/Kg fat and 0.045 and 0.032 mg malondialdehyde (MDA)/ 100 g of samples for TBA value. Based on these figures the main nutritional values were comparable in both groups, even if some differences have been observed for CF, NDF and Ash. A wide difference has been observed between CF and NDF values measured in NIDs, by contrast the same variables were closer in PDs. Of note, NDF and FG values were not correlated. PV results indicated that: in both groups high peroxidase values (>100 meq O_2/Kg fat) have been measured in low fat content diets (2.81 up to 5% of ether extract). By contrast, when the highest fat content diets for each group (8.89 and 11.38 for NIDs and PDS, respectively) were considered, peroxide value was lower in PDs diet than in NIDs one (25.90 *vs.* 120.60 meq O_2/Kg fat). The TBA means was respectively 0.045 and 0.032 mg MDA/ 100 g of sample for NIDs and PDs, ranged from 0.017 to 0.092 mg MDA/ 100 g of samples. Results obtained have indicated that: 1) in lab animal diets CF and NDF can be extremely different also for the same sample; different raw materials, in the case of NID, and sources of fibre, for PD, seems to be the main reason of that. To measure both values is therefore recommended; 2) in lab animal diet low fat content do not prevent lipid peroxidation, while fat sources (olive oil *vs.* soybean oil) as well as technological treatments (i.e. irradiation, sterilisation) may affect fat quality and stability. However, it should also be considered that, not only this study has been done on limited number of samples, but also peroxides are transitory products that represent just an indication about fat quality.

P-150

A proteomic approach in biomarker discovery for paratuberculosis, in sheep and goats

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Paratuberculosis (Johne's disease) is an intestinal inflammation associated with infection by Mycobacterium avium subsp. paratuberculosis (MAP). Even among small ruminants, the clinical forms and strains differ substantially between sheep and goats, resulting granulomatous enteritis, mesenteric lymphadenitis, decay and death, with severe economic losses for farmers. The PON EPISUD project n° PON01_ 01841, project funded by MIUR (Ministero dell'Istruzione, dell'Università e della Ricerca) aimed to study paratuberculosis in sheep and goats in Southern Italy. One of the objectives of the PON EPISUD is the identification of some protein biomarkers to be used for the production of diagnostic kits able to identify infected and infectious animals before overt clinical phase, during which the ELISA test, currently available, is not effective in the infection diagnosis. To this end, an MS-based proteomics approach to evaluate the changes in the protein constituency of cells, as a cause or consequence of the disease, was proposed. In this work, the traditional proteomic techniques such as two-dimensional electrophoresis (2DE) and mass spectrometry (MS) are applied to identify possible protein biomarkers that could be used in distinguishing healthy from ill subjects. Particularly, 388 blood sera of sheep (110 from ill, 278 from healthy sheep), and 116 blood sera of goats (45 from ill, 71 from healthy goats) were processed with 2DE analysis. The 2DE maps were firstly studied with the software Image Master 2D Platinum (GE Healthcare); then compared with the human plasma map on the ExPASy, Bioinformatics Resource Portal so that, based also on the relevant literature, different groups of proteins with specific physiological function were identified. The statistical analysis of protein spot parameters of the 2DE map, highlights significant differences between healthy and ill subjects. Infact, in some areas corresponding to the groups of Apolipoprotein A-I (pI range 4,5-7; molecular weight 20-28 kDa), Alpha-1-antitrypsin (pI range 4,5-5,5; molecular weight 50-60 kDa), and Immunoglobulins heavy chain gamma (pI range 5,5-7; molecular weight 55-40 kDa) there are variations in the number, intensity, volume and area of spots. The most variable spots were analyzed by in gel tryptic digestion and LC/MS-MS, and the results were submitted to Mascot Uninterpreted MS-MS database search in order to identify the proteins more related to presence/absence of disease.

P-151

Effects of dietary microalgae (*Phaeodactylum tricornutum*) supplementation on some nutritional characteristics of tilapia (Oreochromis niloticus)

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The study evaluated the inclusion of microalga Phaeodactylum tricornutum (2% and 4% on dry weight) in the commercial feed for Nile Tilapia (Oreochromis niloticus) as finishing diet. The study evaluated the inclusion of microalga Phaeodactylum tricornutum (2% and 4% on dry weight) in the commercial feed for Nile Tilapia (Oreochromis niloticus) as finishing diet. *P. tricornutum* is a diatomaceous microalgae known as one of the most promising EPA source. Fish were divided in 3-tanks of 1 m^3 each one (42 fish/tank: Control, Pt2% and Pt4%) with a density of 10 kg/m^3. Lyophilized *P. tricornutum* powder was included in commercial pellets using agar as a coating agent; pellet coated only with agar was used as a control diet. The initial total body weight of fish was 240.3±40 g. Fish were fed twice a day for 45 days. Zootechnical parameters (weight, length, SGR) were assessed at the 15th, 30th and 45th day of the experiment and on 5 fish from each treatment chemical composition, fatty acid profile, colour and lipid oxidation (TBARS) of fillet were evaluated. Colour and TBARS were performed at 0, 3 and 5 days of cold storage (4 °C) of fillets with and without vacuum. The Pt2% group showed: (i) after 45 days a 50% increase in weight compared to 44 % of the Pt4% group ($P<0.01$) and 35% of the control group ($P<0.05$); (ii) in the first 15 days an increase, although not significant, of intramuscular lipid level (from 1.23 % to 2.79%) and of protein (from 14.3% to 16.6%). The level of 2% of microalgae modified also the fatty acid composition of lipids, making a statistically significant decrease of total saturated fatty acids ($P<0.01$), and an increase of the total unsaturated FA ($P<0.01$) and particularly of ⇨-linolenic acid ($P<0.01$). The latter is of extreme importance as a precursor of EPA and DHA. The best lipid profile in relation to time was obtained after 30 days for the highest value ($P<0.05$) of n3-PUFA, EPA, and n3/n6 ratio. Moreover no significant differences in colour profile were detected among all groups and at this level there were no appreciable differences in colour of fillet with two kind of storage (under vacuum or not). The present study suggests that P. tricornutum in dose of 2% of dry weight for 30 days may enhance the nutritional profile of Tilapia without detrimental effects on lipid oxidation and colour during cold storage of fillets.

P-152

Productive and nutritional traits of recent released varieties of cotton grown in Southern Italy

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The aim of the present work was to evaluate productive traits and oil content of 15 recent released varieties of cotton for a possible reintroduction in the cropping systems of Southern Italy and use the seed as ingredient in animal feeding. The trial was carried out in 2011 in Gela Plain on a clay soil on 14 varieties of Gossipium hirsutum and 1 hybrid of Gossipium barbadense (5A) adopting a randomised block design with three replications. Sowing was performed on the 6th of May with a plant density of 12 plants m^{-2}. The fatty acids methyl esters (FAMEs) were analyzed by GC-FID. Individual fatty acids were expressed in g 100 g^{-1} of the total fatty acid methyl esters identified. All data were submitted to one-way ANOVA (percentage data were previously arcsine transformed) and Tukey's HSD was adopted as multiple comparison test. Fibre yield was on average 1.05 t ha^{-1}, with a group of seven varieties that showed the highest fibre production, among which emerged, 'Elsa' and 'Claudia' . As far seed production, the most productive varieties resulted '5A' and 'Elsa', with a seed yield of 1.83 t ha^{-1}, on average. Oil content was on average 18.5 g 100 g^{-1}. Among the fatty acids of nutritional interest, results showed that the fatty acid profile was little influenced by varieties, less than the agronomic traits.

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P-153

Qualitative traits of different varieties of guar grown in Southern Italy

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Inadequate availability and increasing prices of quality feed ingredients have made it increasingly difficult to formulate a balanced and least cost animal feed. This situation advocates the exploration of non-conventional feed resource. Guar (*Cyamopsis tetragonoloba* L.) is an interesting summer legume crop used in many industrial activity, which by-product named guar meal, have a well-recognised application as animal feeding due to its high protein content. The aim of the present research was to assess the qualitative traits of eight varieties of guar with different origin: India, South Africa and USA (Kinman, Lewis Matador and Monument) grown in Southern Italy. The trial was carried out in 2012 in Gela Plain on a clay soil adopting a randomised block design with three replications. Seeds were analysed for the main qualitative traits (protein, oil, fibre, ash) and polyphenols. The amount of total phenols was measured by using a UV-VIS spectrophotometer (UV-2600 Shimadzu), at the absorbance of 760 nm. Gallic acid was used as a positive control. Total phenol values were expressed in terms of gallic acid equivalent-GAE (mg/g of extracted compounds). All data were submitted to one-way ANOVA (percentage data were previously arcsine transformed) and Tukey's HSD was adopted as multiple comparison test. The mean values of dry matter for the eight varieties of guar was of 89.8%, on average. Protein content (g 100 g^{-1} as fed) of the whole seed ranged between 21.2 of Matador and 32.1 of India 2, with an average value of 26.8 and a CV of 17.8. Oil content (g 100 g^{-1} as fed), as reported in literature was quite low, with only South Africa variety that overpassed 3.0. Crude fibre (g 100 g^{-1} as fed) was on average 10.3 showing the lowest variability (4.2). Ash content (g 100 g^{-1} as fed) ranged between 5.32 of Matador and 3.62 of India 2, following the trend already seen for protein content. Polyphenol content (mg g^{-1} of GAE) was very high in Kinman (6.63) and very low in Indian varieties (1.61, on average for the three varieties) with a very high variability (52.2). Results of qualitative parameters indicate guar as a possible cheap and valuable feed source.

P-154

Infra-vitam and post-mortem performance of Italian Merino derived lambs fed with polyphenols; preliminary results

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Antioxidants supplementation, such as polyphenols, in animal nutrition may improve the quality and the shelf-life of the meat. The aim of this study was to evaluate polyphenols integration on infra-vitam and post mortem performance in lambs. The trial was performed on 30 male lambs of Italian Merinos bred over a total period of 41 days. The lambs were weaned at 57±5 days of age and were fed with hay *ad libitum* and concentrate to meet growing requirements. The animals were divided into 3 homogeneous groups, according to age and weight at weaning: Group A (n=10) as control group; Group B (n=10) supplemented with 3.2 mg of polyphenols as powder and Group C (n=10) supplemented with 6.4 mg of polyphenols as capsules on alternate days. The lambs were weighed every 10 days and the daily weight gain (DWG) was calculated. After 40 days the animals were slaughtered and carcass yield and pH were recorded after 45 minutes and 24 hours. Samples of longissimus dorsi were collected from each lamb to evaluate the chemical composition of the meat. Finally, samples of forestomachs were collected to evaluate the histological characteristics by haematoxylin-eosin staining and light microscopy analysis. Data were analyzed by ANOVA for repeated measures. Although a similar live weight was recorded in the three Groups at the end of the trial (27.8±0.7, 26.9±1.1 and 29.2±1.3 kg, in Group A, B and C, respectively), the mean DWG was higher ($P<0.05$) in Group C compared to Group A and B (0.21±0.0, 0.18±0.0 and 0.24±0.0 kg/day in Group A, B and C, respectively). Carcass yield was similar among groups either at 45 minutes (15.1±0.5, 14.7±0.7 and 16.1±0.8 kg in Group A, B and C, respectively) and 24 hours (14.8±0.5, 14.4±0.7 and 15.7±0.8 kg in Group A, B and C, respectively). Similarly, no differences were present in pH values (ranging from 6.6 to 6.7 at 45 minutes and 5.7 to 5.9 at 24 hours) and . meat chemical composition. The ultrastructural microscope analysis of forestomachs mucosal layers observed in Group B and C was more organized than that observed in Group A. In particular, the epithelial mucosa thickness of lambs in Group A was lower than those observed in Groups B and C. Furthermore, lambs fed with polyphenols supplementation showed a more developed and differentiated keratinized sheets of the epithelial mucosa. It can be hypothesized that polyphenols supplementation may improve the ultrastructural organization of forestomachs, improving growth performance in lambs.

P-155

Plant extracts against transport stress in newly received beef cattle

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Newly received beef cattle experience prolonged stressful conditions impairing oxidative and immune status and predisposing to disease. Plant extracts are known for antinflammatory and antioxidant properties. The study aimed to test the effectiveness of a plant extracts blend in reducing oxidative stress, improve immune function and reduce inflammatory status of newly received beef cattle. 100 newly received Charolaise and Charolaise X Limousine male cattle were divided into two homogenous groups, allotted in two big paddock, fed the same diet and monitored for the entire arrival period (50 days). Treated group was supplemented with 15 g/head/day of a blend of Spirea ulmaria, Curcuma longa, Ribes nigrum, Aloe ferox Mill e Silibum marianum extracts (Spireasol®, Fitoterapy). At the arrival (day 0) animal were dosed for internal and external parasites and vaccinated against respiratory disease. Animals were weighed and blood samples were collected from 15 healthy animals per group at days 0, 21 and 50 to evaluate whole blood (WB) and red blood cells (RBC) antiradical activity (KRL test), specific (BHV-1 antibody titer) and aspecific immune status (serum bactericidal activity), serum total protein (STP), albumins, globulins and positive (Serum amyloid A-SAA, Haptoglobin-Hp) and negative (Paraoxonase-1-PON-1) acute phase proteins (APP). Animal was the experimental unit and ANOVA was performed through a mixed model accounting for the fixed effects of plant extract supplementation (PE), sample day and their interaction and random effect of animal within experimental group. PE tend to increase the average daily gain (ADG) ($P=0.08$), while did not improve WB and RBC antiradical activity, likely due to the short supplementation period. Antiradical activity increased for WB ($P<0.001$) and decreased for RBC ($P<0.001$) during the experimental period. PE tend to improve immune reaction ($P=0.10$), without affecting serum bactericidal activity. Both of them increased during adaptation period ($P<0.001$). Treated animals showed lower STP ($P=0.04$) and SAA ($P=0.02$), that decreased during experimental period ($P=0.05$ and $P<0.001$ respectively). PON-1 was not affected by dietary treatment and decreased with time, following an unexpected trend as negative APP. At the present conditions newly received cattle showed a progressive recover from transport stress and the tested PE blend exerted an anti-inflammatory effect, reducing positive serum APP.

P-156

Chemical and microbiological characterization of fruit and vegetable by-products silage

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Organic waste from markets represents about 10-20% of the total waste of a city. A large proportion comes from the overproduction of fruits and vegetables. An alternative for the use of these by-products is the production of animal feed. The valorization of food by-products presents two main problems, the microbiological contamination and its limited life time. A method to inhibit the growth of many bacterial strains could be the ensilage. The aim of this work was to produce a silage from vegetable and fruit by-products, collected from a Despar food store supermarkets (Messina, Italy), for animal feeding. Approximately 8 tonnes of food by-products were collected and transferred to a farm for silage. Vegetables and fruits, with the addition of 20% straw, were homogenized by a mixer wagon. The final product has been stored for 40 days to obtain a stable compound. The samples were taken from different points and depths to prove the product homogeneity. Every 10 days, pH, moisture, ash, crude protein (CP), ether extract (EE), crude fiber (CF), neutral detergent fibre (NDF), acid detergent fibre (ADF), sulphuric acid lignin (ADL) and sugars were quantified. To check the silage health quality and fermentation process, the following parameters were carried out: aflatoxins; Salmonella spp.; *Escherichia coli* O157:H7; Listeria monocytogenes; total coliform (TC); acid lactic bacteria (LAB); yeast and mold (YM). This study has showed the suitability of food by-products to silage process. In the silage, pathogenic strains and aflatoxins were not found in the course of trial. The mainteinance of LAB at value > 6.4 log CFU/g in all period, the decrease of TC at <10 CFU/g, pH (3.84), glucose and fructose (both ,â§ 0.006% dm), just after 10 days, confirm the efficacy of fermentation process. Chemical analysis showed a content of CP and CF respectively of 6% dm and 35% dm comparable to corn and triticale silages, commonly used in animal feed. NDF showed higher values in the silage containing fruits and vegetables (70.26% dm), respect to the corn stalks and triticale silages as reported in the literature (60% dm). In view of the results in this study, it may be concluded that fruit and vegetable by-products silage can be used in animal feeding, allowing to decrease the waste in landfill, reducing the environmental impact, and at the same time to lower the costs of business management.

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P-157

Production of single cell protein from Orange Molasses using indigenous Candida krusei

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Single cell protein (SCP) is defined as a kind of protein source from microbial cultures for feeding animals and even humans. In the recent years, agro-industrial wastes, such as citrus pulp or molasses, are used in animal nutrition to reduce feed cost and environment pollution. Orange molasses is obtained as a waste product in the course of orange juice production and is a product dense and rich in sugars. The aim of this study was to produce single cell protein from orange molasses using Candida krusei. On Citrus molasses a preliminary count of yeast in according to ISO 21527-1:2008 was made. The yeast identification was made with Matrix-assisted laser desorption/ionization - time of flight (MALDI-TOF-MS) mass spectrometry. The following yeast strains were found: Candida krusei, Saccharomyces cerevisiae, Kluyveromyces marxianus. Candida krusei was chosen because it was the predominant strain and for its attitude to produce biomasses. The molasses was also characterized chemically with the following results: crude protein (2.81% on Fresh Matter -FM), crude fat (0.04% FM), crude fiber (0.34% FM), pH (3.07). The concentration of total reducing sugars was 22.14 g/L. The major sugars in molasses were sucrose (7.4 g/L), fructose (12.31

g/L), glucose (12.87 g/L). The effect of orange molasses concentration (7%, 10%, 12%) on SCP production was conducted in 1000 ml flasks cultivated in a vibrating shaking incubator at 30°C, pH 5.5 for 72h. The initial amount of C. krusei for each concentration was 2.05±0.11 Log CFU/ml. At 0, 24, 48 and 72 hours chemical and microbiological analysis were carried out. Cell dry weight of cultures was analyzed by centrifuging, washing the pellet and drying at 105°C to constant mass. Protein content in drying cells was measured and sucrose, fructose and glucose were analyzed using enzymatic assay. For the yeast count Dichloran Rose-Bengal Chloramphenicol Agar plates were used. After 72 hours the yeast reached: 7.90 Log CFU/ml at 7%, 7.09 Log CFU/ml at 10% and 6.80 Log CFU/ml at 12%. Cell dry weight was 3.15 g/L at 7%, 7.65 g/L at 10% and 4.16 g/L at 12% and the protein content in dry cells of the strain was 37.55% on Dry Matter - DM, 30.42% DM and 34.02% DM respectively. These results suggested that C. krusei might be applied effectively to produce SCP using orange molasses as a low-cost substrate. Further investigations are currently underway.

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P-158

Nutritional traits and phenolic content of dried Olive Cake from three different cultivars of Olea Europea grown in Sicily (Italy) for livestock feed

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Olive tree culture has a great economic and social importance in the Mediterranean area. In addition olive oil industry generates large amounts of by-products such as olive cake, vegetation water, twigs and leaves. Olive Cake (OC) has a little economic value and always it is used as animal feed. Aim of this work was to characterize three varieties of stoned and dried olive cake (Biancolilla, Cerasuola and Nocellara) cultivated in the same area (Trapani, Sicily, Italy), from the 2012-2013 campaign, and milled in the same oil-mill. In this regards were quantified: moisture, ash, crude protein (CP), ether extract (EE), crude fiber (CF), neutral detergent fibre (NDF), acid detergent fibre (ADF) and sulphuric acid lignin (ADL), total extractable polyphenols (TEPP) and the degree of acidity. The results do not show significant differences in the three varieties. Protein content of the olive cake varied from 7.65±0.54% DM (Cerasuola) to 9.05±0.14% DM (Nocellara). Ash content varied from 4.04±0.14% DM (Biancolilla) to 5.00±0.96% DM (Nocellara). The use of stoned olive cake determines low values of ADL in the range of 15.95±0.85% DM (Cerasuola) to 16.90±0.12% DM (Nocellara) compared to samples with kernel. This result is important because the lignin is indigestible and therefore this product is better for the ruminant nutrition. However, an improved processing could lower further the lignin value. The analysis of TEPP showed values between 4.58±0.66 DM (Biancolilla) ‚Äì 6.00±1.22 DM (Cerasuola). These values are not very high because probably the polyphenols are highly sensitive to increased of temperature and light. The phenolic compounds in olives are recognized as potentially bioactives and may have antioxidant and therapeutic properties. OC is considered a rich source of phenolic compounds with a wide array of biological activities, then, it could be very important to find a conservative drying process. The amount of crude lipids, from 29.05±3.86% DM Cerasuola to 29.62±3.01% DM Nocellara, is another important result of these varieties since the values are much higher than the data present in the literature. A similar product can be considered of great interest in animal feed industry, although the stoned and dried processes should be optimized to improve and preserve its nutritive and functional values.

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P-159

Farming insects for feeding pigs: constraints and opportunities

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Farmed insects are among novel protein sources for pig feeding. In Europe, insect producers and pig breeders have to comply with rather complex rules and legal requirements, mainly related to the feed (or "substrate) fed to the insects. Annex III of Regulation (EC) 767/2009 lists a number of materials that are prohibited as substrate for insects, such as feces and "household waste". Substrates have to comply also with EU regulations on animal proteins (Regulation 1069/2009 and the implementing 142/2011): according to these regulations, some (animal protein) sources such as manure, gut content, dead-in-shell poultry, and fallen stock are prohibited as substrate for insects. Insects are expected to be increasingly used in Europe as protein replacers in animal nutrition, and the potential species for use in pig diets are Hermetia illucens (black soldier fly), larvae of Musca domestica (common housefly), and Tenebrio molitor (yellow mealworm). Black soldiers larvae meal is a suitable ingredient in growing pigs diets, being valuable in particular for its protein, lipid and Ca content and palatability (Newton et al., 1977). The unbalanced aminoacid content of prepupae meal may be a limiting factor in diets for early weaned piglets; additional refinement, such as cuticle removal and rendering, may be necessary to make it more suitable for piglets. The common housefly (M. domestica) maggot is of particular interest because it can grow on a large range of substrates and transform wastes into a valuable biomass rich in protein and fat. Sows and piglets fed maggot meal did not show any adverse effect on performances and health, and on sensorial property of meat (Bayandina and Inkina, 1980). Positive results were observed on weaned pigs fed a soybean based diet supplemented with 10% maggot meal to replace fishmeal (Viroje and Malin, 1989). Also yellow mealworm (Tenebrio molitor) could be suitable in animal feeding due to its high content of crude protein (47-60%) and fat (31-43%), but at the moment no information is available for pigs and ruminants. Future research on insect meal is needed, focused in particular on safety hazards helping EU to assess conclusive laws on the use of insect meals in pig diets.

P-160

Insects as innovative protein source for fish feeds: a brief review

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Global fish production has grown steadily over the last 5 decades reaching a total of 158 million tons in 2012 with more than 42% coming from aquaculture. From 1980 to 2012, the global aquaculture production grew at an average annual rate of 8.6% and this increasing trend is expected to continue. In order to feed the world population (9 billion in 2050), food production must increase by 70% and aquaculture production will need to increase by 133%. An increased availability of quality aqua feeds is required for sustaining such rates of increase in aquaculture production. Fish meal (FM) is the optimal protein ingredient in fish feeds and still widely used. However, aqua feed production is under increasing pressure due to limited supplies and increasing price of FM. This means that FM will likely continue to be an important ingredient, but it will increasingly be used in combination with other ingredients. Plants already deliver the majority of the protein to diets for farmed fish due to the abundance, potential for increased production and low cost. However, inclusion of vegetable meal in aqua feeds (mainly soybean meal ‚Äì SBM) is limited since a number of adverse effects are observed. Furthermore, the massive utilization in animal feeding of vegetable meal poses severe environmental issues. Therefore, scientific research must focus on new protein sources able to save biodiversity and to guarantee the sustainability of aquaculture productions. FAO indicates insects as innovative source to be employed in feed, due to their high nutritional value, especially in terms of crude protein (CP) content. In aquaculture, trials have shown that their use is possible with good growth performances even if some amino acid limitations have been highlighted. Insect meal could thus make a significant contribution to the sustainable development of the aquaculture industry. In this scenario, the Committee on ‚ÄúUsing innovative sources of protein in animal feed‚Äù, appointed by the ASPA, is studying and discussing recent advances in feed research towards innovative new high protein feedstuff to be included in feeds. Different topics are focusing on insect meal, microalgae and animal by-products processed with innovative techniques that can be more sustainable and available on a global basis.

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P-161

Role of resistant starch from different sources on the *in vitro* production of short-chain fatty acids in a pig model

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There is increasing interest in incorporating nutrients that may act as potential prebiotic sources in pig diets, including resistant starch (RS). Pig colonic bacteria ferment RS to short-chain fatty acids (SCFA) that exert several physiological effects related to energy supply and renewal of intestinal cells. The aim of this work was to evaluate whether the fermentation of RS from different starches may influence SCFA fermentation patterns and related kinetics. An *in vitro* experiment based on enzymatic digestion followed by fermentation with faecal inoculum was conducted and 5 native purified starches were tested. Each ingredient was pre-treated with a pepsin-pancreatin hydrolysis and 200 mg of each hydrolysed RS residue was then incubated in glass bottles in a buffered mineral solution with fresh pig faeces. Two fermentation runs were set up in two different days and bottles within runs were considered repetitions, with bottles between runs as replicates. One ml aliquot was removed at 0, 4, 8, 24, 48 and 72 h for SCFA determination by gas chromatography. The maximum rate of production (Rmax) and the time at which it occurs (Tmax) were calculated (Groot *et a*l., 1996). Data were subjected to ANOVA using the mixed procedure of SAS® and the minimum significant difference (MSD) was generated from Tukey's test and used for multiple comparisons among means. The significance level was $P<0.05$. The average SCFA productions, as well as the molar ratios (MR) of acetate, propionate and BCFA, were similar among starches. The MR of butyrate was affected by the RS sources ($P<0.05$), showing that different types of RS may favour butyrate production. Butyrate is the major fuel for colonocytes and can inhibit apoptosis of colon crypt cells *in vivo*. Differences were also recorded for Rmax and Tmax values ($P<0.05$). In conclusion, current data could facilitate the selection of ingredients to manipulate pig fermentation activities and to potentially enhance butyrate production.

P-162

Hyperlipidemia in African Grey parrots; case series with Isikli, Aydin experience and limited literature review

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Parrots are prone to a condition known as hyperlipidemia, meaning high levels of fat leading life threatining conditions. Even if fats such as cholesterol and lipoproteins accumulate inside the blood vessels, atherosclerosis exist. In this study, the aim was to analyze lipid profile of African grey parrots which presented with complaints of in appetence, lethargy and exercise intolerance then were compared to healthy control. The importance of hyperlipidemia due to one way nutrition in psittacines were investigated by review of limited literature in veterinary internal medicine. Four different African grey parrots with related clinical signs which ages ranging from 3 to 6 years and a healthy 3-year-old were included in the study. Serum biochemistry analysis (cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides) was determined on examination dates. Hyperlipidemia was evident based on analysis. As a result, it is concluded that hyperlipidemia can develop in parrots feeding with consistently one-way and same type nutrients (sunflower seed). Hyperlipidemia should be considered on examination and diagnosis of parrots with related clinical signs and treatment approach can also require dietary modification.

P-163

Insect meal (*Tenebrio molitor larvae*) as protein source in broiler: effect on growth rate from 30 to 62 days of age

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The aim of our study was to assess whether Tenebrio molitor larvae meal can be suitable for the complete replacement of soybean meal in broiler diets from 30 to 62 days of age. To this purpose, 80 thirty days old male Shaver brown broilers (BW 1.76 ± 0.19 kg) were homogenously divided into 2 groups (40 birds per group, each consisting of 8 replicates of 5 birds). The groups were fed two isoproteic and isoenergetic diets differing for the ingredient used as protein source: the control group (SBM) fed a corn-soybean meal based diet (CP 20.36 %; ME 2,812 kcal/kg), while in the other group (TML) the soybean meal was completely replaced by Tenebrio molitor larvae meal (CP 20.19 %, ME 2,849 kcal/kg). Up to 62 days of age, body weight and feed intake were recorded weekly and body weight gain (BWG), feed conversion ratio (FCR), protein efficiency ratio (PER) and European efficiency factor (EEF) were calculated. Feed intake was not different between groups considering the entire period of the trial. The FCR was more favorable in TML than SBM group ($P<0.05$) from 46 days of age and in the entire period of the trial (4.13 *vs.* 3.62, $P<0.05$). The PER was higher in SBM than in TML group (1.92 *vs.* 1.37, $P<0.05$) while the EEF was higher in broilers fed TML diet (132.6 *vs.* 156.2, $P<0.01$). Our results are in line with the finding of Ballitoc and Sun (2013) who showed a decreasing trend in FCR values of broiler fed TML from 0 to 10 % of inclusion level in the diet. Feed intake in the period 30 – 62 days of age was not different between groups suggesting that dried mealworms had a sufficient palatability for broiler also when the inclusion level was near to the 30 % of diet, as in our trial. A previous study (Ramos-Elorduy et al., 2002) showed no rejection due to texture or palatability of TML meal in broiler diet up to the inclusion level of 10 %. Since variables included in the EEF calculation are related to growth performance (livability, live weight, days of age and feed conversion ratio which includes feed intake and body weight gain), it is possible to affirm that feeding TML meal had a positive effect on broiler growth performance when compared to the SBM diet. TML meal can be used to completely replace soybean meal in broiler diet during the growing period without negative effects on diet palatability. In addition, positive effects can be observed on feed conversion ratio and European efficiency factor due to the use of TML diet.

P-164

Effect of dietary linseed supplementation on milk coagulation properties of dairy goats with different CSN1S1 genotype

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In this study, the effects of dietary supplementation with extruded linseed on milk coagulation properties of dairy goats with different alpha-s1 casein (CSN1S1) genotype are investigated. Twenty-four Saanen goats selected on the basis of the CSN1S1 genotype were divided in two groups: one with low (Low group; goats with FF, 0101, and F01 genotype) and the others with high (High group; goats with AA genotype) level of CSN1S1. Goats of each genotype were allocated into two dietary treatments: one was the control (CON) and one was supplemented with 200 g/d of extruded linseed (LIN). The trial lasted 4 weeks. Milk samples of morning milking were collected weekly and analyzed for fat, protein, lactose, and somatic cell count (SCC). The milk coagulation properties (RCT rennet coagulation time, K20 curd firming time, A30 curd firmness) were determined with a Formagraph. Data were analyzed using a mixed linear model that included the period, diet, genotype and diet × CSN1S1 genotype interaction as fixed factors, and the goat as random factor. The CSN1S1 genotype did not significantly influence milk composition, even in terms of protein percentage (3.22 and 3.24% in Low and High groups, respectively). The RCT (12.3 *vs.* 12.1 min) and A30 (25.3 *vs.* 26.6 mm) did not vary significantly ($P > 0.05$) between the Low and High CSN1S1 genotypes, whereas the K20 tended to be lower in Low than High groups (2.8 *vs.* 3.6 min; $P<0.10$). No significant effect of extruded linseed was observed on milk composition and milk coagulation properties. In conclusion, no interaction between CSN1S1 genotypes and dietary linseed was found on milk composition and milk coagulation properties. Acknowledgements: Research supported by Fondazione Banco di Sardegna.

P-165

Effect of feeding extruded linseed (*Linum usitatissimum* L.) and oregano (*Origanum vulgare* L.) on meat production and quality in Jonica kids

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The autochthonous Jonica goat breed has suffered a considerable genetic erosion in the last decade in Apulia. This research was planned in order to exploit feeds and animal resources available in our region, such as extruded linseed, with or without oregano supplementation, a natural Mediterranean essence with antioxidant properties, on meat production and quality in Jonica kids. Male kids (N.=18) were divided into 3 homogeneous groups and fed *ad libitum* with the following treatments beginning on 20 days of age: C, control group; L, feedstuff containing extruded linseed (3%); L+O, feedstuff containing extruded linseed (3%) and sun dried oregano plants in bloom (0.6%). During the trial kids suckled goats' milk, therefore the 3 feedstuffs were offered to kids' respective mothers. Once a week kids were individually weighed to determine the average daily gain (ADG) and the amount of feed given and refused was recorded to evaluate the voluntary feed intake. Kids were slaughtered at 60 days of age. Carcasses were evaluated and sectioning data were recorded. The Longissimus lumborum muscle was analyzed for chemical composition and lipid peroxidation by performing the TBARS (thiobarbituric acid reactive substances) test. Data were processed by ANOVA using the GLM procedure, and means were compared by Student's t test. The final live weight (11.39, 11.66, 11.42 kg) and ADG (0.119, 0.118, 0.127 g/d) were very comparable among groups, respectively for C, L and L+O. Diet had no influence on performances at slaughter. The sectioning data showed a lower incidence of the brisket in the L+O group (6.15%) as compared to L (6.27%) and even more to the control (6.87%; $P<0.05$). Supplementation with oregano determined a significant reduction in the amount of fat (6.75%) of the loin, in comparison with L (9.90%; $P<0.05$) and C groups (10.92%, $P<0.01$). Similarly, the dissection of the hind leg showed a reduction of the amount of fat following the L+O diet (4.80%) in comparison with the L group (6.30%) and especially with the control one (7.73%; $P<0.05$). The beneficial effects of oregano supplementation found in this trial were also confirmed by the TBARS test by a lower lipid peroxidation (0.247 *vs.* 0.398 and 0.434 mg malonaldehyde/kg meat, respectively for L+O, L and C; $P<0.01$).

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P-166

Performances, carcass and some meat quality traits in lambs fed with extruded linseed (*Linum usitatissimum* L.) and oregano (*Origanum vulgare* L.)

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Extruded linseed has been extensively used in ruminant feeding due to its positive effects on the gastro-intestinal function, content of proteins characterized by high solubility and ω-3 fatty acids. The aim of the research was to evaluate the effects of feeding diets containing extruded linseed, with or without the association of oregano as a natural antioxidant, on lamb growth, meat production and quality. The trial was carried out on 18 Gentile di Puglia breed male lambs, divided into three groups of six subjects each, according to age and live weight, and fed *ad libitum* with the following treatments beginning on 20 days of age: C, control group; L, feedstuff containing extruded linseed (3%); L+O, feedstuff containing extruded linseed and sun dried oregano plants in bloom (0.6%). During the trial lambs suckled also ewes' milk, therefore the three feedstuffs were offered simultaneously to lambs' respective mothers. Once a week lambs were individually weighed to determine the average daily gain (ADG) and the amount of feed given and refused was recorded to evaluate the voluntary feed intake. Lambs were slaughtered at 40 days of age. Carcasses were evaluated and sectioning data were recorded. The Longissimus lumborum muscle was analyzed for chemical composition. Data were processed by ANOVA using the GLM procedure, and means were compared by Student's t test. Diet did not affect lamb final live weight (13.94, 14.64, 13.93 kg, respectively for C, L and L+O). However, both the diets containing extruded linseed slightly improved the ADG (0.228, 0.212 g/d, respectively for L and L+O) in comparison with the control group (0.205 g/d). Hot and cold carcass weights and net dressing percentages did not significantly differ among treatments. Lower amount of fat was recorded in the L and L+O groups for the dissection of the loin (10.30-10.59 *vs.* 11.13%) and hind leg (4.92-5.39 *vs.* 5.84%). As for meat chemical composition, feeding extruded linseed in association or not with oregano faintly lowered the meat intramuscular content of fat in the loin in comparison with the control group (3.56-2.98 *vs.* 3.87%). Based on the results obtained in this trial, it would be interesting to extend the length of the dietary treatments in lambs by increasing their age at slaughter.

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P-167

Improving sustainability of poultry production by feeding insects: state of the art and perspectives

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The market for poultry products (egg and meat) is growing worldwide owing also to the absence of cultural or religious obstacles. Dietary protein sources represent the primary production costs. Soybean meal is the most used protein source in the diet formulations for broilers and laying hens. However due to its ever-increasing price, the sustainability of this production chain is becoming critical, in particular in some developing countries. Therefore, alternative feed protein sources, locally available and sustainable are required. The use of insects as an alternative source of protein in animal feeding is becoming globally more appealing, especially for its sustainability. In addition, insects (adult, larval and pupal form) are consumed naturally by wild birds and free-range poultry. The rural poultry farming in the economy and food security of developing countries has been momentous. Studies have shown the feasibility of using insects as an alternative feed source or as complementary feed source for poultry. Grasshoppers, darkling beetle, crickets, cockroaches, termites, lice, stink bugs, cicadas, aphids, scale insects, psyllids, beetles, caterpillars, flies, fleas, bees, wasps and ants have all been used as complementary food sources for poultry. Soybean or fish meal in broiler chicken or laying hen diets have been successfully replaced at low inclusion levels by black soldier flies (Hermetia illucens), housefly (Musca domestica), mealworms (Tenebrio molitor) and silkworm (Bombix mori) pupae. Insect inclusion in poultry diets ranged between 0.2 to 20 % without affecting growth performance or, in some cases, by improving it. An obstacle to the use of insect meals in poultry diets is the lack of information about nutrient digestibility in commercial poultry farms. Recently, Schiavone et al. (2015), have estimated the nutrient and apparent metabolizable energy values for mealworms and black soldier flies pupae used in chickens feeding. The use of insects as sustainable protein-rich feed ingredient in poultry diets is technically feasible. Insects can be reared on low-grade bio-waste substrates transforming them into high-quality proteins. Furthermore, invertebrates are a raw material included in the Feed Register Material of the EU. Although they are currently authorized only for pet feeding, insect-derived feed could represent in the near future a suitable ingredient for poultry, too.

P-168

Use of a whey-based milk replacer and an high-fiber starter concentrate during the weaning period of buffalo calves.

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The effects of two different milk replacers (based on milk or whey) and two starter concentrates (at low or high fiber content) were evaluated during the weaning period of buffalo calves. Twenty calves were divided in two groups. The control group (CON) was fed a milk replacer based on milk power (26.0% CP, 20.0% Fat, and 7.3% CF), the experimental group (EXP) was fed a milk replacer based on whey (26.0% CP, 22.5% Fat, and 0.05% CF). Groups were homogenous for sex (1 male and 9 females), age (10.0±6.5 and 9.4±7.5 d for CON and EXP, respectively) and live weight (LW; 45.3±7.7 and 44.8±7.7 kg). The 11-week experimental period was divided in two phases. In the first phase (week 1st-5th), calves were kept in individual box and were fed milk replacer. In this period, fecal scoring was evaluated twice weekly, and LW was recorded every week. In the second phase (week 6th-11th), calves were kept in collective pens (5 calves/box) and fed, beside milk replacers, solid feeds. CON group was fed a starter at 3.4% DM of CF and meadow hay; EXP group was fed a starter at 14.6% DM of CF. DM intake (DMI) and LW were measured weekly. At 11th week, in vivo digestibility was measured by using acid-insoluble ash as an internal marker. Data on fecal score, average daily gain (ADG) and DMI were analyzed by the mix procedure of SAS with week as repeated factor and treatment (CON and EXP) as non-repeated factor. Final LW, DMI and digestibility coefficients were analyzed by one-way analysis of variance with treatment as factor. In the first phase of the experimental period (1st-5th weeks), no differences were found between treatments for fecal score, milk replacer intake, final LW, and ADG. In similar way, in the second phase of the experimental period (6th-11th weeks), ADG and final LW were similar between groups. Total DMI (milk replacer + starter concentrate and milk replacer + starter concentrate + meadow hay for CON and EXP groups, respectively) was higher in CON group, but, obviously, the intake of starter was higher in EXP group. NDF digestibility of the EXP animals was significantly higher than that of the CON calves. In addition, digestibility of DM, OM, tended ($P \leq 0.10$) to be higher in the EXP. Probably the fiber of EXP starter was more digestible as compared to the hay. We conclude that whey-based milk replacers and high fiber concentrates, commonly used in dairy cattle farming, may also be utilised during the weaning period of buffalo calves without adverse effects.

P-169

Effect of controlled release NPN on beef performance and meat quality

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The goal of controlled-release NPN sources (CR-NPN) is to guarantee a constant amount of NPN into the rumen, essential for bacterial growth, avoiding at the same time excessive ammonia production. The effect of NPN on beef quality has never been tested on continental breeds. Therefore, the study aimed to evaluate the effect of partial replacement of soybean meal with CR-NPN (Beefsync®, Alltech Inc.) on beef performance and meat quality. 100 Charolaise heifers were divided in two experimental groups (10 pens of 5 heifers each, balanced for initial weight and conformation), fed two isoenergetic and isonitrogenous diets for all the entire fattening period (118 days), in which soybean meal of the control diet was partially replaced with CR-NPN for treated group. Individual live weigh (LW) was recorded at the enrolment, at day 49 and the day before slaughter, average daily gain (ADG) was then calculated. Pen feed consumption (DMI) was weekly recorded for feed conversion ratio (FCR) calculation. Carcass characteristics and meat samples were taken from 1 animal/pen (20/group) to evaluate cold carcass weight (CCW), dressing percentage (DP) and longissimus dorsi chemical composition, physical traits (thawing and cooking loss, shear force) and colour stability during 5 days of natural atmosphere refrigerated storage in dark room (L*, a*, b*, C*, h measured at days 0 and 5). ANOVA was carried out through a mixed model (SAS 9.3, SAS Inc.) considering the fixed effect of treatment, time and their interaction (when available) and the random effect of the experimental unit. Pen was the experimental unit for rearing performance and feces composition, while animal was the experimental unit for carcass traits, meat physical properties, chemical composition and colour stability. CR-NPN administration improved ADG ($P<0.001$) and FCR (<0.001), without affecting DMI. Treated group showed a lower fecal starch ($P=0.003$) and CP ($P=0.02$) content. No differences were found for DP, while the higher live weight at slaughter, due to the better ADG, determined an higher CCW ($P<0.001$). Treatment did not affect meat chemical composition and physical properties, as well as colour stability. At the present experimental conditions, the partial replacement of soybean meal with CR-NPN source increased feed efficiency and weight gain due to a more efficient nutrient utilization, as demonstrated by the lower protein and starch excretion, without affecting meat physical and chemical properties.

P-170

Vegetable food waste valorization to earthworms production: preliminary evaluation for strategic decision-support in rearing system

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In food vegetable processing plants, green wastes not suitable to be transformed in animal feed, are buried in a landfill (increasing GHG in the form of methane emission) or are composted by earthworms. From the compost process, the earthworms can be utilized as novel protein source. However microorganisms present in the vegetable waste can be a source of contamination in the earthworms rearing system. Considering that the rearing production technology is still in a developmental stage, the assessment and management of such microbial pollution is thus an important preliminary step to be considered. With the HACCP approach in the rearing procedure and meal production, effective and convenient methods to reduce or eliminate pathogens needs to be addressed to guarantee this potential novel protein source as healthy feed or food. To establishing critical limits at critical control points which separate acceptability from unacceptability for the prevention, elimination or reduction of identified hazards, we evaluated the microbial contamination of earthworms (Eisenia foetida) reared on food green wastes. Approximately 500 earthworms were separated from the substrate and washed in running controlled water to remove dirt from the body surface. The samples obtained were analyzed according to AFNOR and ISO methods for total bacterial count, Enterobacteriaceae, coagulase-positive Staphylococci (CPS), Bacillus cereus, total coliforms, Listeria monocytogenes, sulphite reducing clostridia, *Escherichia coli* and Salmonella spp. Bacterial counts were expressed as Log CFU/g sample. Salmonella spp. and Listeria monocytogenes were absent in 25 g in all the samples. *Escherichia coli* and CPS were below the detection limit (1 Log CFU/g). The samples were characterized by levels of microflora above 6 Log CFU/g mainly composed by Enterobacteriaceae, total coliforms, Bacillus cereus and sulphite reducing clostridia, derived from the substrate where they grew. The results ranged above common benchmark and threshold values. In conclusion, the development of this new process needs to be implemented by the expertise on the engineering process and on the scientific research. Furthermore in this developmental stage, the safety assessment, the economical evaluation, the life cycle analysis (LCA), the benefits and the social impact, will be assessed.

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P-171

Vegetable food waste bioconversion to earthworms as novel protein source

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Earthworms offer potential thanks to their ability to convert waste streams into usable nutrients for food and feed production. The industrial processing of fruits and vegetables, produces many tons of waste. In food vegetable processing plants, the wastes not suitable to be transformed in animal feed, are buried in a landfill (increasing GHG in the form of methane emission) or are composted by earthworms. From the compost process, earthworms can be utilized as novel protein source. In EU and globally, creative approaches are required to fit alternative protein sources for animal nutrition with no or limited competition with human food. Earthworm meal could reduce the environmental impact of food production and could be considered a sustainable and viable feed protein supplement as well as food resources for humans. As a part of studies on the feasibility to transform vegetable food wastes in this novel protein source, we evaluated the protein content and the amino acids profile of earthworms (Eisenia foetida) reared on food vegetable wastes. Briefly, approximately 800 earthworms were separated from the substrate, washed in running controlled water to remove dirt from the body surface, kept in saline solution at room temperature changing the solution until the digestive systems were clean, and then freeze-dried for later analyses. Samples of earthworm were analyzed for CP and protein quality was measured by amino acid profile. The CP was 67.15% of the dry matter. The earthworm have been shown to contain all the essential amino acids with high lysine and methionine level. From the results, earthworms can provide an appropriate protein source of high biological value. Amino acids content meets the requirements of the animals allowing the precision feeding approach, as part of the precision livestock farming, reducing the protein supplied to the animals and nitrogen excretion. Earthworms grown and reared on food waste in compliance with EU regulations for feed producing animals, contribute to reduce the environmental impact. Furthermore we will not only focus on nutritional profile of novel protein sources, but also on economic, environmental, social, strategic and management, decision-sup-

port system innovations, that are of relevance for more sustainable, responsible and responsive livestock farming systems.

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P-172

Vegetable food waste bioconversion to earthworms as novel feed/food: earthworms fatty acid profile

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The ability and commitment to produce food from animals under socially and ethically sound conditions is the central question for the upcoming decades. This requires innovative and holistic sectorial concepts for innovations in animal husbandry. The aim of the SMARTLIVESTOCK project is to assess and identify the most important and influential factors responsible for improving sustainability and productivity of livestock production systems. Efficient livestock production chains should also include alternative feed sources whose production does not compete with food production for humans, or lead to significant changes in the land use. As a part of the SMARTLIVESTOCK project this study aim to evaluate earthworms as novel feed/food. The fatty acid profile of earthworms (Eisenia foetida) reared on food vegetable wastes were evaluated. Briefly, approximately 800 earthworms were separated from the substrate, washed in running controlled water to remove dirt from the body surface, kept in saline solution at room temperature changing the solution until the digestive systems were clean, and then freeze-dried for later analyses. Samples of earthworm were analyzed for fat content and fatty acid profile. The fatty acid profile were determined using reference FAME-GC-FID analysis. The ether extract was 10.84% of the dry matter. The fatty acid composition of earthworms revealed a great amount in terms of PUFA proportion (43.91%). Among PUFA class ω3 series and ω6 series fatty acids were well-represented (8.22% as sum of ω3 and ω6 series). With this quantity and quality of lipid content, earthworms are promising sources of lipids of high biological value for novel products and applications, and not only for their high protein content.

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P-173

Secnidazol treatment to improve milk yield in sheep with giardiasis

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The purpose of this multidiciplinary (agricultural and veterinary fields) study was to assess the effect of single secnidazol treatment on milk production in dairy ewes naturally infected with Giardia duodenalis. Thirty dairy ewes with Giardiasis, were enrolled into 3 equal groups and Groups I and II were treated with secnidazole at a single dose rate of 10 mg/kg or 30 mg/kg, respectively, perorally and G III ewes were controls. Throughout the study ewes in G III remained positive for Giardiasis, with some of them showed an increase without statistical significance in cyst counts on day 10 (ranged 1300-241650) compared to the initial values (ranged between 5600-274600). The least square means and standard error of means of cyst excretion on D0 and D10 revealed that there was a significant reduction ($P<0.001$) in cyst reduction in GI and GII animals. Both group GI and GII ewes produced significantly more milk than group G III ones ($P<0.001$). The change of mean milk yield over time was statistically significant ($P<0.001$) among group GI and GII ewes; besides a statistically significant ($P<0.001$) reduction in the mean milk yield of group GIII ewes was observed. Given the efficacy of secnidazol treatment for cyst reduction and increased milk yield, it may be safely suggested that Giardiasis adversely affects the production of the infested animals.

P-174

Use of fresh forage in buffalo cows' feeding: effect on sensory properties of Mozzarella cheese

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The effect of dietary inclusion of fresh fodder on sensory properties of mozzarella cheese was evaluated. In a dairy buffalo farm of Volturno plain two homogenous groups of 15 lactating buffaloes (on average, milk yield 13.3±1.5 kg/d, days in milk 110±13 d, parity 3.0±1.3) were fed the same total mixed ration containing (FRESH group) or not (DRY group) 20 kg of fresh sorghum. Three batches of mozzarella from the two groups were produced and were tested in a quantitative descriptive analysis. In each session, additional four commercial mozzarella samples were evaluated. All samples were produced according to the PDO Mozzarella di Bufala Campana regulation. A trained panel of ten assessors rated the intensity of 19 attributes to describe the aspect (three attributes), odor and flavor (six), taste (three), and texture (seven) of the products. Data were subjected to analysis of variance using product, replication, assessor and the corresponding first order interactions as factors. The interactions product x replication and product x assessor were never significant ($P>0.05$), thus indicating a high reliability of the panel performances, as products were not evaluated differently either in different replications or by different assessors. The two experimental products (FRESH and DRY) significantly differed from commercial samples, being brighter (72.2 *vs.* 65.3 *vs.* 54.0, respectively for FRESH, DRY and the mean of the commercial samples), smoother (74.0 *vs.* 61.2 *vs.* 55.1) and with a more intense color (75.5 *vs.* 74.2 *vs.* 61.6). The product FRESH was significantly brighter and smoother than the product DRY. The DRY product also showed significantly more intense odor/flavor (81.5 and 65.2) compared both to the commercial samples (67.4 and 61.0) and the product FRESH (74.8 and 57.7), although it was scored as the most tender (78.7 *vs.* 55.4 *vs.* 69.7). Based on the sensory characteristics of the products, trained panelists were able to discriminate the two experimental products from each other as well as from the commercial ones. Further studies are needed to assess product acceptability in order to meet the sensory requirements of particular segments of consumers.

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INDEX OF AUTHORS

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