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ASPA 20th Congress

Bologna, June 11-13, 2013

Book of Abstracts

Guest Editors: Andrea Piva, Paolo Bosi (Coordinators)
Alessio Bonaldo, Federico Sirri,
Anna Badiani, Giacomo Biagi,
Roberta Davoli, Giovanna Martelli,
Adele Meluzzi, Paolo Trevisi

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ASPA 20th Congress

Bologna, June 11-13, 2013

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ASPA 20th Congress

Bologna, June 11-13, 2013

Organised by

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Dipartimento di Scienze Mediche Veterinarie
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ASPA 20th Congress
Bologna, June 11-13, 2013

Programme of the Congress

Tuesday, June 11

08.00-10.00	Arrival and registration of participants
10.00-11.00	Plenary session
11.00-11.30	Coffee break
11.30-13.00	Scientific sessions
13.00-14.00	Lunch
14.00-17.00	Scientific sessions
17.00-18.00	Transfer to Bologna downtown
18.00-20.00	Opening ceremony (Hall *Stabat Mater*)
20.00	Welcome cocktail

Wednesday, June 12

09.00-10.00	Plenary session
10.00-11.00	Scientific sessions
11.00-11.30	Coffee break
11.30-13.00	Scientific sessions
13.00-14.00	Lunch
14.00-17.00	Scientific sessions
17.00-18.00	Poster viewing
18.00-20.00	Meeting of ASPA Members
20.30	Social dinner

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09.30-13.00	Workshop
13.00-14.00	Lunch
14.00-17.00	Scientific sessions

	Tuesday, June 11th	Wednesday, June 12th	Thursday, June 13th
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11.30-13.00	**AB** (plenary room) **NF** (room 1) **MQ** (room 2)	**AB** (plenary room) **NF** (room 1) **DP** (room 2) **PR** (room 3)	
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14.30-15.30	**AB** (plenary room) **NF** (room 1) **MQ** (room 2)	**AB** (plenary room) **NF** (room 1) **DP** (room 2) **AW** (room 3)	**Malcolm A. Mitchell**. Quantifying physiological stress in livestock transport: understanding the implications for welfare and meat quality (plenary room)
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17.00-18.00	*Transfer to Bologna downtown*	Poster viewing	
18.00-20.00	***Opening Ceremony*** (Hall *Stabat Mater*)	***Meeting of ASPA members*** (plenary room)	
Evening	*Welcome cocktail*	*Social dinner (Savoia Hotel)*	

Legend

AB	Animal breeding and genetics
AW	Animal welfare, health and behaviour
AQ	Aquaculture
DP	Dairy production
MQ	Meat quality
NF	Nutrition and feeding
PR	Poultry and rabbit production

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Agenda for oral presentations

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Chairpersons: Nicolò P.P. Macciotta - Paolo Zambonelli

Tuesday, June 11 - Animal breeding and genetics - Plenary Room

Chairpersons: Roberta Davoli - Alessio Valentini

Tuesday, June 11 - Nutrition and feeding - Room 1

Chairpersons: Paolo Bosi - Francesco Masoero

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Different farming techniques for Crassostrea gigas (Thunberg,793) in a Sardinian coastal lagoon (Italy) C-084

Wednesday, June 12 - Dairy production - Room 2

Chairpersons: Andrea Summer - Paolo Trevisi

11.30-11.45 Andrea Summer, Massimo Malacarne, Piero Franceschi, Andrea Criscione, Salvatore Bordonaro, Donata Marletta
Mineral contents in ass milk........C-085

11.45-12.00 Valentina Toffanin, Massimo De Marchi
Prediction of calcium, phosphorus and titratable acidity, and relationships with coagulation traits and chemical composition of bovine milk........C-086

12.00-12.15 Massimo Malacarne, Piero Franceschi, Paolo Formaggioni, Maria Simona Mariani, Sandro Sandri, Primo Mariani, Andrea Summer
Salt equilibria and milk rennet coagulation aptitude........C-087

12.15-12.30 Stefania Chessa, Omar Bulgari, Rita Rizzi, Luigi Calamari, Anna Maria Caroli
Genetic analysis of milk clotting aptitude in the Italian Holstein-Friesian C-088

12.30-12.45 Claudio Cipolat-Gotet, Alessandro Ferragina, Giorgia Stocco
Effect of somatic cell count on coagulation properties, cheese yield and nutrients recovery of individual milk of Brown Swiss cows C-089

12.45-13.00 Alba Sturaro, Micaela Pivato, Annarita Trentin, Antonio Masi, Martino Cassandro
Validation of RP-HPLC method for separation and quantification of whey protein in dairy industry by-products.........C-090

Wednesday, June 12 - Dairy production - Room 2

Chairpersons: Giulio Cozzi - Andrea Formigoni

14.30-14.45 Marta Brscic, Luigi Zago, Giulio Cozzi, Barbara Contiero, Flaviana Gottardo
Evaluation of the relationship between mastitis problems and subjective judgments of facilities and animal cleanliness in dairy farms C-091

14.45-15.00 Francesco Zendri, Elisa Marchiori, Enrico Sturaro
Milk yield and quality of dairy and dual purpose cows reared in single breed and mixed breeds farms in mountain C-092

15.00-15.15 Rossella Di Palo, Diana Neri, Barbara Ariota, Rosanna Serafini, Marcella Spadetta, Fabio Zicarelli.
Milking technique in jenny: effects on milk production C-093

15.15-15.30 Adriana Bonanno, Antonino Di Grigoli, Francesca Mazza, Massimo Todaro
Effects of αs1-casein and k-casein genotypes on nutrient intake and milk production in Girgentana goats: a further investigation C-094

15.30-15.45 Alessandro Ferragina, Claudio Cipolat-Gotet
Comparison among different FT-MIR spectra treatments for the prediction of coagulation properties of individual milk of Brown Swiss cows C-095

15.45.16.00 Massimo De Marchi, Valentina Toffanin, Mauro Penasa
Prediction of coagulating and noncoagulating milk samples using mid-infrared spectroscopy C-096

16.00-16.15 Federico Righi, Yesid Orlando Gonzáles Torres, Francesca Martuzzi, Marco Renzi, Afro Quarantelli
Effect of the extent of digestion of dry matter and fiber on fecal particle distribution in cows feces C-097

16.15-16.30 Alberto Palmonari, Mattia Fustini, Giorgia Canestrari, Ester Grilli, Andrea Formigoni
Evaluation of the neutral detergent fibre indigestible fraction in forages C-098

Wednesday, June 12 - Poultry and rabbit production - Room 3

Chairpersons: Adele Meluzzi - Gerolamo Xiccato

10.00-10.15 Eirini Filiou, Duilio Majolini, Angela Trocino
Reactivity of growing rabbits under different housing systems C-099

10.15-10.30 Antonino Nizza, Francesco Iannaccone, Carmelo Di Meo, Giovanni Piccolo, Fulvia Bovera
Effect of a differentiated access to water along 4 weeks post weaning on rabbit caecal fermentation C-100

10.30-10.45 Marco Tazzoli, Duilio Majolini, Eirini Filiou, Marco Birolo, Gerolamo Xiccato
Effect of increasing soluble fibre and starch and reducing acid detergent fibre in diets at different protein level for growing rabbits C-101

10.45-11.00 Nicolaia Iaffaldano, Michele Di Iorio, Martina Rocco, Angelo Manchisi, Maria Pina Rosato
Effects of two different concentration of dimethylsulfoxide on the quality of turkey semen cryopreserved in straws over liquid nitrogen vapor C-102

11.30-11.45 Federico Sirri, Massimiliano Petracci, Stefano Pignata, Luca Stocchi, Adele Meluzzi
Magnitude of emerging meat quality defects in breast fillets of different commercial meat-type chicken hybrid C-103

11.45-12.00 Massimiliano Petracci, Samer Mudalal, Angelo Bonfiglio, Claudio Cavani
Effect of white striping on quality traits of raw and marinated chicken breast meat C-104

12.00-12.15 Vladimiro Cardenia, Stefania Peluso, Antonietta Funaro, Maria Teresa Rodriguez-Estrada, Massimiliano Petracci, Claudio Cavani
Effect of dietary thymol supplementation on lipid oxidation of chicken legs as related to storage conditions C-105

12.15-12.30 Siria Tavaniello, Andrzej Witkowski, Sebastian Knaga, Marek Bednarczyk, Giuseppe Maiorano
Effects of genotype and sex on carcass traits and meat quality in two Japanese quail generations C-106

Wednesday, June 12 - Animal welfare, health and behaviour - Room 3

Chairpersons: Giovanna Martelli - Leonardo Nanni Costa

Thursday, June 13 - Workshop
Competitività e sicurezza della filiera alimentare europea delle produzioni animali
Plenary Room

Chairperson: Dr. Andrea Rosati, Segretario Generale EAAP-ICAR

09.30-10.00 Prof. Paolo De Castro/Dr. Felice Adinolfi
Produzioni zootecniche europee: crisi, equilibrio, o crescita?

10.00-10.30 Dr. Alberto Allodi (Presidente Assalzoo)
L'industria mangimistica europea: efficienza e competitività

10.30-11.00 Dr. Claudia Roncancio (Head of Feed Unit, EFSA)
Valutazione e comunicazione del rischio nell'Unione Europea

11.00-11.30 Dr. Alessia Di Sandro (Area Ricerca e Trasferimento Tecnologico - ARIC, Università di Bologna)
Priorità di ricerca per la UE

11.30-12.00 Prof. Giuseppe Pulina (Presidente ASPA)
Zoo-etica: cura e sostenibilità delle produzioni zootecniche

12.00-12.30 Dr. Giuseppe Blasi (Capo Dipartimento delle politiche europee e internazionali e dello sviluppo rurale – MIPAAF)
Il ruolo del MIPAAF nelle politiche di competitività e sostenibilità delle produzioni zootecniche

Thursday, June 13 - Animal breeding and genetics - Plenary Room

Chairpersons: Luca Fontanesi - Baldassare Portolano

Thursday, June 13 - Animal welfare, health and behaviour - Room 1

Chairpersons: Giuseppe Bertoni - Giovanna Martelli

Thursday June 13 - Dairy production - Room 2

Chairpersons: Antonello Cannas - Attilio Mordenti

ASPA 20th Congress

Bologna, June 11-13, 2013

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Agenda for poster presentations

Animal breeding and genetics

Aquaculture

Animal welfare, health and behaviour

Dairy production

Meat quality

Nutrition and feeding

Poultry and rabbit production

PREFACE

The 20th Congress of the Animal Science and Production Association (ASPA), is held in the prestigious home of the oldest university of the world: Bologna. The anniversary also celebrates the 40th year of the founding of the Society and is an occasion to remember the past and plan for the future of Animal Sciences in Italy.

In this Congress, 137 oral presentations and 155 posters are presented, for a total of 292 scientific contributions.

The Congress sessions with their relative number of papers are listed as follows: Animal breeding and genetics 109, Nutrition and feeding 45, Dairy production 42, Animal welfare, health and behaviour 39, Meat quality 35, Poultry and rabbit production 11 and Aquaculture 11.

I would like to point out that 3 main lectures will be presented in the Animal breeding and genetics, Nutrition and feeding and Animal welfare, health and behaviour sessions, respectively.

I would thank, first of all, Prof. Vincenzo Russo, Honorary President of the Congress and Emeritus of ASPA, for the contribution of the University of Bologna in organizing this event. I would also thank all the members of the Scientific Committee, Prof. Andrea Piva (Chairperson), Prof. Anna Badiani, Prof. Giacomo Biagi, Dr. Alessio Bonaldo, Prof. Paolo Bosi, Prof. Roberta Davoli, Prof. Luca Fontanesi, Prof. Giovanna Martelli, Prof. Adele Meluzzi, Dr. Attilio Mordenti, Prof. Luca Sardi, Dr. Federico Sirri, Dr. Paolo Trevisi, and of the Organizing Committee, Prof. Claudio Cavani (Chairperson), Prof. Stefania Dall'Olio, Prof. Andrea Formigoni, Prof. Pier Giorgio Monetti, Prof. Leonardo Nanni Costa, Dr. Massimiliano Petracci, Prof. Giuliano Zaghini, Dr. Paolo Zambonelli.

A special thanks to the secretary of the Congress, MV Congressi.

Indeed, I would like to congratulate and thank all those who have organized the meeting and have collaborated in reviewing the summaries for what they have done for ASPA and the Italian Animal Science. A special thanks to Prof. Rosanna Scipioni, Editor-in-Chief of the Italian Journal of Animal Science, for having contributed to the edition of these proceedings.

Finally, I acknowledge the University of Bologna and the institutional agencies, in particular the Ministry of Agricultural, Food and Forestry Policies (MIPAAF), the Regione Emilia Romagna and the Comune di Bologna, and the main sponsors, namely Associazione Italiana Foraggi Essiccati, DSM, Vetagro and Fatro.

Prof. Giuseppe Pulina
President of Animal Science and Production Association

ORAL COMMUNICATIONS

C-001

Recent innovations and tools of molecular genetics applied to animal breeding for the improvement of animal productions

Marinus F.W. te Pas, Mari A. Smits, Roel F. Verrkamp, Mario P.L. Calus, Ina Hulsegge, Sipke-Joost Hiemstra, Marike Boesr, Jack J. Windig

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Traditionally, animal breeding improved production traits by using the performance of livestock animals, and including the performance of the ancestors and/or siblings of the animal to estimate the genetic component of the performance to select the best parents to produce the next generation. It has long been recognized that variation at the genomic level was responsible for the observed variation in the traits. With the onset of the genomics era the number of livestock species with a known genome sequence rapidly increases. Thus, the tools to investigate the biological background of variation in the production traits and the use of this information to speed up genetic improvement of production of livestock became available. During the last decade the research on the regulation of production traits and new traits (*e.g.* animal health) has resulted in new insights and has developed several new tools that can be used by the breeding companies. The regulation of many production traits proved to be highly complex. As expected genetic variation in the coding sequences of the genes was found to be important. However, a number of other mechanisms were also recognized as important regulators determining the production. Among these are the regulation of the expression of the genes at several levels. A first regulatory level is the epigenetic level – chemical changes made to the chromosomes to make active or inactive areas, directly affecting gene activity. Once a gene is expressed the resulting mRNA can be inactivated, or translated into proteins. These proteins are important as building blocks for the body of the animal and for the livestock product. The activities of the proteins are important for producing the metabolites determining the quality of the products. New omics technologies have been developed to measure all these levels of expression of the genome and develop new tools to improve livestock production. During the presentation we will explain the new tools and show results that can be used by the breeding industry. At present, some of these results are already actively used by the breeding industries, and we will indicate how the new tools are used.

C-002

Genomic developments in the Italian Holstein Friesian

Raffaella Finocchiaro, Stefano Biffani, Johannes B.C.H.M. van Kaam

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Since December 2012, Holstein genomic evaluation in Italy has become official for females, following bulls in December 2011. Anafi entered a consortium with USA, Canada and UK, in which genotypes of bulls are exchanged. Currently the training population consists of 18700 bulls. After identity control and single nucleotide polymorphisms (SNP) data editing, imputation is done so all SNP chips can be included. Full pedigree is used to derive deregressed proofs (EDPs) from national or MACE EBVs. For genomic validation a reduced dataset is created and then deregressed. A linear SNPblup model with 41,501 autosomal (SNPs) and a residual polygenic component of 10% is used. For sires with daughters and cows with phenotypes, direct genomic information is blended with traditional information to increase reliability. The final result is the Genomic Enhanced Breeding Value (GEBV). The weight of the two indexes (direct genomic and traditional EBV) depends on the number of Equivalent Daughter Contribution (EDC): as the EDC on phenotypes increases, the relative weight of the direct genomic index decreases. With the increase of the number of lactations per cow, the weight of the Direct Genomic Value (DGV) for milk decreases on average from 72% to 63%. GEBVs of young bulls and heifers are based on DGVs, because they have no daughters or own performance information. DGVs and GEBVs are computed for all single traits. DGVs and GEBVs of composite traits are derived from single trait proofs. Pedigree indexes are now based on the most reliable indexes of the parents, hence GEBVs if parents are genotyped. One of the greatest advantages of genomic evaluation is the superior reliability of the indexes obtained. Using genomics, indexes can be obtained with a reliability of approx. 65%-70%, which is approximately double the value obtained with the pedigree index. In the first three years of genomic selection AI centers achieved a genetic progress equivalent to 10 years. Additional progress is expected from genomics on females, further increased preselection intensity on males and the use of young bulls as sires of sons. Starting April 2013 more services are going to be available to the farmers both for males and females.

C-003

Animal identification and parentage testing by bovine beadchips

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Bovine SNP chips are available with different densities, and panels with 3k, 7k, 54k or 800k SNPs are currently used for genomically enhanced breeding value estimation; in addition, a very reduced set of SNPs, which may be included in large panels or used alone, is suggested for parentage testing.

We propose a very simple, lossy and compressed coding of genotypes, in order to hide the laboratory results and to protect the property of the data from HD panels analysis, but preserve their usability for animal identification and parentage testing. The proposed coding has been tested in three simulations with 100,000 animals each, consisting of three pure breeds and their F1 crossing: all animals were genotyped, using a different mixture of 3k, 7k, 54k or 800k panels according to their sex, then their genotypes at a pair of independent *loci* were coded together asymmetrically. Using this coded data, there was no error in dividing the 270,184 pure breed animals from the 29,816 F1 crossings; in fact, the values of a dedicated homozygosity index calculated by 3k, 7k, 54k and 800k SNPs panels were 704±14, 1618±45, 12,668±318 and 188,331±4197 respectively for pure breed animals, and 417±17, 964±26, 7532±74 and 112,349±264 for crossings. A model, where the dedicated homozygosity index was used to predict the inbreeding coefficient, calculated from the pedigree file, showed a determination coefficient of 0.62. The coded data were also useful for parentage testing; using 600,000 parent-progeny pairs, an incompatibility index was calculated; the 1th-99th percentile ranges of this incompatibility index were 0-6 (3k), 0-11 (7k), 17-53 (54k) and 351-672 (800k): these ranges respectively raised to 50-198, 125-443, 1027-3397 and 16,663-51,970 when the 600,000 couples of animals were not a parent-progeny pair. We speculate that the proposed coding of genotypes could improve the exchange of data between breeders' organizations.

C-004

Comparison between 800K and 54K in Genome Wide Association Studies for production and morphologic traits in Italian Holstein

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Genome Wide Association Studies (GWAS) exploit linkage disequilibrium (LD) and a high number of markers to explain the variation of productive and linear traits in livestock. LD varies across cattle breeds depending on their genetic management and demographic history. As a consequence of wide use of artificial insemination (AI), the effective population size of the Italian Holstein dairy breed is estimated to be quite low, less than 100 individuals. Under these conditions LD is expected to be high, so that marker-trait associations can be detected at a distance of several hundred kb down and up stream of a causative mutation. However LD depends also on the genomic landscape and in particular on the distribution of recombination hotspots along chromosomes. To investigate the effect of local LD on GWAS results, we have genotyped 916 Italian Holstein bulls with the 800K SNPchip and analyzed them at 54K and 800K resolutions. Single SNP mixed model, estimation of haplotype blocks and Bayesian approaches were evaluated for milk, fat, and protein yield as well as for height and udder conformation. Preliminary results indicate that 800K SNPchip confirms significant regions detected with the 54K panel but better define the QTL position, possibly facilitating the downstream search for causative mutations.

C-005

Genomic structure of Italian Holstein assessed by high density single nucleotide polymorphisms

Paolo Ajmone Marsan[1], Ezequiel Luis Nicolazzi[2], Johannes B.C.H.M. van Kaam[3], Lorraine Pariset[4], Alessio Valentini[4], Cesare Gruber[4], Maria Gargani[4], Nicola Bacciu[5]

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Since a few years the 50K SNPchip panel (54,001 SNPs) is used for a number of applications in animal breeding, spanning from the estimation of genomic relationship between animals, to genome wide association studies, detection of selection signatures and genomic selection. A new high density SNP panel has recently become available, containing 777,962 SNP (800K) evenly spread along the genome and discovered in a much wider pool of breeds, compared to the 50K. Genotyping 800k is more expensive than genotyping 50k, but the higher density of the panel potentially permits a more accurate study of the genetic structure of populations. In this study we compared the genetic structure of the Italian Holstein populations as inferred from 50K and 800K analyses. Haplotypes and LD were estimated using a combination of in-house software, FastPHASE and R genetics packages. Results are reported with particular reference to BTA6 where casein genes are located. In BTA6, the 50K SNP panel detected 67 haplotype blocks of 2 to 6 consecutive markers, while the 800K panel identified 1919 blocks of 2 to 96 markers. Two regions, a gene-rich region around the casein genes, at 87Mbp and a gene-poor region at 55.5Mbp were compared. In these regions adjacent pairs of 50K SNPs contained between 3 and 19,800K SNPs that defined 1 to 38 different sub-haplotypes. The highest value of LD was found in the alpha-S2 casein region (r^2=0.81). The average LD detected by the 50K panel along BTA6 (r^2=0.16) was lower than that detected by the 800k panel (r^2=0.22), indicating that the latter permits the investigation of the BTA6 structure at a higher level of resolution. In fact a higher number of haplotypes and LD blocks of smaller size were revealed by the higher density panel. In spite of this, the literature reports only a slight gain of the reliability of genomic estimates of breeding values when using the 800K compared to 50K SNPs, suggesting that *loci* of higher effect preferentially occur in regions of high LD.

C-006

Evaluation of direct genomic values by using the maximum difference analysis to reduce the number of markers

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Computational difficulties are one of the most important constraints that limit a wide diffusion of genomic selection in animal breeding. To partially overcome these problems, we proposed a new procedure, named maximum different analysis (MDA), able to select a pool of markers significantly associated (both positively and negatively) with a particular trait. The retained SNP were used to evaluate the direct genomic value (DGV) of the involved animals. Data consisted of genotypes belonging to 552 Simmental bulls genotyped with the Illumina's 50K BeadChip. Phenotypes were polygenic breeding values for milk, fat and protein yield. At the beginning, animals were ranked according to a particular trait (T). The best 35 (B) and the worst 35 (W) animals were then selected and the genotypic frequencies were evaluated. The procedure to select markers positively associated with T started by considering, for each SNP, the maximum genotypic frequency in B. In correspondence, the frequency for the same genotype was recorded in W and the difference between the two frequencies was calculated. This value represented the maximum difference (MD) frequency for a SNP. A bootstrap procedure was then implemented to derive a posterior probability distribution that was used to declare a SNP positively associated with T. Markers negatively associated with T were detected through the same procedure with the only difference that, for each SNP, the maximum genotypic frequency was recorded in W. For each trait, a pool of SNP was selected and used to evaluate DGVs. Results were then compared with DGVs obtained by using all markers. The MDA selected 155, 177 and 217 different markers for milk, fat and protein, respectively and, for the same traits, DGV accuracies were 0.35, 0.39 and 0.41. Accuracies evaluated with all SNPs were 0.20, 0.27 and 0.24 for milk, fat and protein, respectively. These results suggested that a customize assay containing only the MDA selected SNPs could be developed to genotype animals thus reducing costs and computational resources.

C-007

Use of the partial least squares regression to impute single nucleotide polymorphism genotypes from low to high density single nucleotide polymorphism platforms

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High-density marker platforms (HDP) of different SNP densities are currently used to genotype animals involved in genomic selection programmes. However, to reduce the genotyping costs, several low-density SNP platforms (LDP) have been recently proposed. Genotypes obtained from a LDP could be then imputed to HDP by using suitable algorithms. In the present study, the ability of the partial least squares regression (PLSR) to impute genotypes from LDP, with 3K or 7K SNP, to HDP, with 50K SNP, was tested. PLSR results were compared with prediction obtained by using Beagle, one of the most widely used imputation software. In all scenarios, no pedigree information was used. Data consisted of 2093 Holstein, 749 Brown Swiss and 479 Simmental bulls genotyped with the Illumina 50K Beadchip. Two approaches were developed: the single-breed, with only data from Holstein animals and the multi-breed where data from the three involved breeds were joined. The impact of genotype imputation on breeding value evaluation was tested for milk yield, fat and protein content. In the single-breed approach, the accuracy of imputation using PLSR was around 90% and 94% for 3K and 7K platforms respectively, whereas the corresponding accuracies obtained by using the Beagle package were around 85% and 90%. Moreover, computing time required by PLSR to impute SNP genotypes was on average around 10 times lower than computing time required by Beagle. The multi-breed approach did not improve the ability of PLSR in genotypes prediction. In this case imputation accuracies were lower than values obtained in the single-breed approach. The impact of the SNP-genotype imputation on direct genomic value accuracy was small. The correlation between the genetic merit obtained by using imputed and actual data was around 0.96 for the 7K chip. Results of the present research suggested that the PLSR imputation method could be useful to impute SNP genotypes especially when family and pedigree information is not available.

C-008

Effect of reference population composition and number of principal components on the accuracy of multiple-breed genomic evaluation

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The use of multiple breed (MB) reference populations might affect genomic selection (GS)accuracy. In this study the effects of breed composition and number of principal components (PC) on the accuracy of MB GS are investigated. A total of 3559 bulls (2093 Holstein, 749 Brown and 717 Simmental) were genotyped at 54,001 Single Nucleotide Polymorphism (SNP) and used to estimate direct genomic values (DGV) with either single breed (SB) or MB approach. Seven datasets were used: Holstein (H), Brown (B), Simmental (S) and different MB composition (HBS, HB, HS, BS). Editing was performed for each breed and for MB populations. SNPs out of Hardy-Weinberg (HW) equilibrium in the SB were removed, whereas in the MB were either kept

(HWk) or removed (HWr). SNPs retained after data editing ranged from 30,326 to 41,360 for HWr and HWk respectively. PC explaining increasing amounts of variance (50%-99%) were extracted from actual genotypes. PC effects were estimated using BLUP. The random effects of PC scores on standardized deregressed proof (DRPF) were estimated in the reference population and then used to predict DGVs of validation bulls (DGVVAL). Four traits were analyzed: milk (MY), fat (FY), protein (PY) and somatic cell score (SCS). The bulls in the MB reference population differed according to breed composition, whereas the validation bulls were the same for all scenarios. Two analyses were performed: a) individual estimation of PC effects, computed in SB; b) joint estimation of PC effects from MB population. Correlation (rDRPF,DGV) between DRPFVAL and DGVVAL were used to evaluate accuracy of predictions. PC explaining from 70% to 99% were considered here. For example, rDRPF,DGV of MY increased passing from 70% to 99% of variance both for SB or MB. For MB HBS reference the accuracy of HWr or HWk did not vary anymore when variance explained by PC was greater than 80%. rDRPF,DGV using HBS reference in comparison with SB did not vary in S, whereas rDRPF,DGV was greater for PC<80% in H (+8% for PC70%,+0% for PC99%) and always increased in B (+9% for PC70%,+5% for PC99%). A multibreed reference population has no negative effects on GS acuracy.

C-009

Feasibility of using a sample of the whole population to calculate the genomic breeding values in the *Sarda* sheep breed

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The aim of this paper was to evaluate the ability of a small portion of the whole registered population bred in a genomic flock (GF) to predict the genomic breeding values (GEBV) of the registered *Sarda* population (RSP). The GF consisted of 2,062 ewes corresponding to 4 generations of descendants of around 1,000 back-cross Lacaune x *Sarda* ewes and *Sarda* rams. The RSP was represented by 141 artificial insemination rams (AI) with a high genetic impact on RSP. Forty-seven out of them had at least 7 daughters in GF (AIgf). The average number of daughters of AIgf in GF was 36, ranging from 7 to 91. All animals were genotyped with the OvineSNP50 Beadchip; milk yield deviations and official breeding values (EBV) were available for GF and AI rams respectively. The genomic relationship matrix (G) was built including GF and AI individuals in order to estimate the level of the genetic link between them and to predict GEBVs of AI by GBLUP (Hayes *et al*., 2009). GEBVs were estimated using just the yield deviations performed in GF. The average genomic relationship coefficient was 0.073 between AIgf and AI without daughters in GF (AI0) and it was 0.085 among AI0 rams. Correlations between GEBVs and EBVs were 0.45, 0.40 and 0.38 for AIgf, AI0 and all AI rams respectively. Such results confirm that genotypes and performances produced in GF can be useful to estimate GEBVs of the whole population. This approach may be important either to increase the accuracy of genetic evaluations for routinely recorded traits or to produce GEBVs for traits costly to record. The crucial point is to identify the best strategy to built the genomic flock taking into account either the need of representing the genomic variability of the whole population or the costs related to breeding, genotyping and trait recording.

Acknowledgments

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C-010

The risk of ascertainment bias in estimating population genetics parameters from single nucleotide polymorphism data

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In all major livestock species, HapMap projects have discovered millions of SNPs permitting the development of arrays assessing in parallel from thousand to almost a million markers. These are currently used in genomic selection, genome wide association studies, identification of selection signatures and in the investigation of population structure and diversity. In a number of these applications, the use of a marker panel affected by ascertainment bias can reduce the power of the analyses, producing false associations and distorting estimates of within and between breed diversity. In this investigation we have analysed the effect of HapMap SNP subsets of different origin (Holstein, Angus, Brahman, Limousine, Jersey, and Norwegian Red) on the population genetics parameters of 9 breeds (Angus, Piedmontese, Holstein, Jersey, Charolaise, *Romagnola*, Limousine, Swiss Brown, N'Dama) in comparison with the version 1 of the Illumina 50K Bovine SNP BeadChip. According to our results, SNP origin has a clear biasing effect on estimates of expected heterozygosity, significantly affecting the relative ranking of the breeds investigated. Conversely, it has different effects on the analysis of population structure: we observed outstanding biases when a few hidden population structures are hypothesized whereas this became less evident considering a higher number of substructures. Our results suggest that this bias is manageable, in the case of comparison between breeds, using less divergent populations. Appropriate correction methods, *e.g.* as those described in Nielsen (Human Genomics 2004, 1:218-224) must be taken into account when analysing genetic parameters in distant breeds.

C-011

Direct and social effects on variation of carcass and ham quality traits in Italian heavy pigs

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Interactions among individuals are common in penned animals as swine and may affect their performance. In traditional pig breeding programmes the impact of the genetic effect of an individual on phenotypes of its pen mates, called social genetic effect, is ignored. Heritable social effects, if present, may become part of total heritable variance and affect the response to selection. We investigated the contribution of direct and social effects to variation of carcass and ham quality traits in 9871 crossbred Goland heavy pigs. Animals were raised in 1645 pens containing from 4 to 7 individuals. Final body weight adjusted at 270 d (WGT270), carcass backfat, ham subcutaneous fat thickness near quadriceps and semimembranosus muscles measured by ultrasound device, iodine number, linoleic acid content and scores from subjective appraisal of ham round shape, marbling and fat covering were traits of interest. Estimates of variance component and genetic parameters were obtained by REML. Comparisons between sequential models including, in addition to sex and slaughter group fixed effects, the random effects of the social group and full sibs family (FSF), direct and social additive genetic effects of pigs were performed through likelihood ratio tests (LRT). The model including social group, FSF, and direct genetic effects provided the best fit. The estimated heritability did not vary substantially when social effects were considered. Likewise, social group and FSF variances were stable across models and accounted for 4% of phenotypic variance. Covariance between direct and social genetic effects was negative with the exception of WGT270. It means that animals with positive direct effects on their own phenotype exert on average negative effects on phenotypes of their group mates. As a consequence, total heritable variance of models including social effects decreased. Social variance estimates were small and explained from 0.15 to 0.7% of phenotypic variance. The LRT showed that addition of social effects did not significantly improve the fit of the model. In conclusion, small heritable social effects affected negatively carcass and ham quality traits of heavy pigs. However, considering the fit of models, prediction of breeding values through a traditional model including direct effects seems still preferable.

C-012

Genomic selection in Italian heavy pigs: a simulation study

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Genomic selection is becoming a routine application in dairy cattle breeding for the advantages it gives in terms of reduced generation interval, increased accuracy and increased selection intensity. Effectiveness and practical implementation of genomic selection on pig breeds is not clear yet due to biological and technically-derived differences between pig and cattle breeding (*e.g.* much shorter generation interval, large litter size, reduced number of semen doses produced per boar in A.I.). However, a few preliminary North European simulation studies have reported that some advantage from genomic selection might also be expected in pigs. However, studies based on North European vision of pig breeding do not take into account the peculiarities of the Italian heavy pig breeding system. Italian pig selection is based on a sib-testing scheme in which 2 full sisters and 1 full castrated brother from the same litter of the candidate boar are performance tested. This scheme opens a wide range of different genotyping strategies to be evaluated. To this aim, a pig breeding population with a 18 chromosome genome marked with 60K bi-allelic single nucleotide polymorphisms and several segregating QTLs was simulated by using the software QMSim. The population was genetically selected over 25 generations based on BLUP estimated breeding values (EBV) for a trait with heritability of 0.40. Culling was based on EBV both in sires and dams. Different genotyping strategies as well as different methods of predicting genomic breeding values (GBLUP, Bayes or Single Step) were compared. Accuracies of genetic values were calculated as correlation with true breeding values. Considering the simulated scenarios, the introduction of genomic evaluations might increase accuracy levels both in young piglets and in selected reproducers. In particular, the choice of the male candidate would be more effective. Other simulations are under way to evaluate if genomic selection could actually give economic advantages to the Italian heavy pig breeding industry.

C-013

Genetic analysis of elbow and hip dysplasia in Italian population of Bernese Mountain dogs

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The objective this study was to estimate genetic parameters for hip (HD) and elbow dysplasia (ED) in Italian Bernese Mountain dogs. Data included screening results provided by two different screening centres of 2051 dogs (1223 females and 828 males) for HD and 1564 dogs (935 females and 629 males) for ED. Screening date ranged from 1995 to 2011 and from 1999 to 2011 for HD and ED, respectively. The outcome variables were: HD score, performed according to the official protocol of the Federation Cynologique Internationale (FCI) and based on a 5-class linear scoring system (from A to E); and ED score, performed according to the International Elbow Working Group (IEWG) protocol and based on a 4-class grading system (from grade 0 to grade III). The pedigree file was provided by the Italian Kennel club (ENCI) and included 5983 dogs. A Bayesian analysis was implemented via Gibbs sampling. Effects of sex, year-month of birth, age of the dog at screening, the experience of the x-raying veterinarian and the screening centre were assigned flat priors; those of kennel and animals were given Gaussian prior distributions. Prevalence for HD (*i.e.* percentage of hips scored C, D or E) was 15.9%, and prevalence for ED (*i.e.* percentage of elbows graded I, II or III) was 20.3%. Marginal posterior medians and the lower and upper bound of the 95% highest posterior density (HPD95) of heritabilities of HD and ED were 0.22 (0.13-0.33) and 0.16 (0.07-0.30), respectively. The genetic correlation between these two traits were 0.53 and the HPD95 did not include zero. These could ensures feasibility of selection programmes aimed to reduce prevalence of skeletal diseases, provided that a regular screening program of Bernese Mountain dogs is performed.

C-014

Joint evaluation of horse and rider in show jumping by an Elo-system

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Many games and sports must face the problem of calculating the value of the competitors because the result depends on their relative, and not absolute, abilities. The rating system developed for chess by A. Elo has been widely adopted: it assumes that the performance is a normally distributed random variable, so that ranking probability for each competitor can be estimated by the rating differences from the others. In show jumping, both the horse and the rider are competitors, and each one contributes to the final result. A dataset with 227,679 official results in 37,373 show jumping, held in Italy between 2005 and 2012, was used to rate 6078 Saddle Horses and 3715 riders according to an Elo model with 950,453 paired comparisons; the classical values of 2000 for performance mean and 200 for performance standard deviation were used, and the update coefficient k was optimized to predict the final rankings: the best k values resulted to be 21.5 for horses and 9.5 for riders. The Elo-performance of the horse was then considered as the trait of BLUP models, in order to estimate its heritability and repeatability and the EBVs; a pedigree of 28,170 horses was used for the random additive effect. Estimates of heritability and repeatability were generally low; values of 0.064±0.007 for heritability and 0.151±0.012 for repeatability were found if the rider was not in the model, and these values slightly changed to 0.064±0.005 and 0.125±0.010 if the rider was included as a random effect; on the contrary, the values raised to 0.104±0.009 and 0.262±0.010 when the Elo-performance of the raider was included as a covariate. Also the cross-validation confirmed the importance of including the Elo-performance of the raider in the estimation of horses EBVs.

C-015

Genetic parameters of linear type traits scored at adult age in Italian Heavy Draught Horse

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The Heavy Draught Horse (IHDH) breed is commonly selected on the basis of linear type traits evaluated at young age on foals born in the same year. A performance test at population level allowed the genetic evaluation of stallion and mares for traits related to the main selection goals, *i.e.*, heavy draught and meat production. However, animals retained for reproduction are scored also at about 30 months of age, *i.e.*, at the official admission to the stud book. This study represents a first contribution on genetic parameters of type traits scored at adult age in the IHDH breed. The initial database consisted in 7133 evaluations of adults scored by 29 classifiers in 19 subsequent years of evaluation (*i.e.* from 1992 to 2011) for 14 linear traits with a 9 point scale system (from 1 to 5 including half points), and a final 5 point judgement score (from fair to excellent). After editing, 4205 horses, 3600 females and 605 males, were retained for further analysis. Data were analyzed using a multiple-trait EM-REML animal model, accounting for the following fixed effects: herd-year-classifier (1297 levels), sex (2 levels), age at evaluation (5 classes, *i.e.*, ≤27, 28, 29-32, 33-47, and ≥48 months of age). The heritability obtained ranged from 0.05 to 0.34. The heritability of the two traits related to outward appearance, *i.e.*, head size expression and temperament resulted 0.28 and 0.17, respectively. On the other hand, the heritability of the traits related to muscularity, *i.e.*, fleshiness, fore and rear diameters resulted of medium-high level, ranging from 0.22 to 0.33. All traits related to limbs showed low heritability values (*i.e.*, from 0.05 to 0.13). The trait with the highest heritability was the frame size (*i.e.*, 0.34), while the final score reached an heritability of 0.29. The highest genetic correlation obtained was between fleshiness and rear diameters (*i.e.*, 0.96). A high genetic correlation was also estimated between fleshiness and fore diameters (*i.e.*, 0.63), and between fore and rear diameters (*i.e.*, 0.68). The more negative genetic correlation observed was between temperament and bone incidence (*i.e.*, -0.73). Accounting for genetic parameters may improve selection in 30-months-animals.

C-016

Effect of stoned olive oil cake on rumen bacterial community: preliminary results of an *in vitro* study

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Understanding the effect of diet on the biohydrogenation (BH) process is important to optimize the production of healthful food from ruminant livestock. From literature, it is well known that the availability of C18:1 trans11 (vaccenic acid, VA) and C18:2 cis9 trans11 (rumenic acid) in ruminant products is limited as consequence of their hydrogenation to C18:0 (stearic acid) by rumen microbial activity. Rumen fermentation can be modulated by presence in feeds of bioactive molecules that are able to affect the structure and diversity of rumen microbial populations. In an *in vitro* trial the effect of stoned virgin olive cake (SVOC), rich in polyphenols, that are potentially able to interfere with rumen fermentation involved in BH processes and methane production, has been investigated. The inclusion of SVOC in feeds at the level of 50 g/kg and 90 g/kg induced changes in fatty acid profile and in microbial populations; in particular, the contemporary disappear of a band (DGGE) related to *Butyrivibrio proteoclasticus* and the accumulation of VA (+50%) has been observed. Moreover, a depression of other microbial groups mainly related to the orders Clostridiales and Neisseriales has been detected.

C-017

Influence of dietary crude protein and rumen protected conjugated linoleic acid on rumination activity, feed intake and rumen fluid parameters in lactating cows

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This trial compared the effects of dietary protein level and rumen protected conjugated linoleic acid (rpCLA) on dry matter intake (DMI), rumination activity, rumen fluid parameters and milk yield. Twenty Holstein-Friesian cows, homogeneous for parity, body weight, body condition score, DIM, and milk yield, housed in pens of 5 were fed 4 diets containing 15% (CP15) or 12% CP (CP12) supplemented or not with 80 g/d of rpCLA, following a 4×4 Latin Square design. Each period lasted 2 weeks for adaptation and one for experimental measurements. The CP12 diet was formulated from CP15 by replacing soybean with barley meal, and they contained similar NDF content (37% DM). Dry matter intake, rumination activity, and milk yield were daily monitored. Rumen fluid was analysed for pH, volatile fatty acids (VFA) and ammonia N (N-NH3), milk was analyzed for proximate composition. Data were averaged by group of cows and analyzed with a general linear model considering period, dietary treatment and group as sources of variation. The reduction of dietary CP content did not influence DMI, increased rumination activity from 16.8 to 19.8 min/kg DMI (+18%; P=0.009), increased rumen fluid pH (P=0.034), decreased rumen fluid N-NH3 (P<0.001), did not influence milk yield (P=0.072) and milk fat content (P=0.896), but slightly reduced milk protein from 3.53 to 3.36% (P=0.026). Addition of rpCLA reduced DMI from 21.4 to 19.6 kg/d (P=0.018), did not affect rumination activity, rumen fluid pH and N-NH3, and VFA contents, milk yield and milk protein content but reduced milk fat content (P=0.003). It was shown that when a reduction of rumen degradable N is applied feed degradation in the rumen would be supported by a compensative increase of rumination activity. The effect of rpCLA on DMI could reflect a reduced energy requirement for milk fat synthesis induced by these bioactive molecules.

C-018

Use of different approaches to calculate the rate of neutral detergent fibre digestion and implication on estimate of neutral detergent fibre digested in rumen

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The rate of NDF digestion (kd, /h) should be considered in nutritional dynamic models for a correct nutritional estimate of feeds used in diet formulation. A first-order mathematical model is used to calculate kd. Generally, inputs required are NDF, lignin and NDF digestibility (NDFD) measured at different rumen incubation times. These parameters are needed to calculate available NDF at time 0 and at each incubation time and indigestible NDF (iNDF, calculated as 2.4 times lignin content of NDF). We previously showed how different calculation procedures (3h fixed *vs* estimated Lag, single *vs* multiple time-point calculations or estimated *vs* measured iNDF) strongly influence kd estimate, suggesting the best way to compute kd valuation is through the use of a multiple time-point calculation with estimated Lag and measured iNDF. In the current work, five different mathematical approaches were used to calculate kd in 4 feeds (*i.e.*, corn silage, wheat bran, alfalfa and ryegrass hays) and being 24h (calculated by using 3h fixed Lag and 24h NDFD), 30h (3h fixed Lag and 30h NDFD), 12-24h (3h fixed Lag and 12h and 24h NDFD), 6-24h (Lag estimated by model and 24h NDFD) or 6-36h (Lag estimated by model and 6h, 12h, 24h, 30h and 36h NDFD). For each feed, the iNDF was measured after 288h of rumen incubation and used to estimate the unavailable NDF pool. As expected, the adopted approaches influenced ($P<0.01$; $\sqrt{MSE}$ 0.00414) kd values with a different response within feeds (sample x model interaction, $P<0.05$). Then, a mechanistic model (multiple NDF pools and multiple concurrent reactions) was constructed to estimate the amount of NDF digested in rumen after 24h or 48h using a computer-aided simulation system, the NDF ingested at time 0 being 7.82 kg. For each approach, a simulation was carried out by using a fixed rate of NDF passage (0.03/h) and kd values calculated for 4 feeds with the different procedures. After 24h, the NDF digested in the rumen ranged from 2.88 kg (30h) to 3.39 kg (12-24h). Similarly, NDF digested in the rumen after 48h ranged from 3.44 kg (30h) to 3.88 kg (12-24h). Intermediate values were obtained by using other models. Concluding, the inappropriate use of model inputs in kd calculation could lead to misleading estimate of NDF digested in rumen.

C-019

In vivo digestibility of different forage species inoculated with *Arbuscular mycorrhiza* spp.

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The aim of this study was to evaluate the effect of inoculation with *Arbuscular mycorrhiza* spp. (AM) on the digestibility of 2 different forage species. The trial, carried out within the Project SOS-ZOOT, was conducted at Bella farm of CRA-ZOE, located in Basilicata region (Southern Italy) at 360 m. a.s.l. For autumn sowing of barley (*Hordeum vulgare*) and clover (*Trifolium alexandrinum*), 2 plots were used for each species: one sown with seeds inoculated (Mycorrhized) with *Arbuscular mychorriza* (commercial products Micosat F®, 1.0 kg/ha) and the other one with seeds not inoculated (Control). Commercial AM products were added to moistened seeds before sowing. The *in vivo* digestibility of barley and clover fresh forage inoculated with AM was evaluated in Rossa mediterranea dairy goats. Twenty-eight mature goats were randomly assigned to 4 groups, homogeneous for age (2.5±0.3 years) and body weight (43.3±1.5 kg), and received the fresh forages produced from inoculated seed or not: Mycorrhized barley group (MBG) and Control barley group (CBG); Mycorrhized clover group (MCG) and Control clover group (CCG). Barley was given fresh *ad libitum* at pre-earing stage, while fresh clover was given *ad libitum* at pre-flowering stage. After 10 days of adaptation, the experimental period has lasted 5 days. Coefficients of organic matter (OM), crude protein (CP), crude fibre (CF) and neutral detergent fibre (NDF) *in vivo* digestibility were measured by using acid-insoluble ash as internal indigestibility marker. Data were analysed by GLM procedure with the forage species as fixed effect. Means were compared by t-test. The results revealed a significant effect of AM inoculation only in grass species both in chemical composition and digestibility. The MBG showed significant increases of the digestibility coefficients in comparison with CBG: OM was 66% *vs* 58% ($P<0.001$), CP 67 *vs* 58% ($P<0.001$), CF 52 *vs* 43% ($P<0.01$) and NDF 54 *vs* 43% ($P<0.001$). The treatment with AM on clover seed did not affect significantly the *in vivo* fresh forage digestibility. The results showed that the artificial inoculation with *Arbuscular mycorrhizal* fungi was able to enhance the digestibility in goat of barley grass forage.

C-020

Comparison between two gas sampling techniques for measuring methane production from *in vitro* fermentation of ruminant feeds

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This experiment compared two techniques of gas sampling to measure methane production from ruminant feeds incubated for 24 h. Gas was sampled by a syringe from: i) headspace of bottles (HB) in which gas was accumulated over 24 h; ii) tight bags (TB) connected to bottles, in which gas was vented by an open-closed valve at a threshold pressure of 6.8 kPa. Bottles (310 mL) were filled with 60 mL of buffered rumen fluid (headspace=250 mL) and 0.04±0.01 g of feed sample, and incubated at 39±0.5°C. Five feeds (mixed and alfalfa hays, corn meal, dry sugar beet pulp, flaxseed expeller) were tested. The experimental design was: 5 feeds × 3 replications × 2 gas sampling techniques, plus 4 blanks (bottles without feed sample), for a total of 34 bottles incubated. Gas production (GP) was monitored by a PC wireless-connected to all bottles, and GP values were adjusted for the amount of solubilised CO_2 (GPa), according to the Henry's law. Gas (2 μL) sampled using HB or TB technique was analyzed for methane by gas-chromatography. Data were submitted to ANOVA considering feed, gas sampling technique and their interaction as sources of variation. On average, no venting of bottles provided GP values at 24 h lower compared to venting at threshold pressure (184±3.8 *vs* 231±4.0 mL gas/g DM for HB and TB, respectively; $P<0.01$). Differences among techniques were also observed for GPa values (216±4.0 *vs* 234±4.2 mL gas/g DM, for HB and TB, respectively; $P<0.01$). Due to partial solubilisation of CO_2, methane concentrations resulted greater with HB compared to TB, for GP values (13.6±0.40 *vs* 8.1±0.51% CH_4 on total GP for HB and TB, respectively; $P<0.01$). When methane concentrations were referred to GPa, differences among two techniques were reduced but not removed (11.9±0.37 *vs* 7.9±0.47% CH_4 on total GPa for HB and TB, respectively; $P<0.01$). In conclusion, the adjustment of GP values was not sufficient to remove differences among techniques. Lower methane concentrations provided by TB technique could be due to gas losses from the connection between bottles and bags or no release of CH4 present in the headspace of bottles at the end of incubation.

C-021

Nutritional profile of high-biomass and forage sorghum silages

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Traditional agricultural systems will be foreseeably influenced by global climate changes, so the study of the nutritional quality of fodder obtained with plants that are resistant to drought and other stress conditions, is becoming increasingly important. The aim of the present study was to assess and compare the chemical composition and nutritive value for ruminants of biomass and forage sorghum silages. In 2008, two Syngenta® breeds of sorghum selected respectively for forage (FS) and biomass (BS) production were sown at two different densities (30 and 44 plants m^{-2}) in a meso-mediterranean climate area (Viterbo, Lazio, Italy) with two watering intensity (0 and 3,000 m-3 ha-1) over the natural rainfall regime. The green fodder was ensiled in vacuum plastic silos, with and without additive (Biotal Wholecrop Legume© at the dose of 1×10^6 U g^{-1} of herbage). At harvest, the productivity (average total weight of biomass produced and average total biomass useful for ensiling) was recorded, and some chemical parameters were determined both on green fodder and silages. All data were analyzed by ANOVA and means were compared with the LSD test. Significance was declared at $P<0.05$. The results showed that both the breed and agronomic factors influenced both the productivity and chemical traits of plants. The BS showed higher biomass production than FS, even without watering. As it is well known for sorghum, water stress did not cause death of the plant but induced only a vegetative stasis. However, CP and EE content of silage were greater ($P<0.005$) in the FS compared with BS (CP: 9.4±0.3 *vs* 7.9±0.4% DM, EE: 2.2±0.1 *vs* 1.7±0.1% DM). The nutritional quality of silages was affected by the DM content at harvest but not by the additive application. The use of BS for animal feeding seems an interesting option, even if it has lower nutritional quality than the forage breed. However, more detailed studies are needed.

C-022

Influences of dietary crude protein and rumen protected conjugated linoleic acid on the efficiency of nitrogen utilization in lactating cows

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In this trial the effects of a reduction of the dietary crude protein concentration, with or without addition of rumen protected conjugated linoleic acid (rpCLA), on DMI, milk yield, nutrients digestibility and N efficiency (milk N/intake N) were examined. Twenty Holstein-Friesian cows in middle lactation, homogeneous for parity, BW, body condition score, DIM, and milk yield, housed in pens of 5 were fed 4 diets containing 150 (CP15) or 120 g of CP/kg DM (CP12) with both CP15 and CP12 supplemented with 0 or 80 g/d of rpCLA, according to a 4×4 Latin Square design. Each period lasted 2 weeks for adaptation and one for experimental measures. The CP12 diet was formulated from CP15 by replacing soybean meal with barley meal to contain similar NDF content (37% DM). DMI and milk yield were recorded daily. Digestibility was evaluated using ADL as a marker, milk was analyzed for its chemical constituents. Individual data, averaged by group within period, were subjected to ANOVA. The reduction of dietary CP did not influence DMI, DM and NDF digestibility but reduced CP true digestibility from 0.82 to 0.77 ($P<0.001$), milk yield from 29.0 to 27.7 kg/d ($P=0.072$), milk protein yield from 1.00 to 0.91 kg/d ($P=0.006$), and increased N efficiency from 0.31 to 0.36 ($P<0.001$). Addition of rpCLA reduced DMI from 21.4 to 19.6 kg/d ($P=0.018$), milk fat production from 0.99 to 0.93 kg/d ($P=0.001$) and improved N efficiency ($P=0.019$). Differently from what observed in literature on beef, no CP × rpCLA interaction was observed. The significant effect of rpCLA on N efficiency suggests that these bioactive molecules would exert influences on N metabolism, irrespectively by the dietary CP density used. The strong reduction of dietary CP had influences on milk and milk protein yields much smaller than those expected, as NRC model predicts for the same CP15 and CP12 diets milk yields of 23.8 and 13.9 kg/d, respectively. This would reflect the existence of some compensating mechanisms, such as N recycling, operating when cows are kept under sub-optimal N supply

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C-023

Effects of rumen protected choline supplementation on milk production in dairy cows fed hay based diets

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Choline has been proposed as limiting nutrient in lactating dairy ruminants especially in early lactation. This situation seems to be exacerbated when silage-based diets are fed, whereas less is known when hay-based diet are used. In light of this the aim of the study was to evaluate milk production in dairy cows receiving a hay-based diet supplemented with rumen-protected choline (RPC). For this purpose ten Italian Holstein multiparous cows, in the second month of lactation (50 days in milk), were divided into two experimental groups: control group (CTR) receiving an empty capsule (no choline), and RPC group receiving 20 g/day of

choline in rumen protected form. Cows were fed a hay-based diet designed according to NRC 2001 requirements. The experiment lasted 60 days. The mean postpartum DMI was unaffected by the treatment (26.0 and 25.2 kg/d for CTR and RPC, respectively). Mean milk yield did not change between experimental groups (33.3 kg/d and 34.3 kg/d for CTR and RPC, respectively), even though RPC supplementation increased milk production ($P<0.05$) in the first weeks of the trial. A slightly increase in milk protein yield (1012 *vs* 1093 g/d for CTR and RPC, respectively; $P<0.05$) has been observed in RCP supplemented animals. No change was observed in milk fat content (3.23% *vs* 3.22% for CTR and RPC, respectively). Dietary treatment did not affect the metabolic profile of experimental cows. Results obtained from the present trial suggested that RPC administration can be effective in optimizing milk production in dairy cow receiving a hay-based diet, even though an exhaustive comparison with a silage-based diet deserves further investigations.

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C-024

Influence of three dietary phosphorous levels during late gestation on metabolic profile of periparturient dairy cows

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The reduction of dietary P levels may be environmentally interesting if cattle physiology and performance are not negatively affected, especially in the transition from gestation to lactation. Our objective was to evaluate the effects of reducing P content within concentrate feeds for dairy cows in late pregnancy on mineral metabolism during the periparturient period. Twenty-four late pregnant Italian Friesian cows were randomly assigned to three groups to be fed isoenergetic and isoproteic diets (0.76% Ca on DM basis) with 0.33, 0.42 or 0.51% P (DM basis) from 4 wk before calving to parturition. After parturition, all cows were fed a common lactation diet. Blood samples were weekly collected from 4 wk before to 4 wk after calving and analysed for plasma minerals (Ca, P, Mg, Na, K, Cl), alkaline phosphatase (ALP) and tartrate-resistant acid phosphatase (TRAP) activities by an automated clinical analyzer. Data from prepartum and postpartum periods were separately analysed as repeated measures by a linear mixed models with diet, week from calving, and their interaction as main factors and random cow within treatment. No cases of postpartum collapse were observed. Dietary P levels had no effect on the considered plasma variables during both periods. The week from calving significantly affected ($P<0.05$) prepartum Na and Cl plasma contents and ALP activity, and postpartum Ca, Na, Cl and K plasma concentrations and ALP and TRAP activities; only a trend ($P<0.10$) for a week effect on plasma Ca content was observed in the prepartum period. During pre and postpartum there was an interaction ($P<0.05$) between diet and week from calving on plasma iP. In conclusion, it is possible to affirm that feeding diets with low P levels during the last 4 wk of gestation has no adverse effects on mineral metabolism and enzyme activities relative to the bone metabolism, this way allowing to reduce P output in the environment.

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C-025

Influence of three dietary phosphorous levels during late gestation on fecal and urinary biochemical features of periparturient dairy cows

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Phosphorous from animal production industry is a topic of growing interest for its environmental impact. Therefore, nutritional strategies must take P into account to be optimised. The objective of this trial was to compare the effects of three different prepartum dietary phosphorus levels during late gestation on foecal P concentration, dry matter content, and pH, urinary pH and biochemical features in periparturient dairy cows. Fifteen late pregnant Italian Friesian cows were randomly assigned to three experimental groups fed isoenergetic and isoproteic diet with 0.33, 0.42 or 0.51% P (DM basis) throughout 4 wk before calving. After parturition, all cows were fed a common lactation diet. Blood, urine, and fecal samples were collected at -5, -1, +1 and +5 wk from calving. Fecal P content was analyzed by ICP-MS, whereas blood plasma and urine minerals (Ca, Mg, inorganic P, K, Na, Cl) and creatinine contents were analysed by an automated clinical analyser. Urine : plasma ratio, urinary mineral : creatinine ratio, and relative fractional excretions of each considered mineral were calculated. Separate ANOVA were conducted for prepartum and postpartum period with prepartum dietary phosphorous as main factor. A significant linear regression ($R^2=0.319$ and $P=0.044$) between average dietary P concentration and individual fecal P concentration (DM basis) was evidenced during the week before calving, without any effect on fecal DM and pH. At the same time, urine pH and urinary concentration, urine: plasma ratio, urinary ratio to creatinine concentration, and fractional excretion of inorganic P, Ca, Mg, Na, K, and Cl were not affected by dietary P level both during prepartum and postpartum period. The decrease of P concentration in prepartum diet for dairy cows substantially reduces manure P content without secondary effects (in the tested range) for kidney metabolism in late-pregnant dairy cow, this way improving the environmental impact.

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C-026

Effect of feeding vitamins and trace minerals to dairy cows after long-term non-supplementation of these nutrients

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Dairy cows are usually fed amounts of vitamins and trace minerals that largely exceed their minimum requirements. This study was conducted at the experimental dairy farm of the University of Bologna and was aimed at evaluating the effect of feeding a vitamin and trace mineral mix to dairy cows that had previously not received any supplementation of these nutrients. Thirty Holstein dairy cows were fed for 8 months a total mixed ratio with no vitamin and trace mineral supplementation. After this period, animals were divided into two groups of 15 cows each (homogenous for milk yield, days in milk and age) receiving for 60 days a total mixed ratio (12.2 kg grass hay, 2.8 kg dehydrated alfalfa, 8.4 kg corn meal, 2.2 kg soybean meal, 0.9 kg molasses, 0.2 kg sodium bicarbonate, 0.1 kg sodium chloride) added or not (control) with a vitamin and trace mineral mix (VTM mix). Control diet met dairy cow minimum requirements for manganese and iron but covered only 50 and 40% of zinc and copper requirements (NRC, 2001), respectively; conversely, all trace mineral requirements were met in cows receiving the VTM mix. Results were analyzed by one-way ANOVA and differences were considered significant for $P<0.05$. During the whole experimental period, compared with data previously collected at the same farm, incidence of health events was not increased by not supplementing cows with vitamins and trace minerals. Feeding the VTM mix did not influence milk yield and composition. Similarly, plasma total protein, albumin, globulins, lysozyme and haptoglobin concentrations were not affected by diet. After 30 days, haemolytic complement activity was higher (50.0 *vs* 42.8 CH50/150 µL; $P<0.05$) in cows fed VTM mix. Oxidative stress as well as the number of leukocytes in blood did not differ between treatments. Plasma levels of copper and zinc were not influenced by treatment but, after 60 days, plasma iron levels were higher in cows fed VTM mix (28.9 *vs* 24.7 µmol/L; $P<0.05$). These data seem to suggest that feeding high levels of vitamins and trace minerals to lactating dairy cows might have only minor influence on animal productivity and immune status.

C-027

Principal component analysis of milk fatty acid: effect of the different feeding regimens

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Feeding regimen may significantly affect milk fatty acid composition, by modulating both rumen and mammary lipid metabolism. Hence, the aim of the present study was to evaluate milk fatty acid (FA) composition as a marker of the feeding regimen in dairy cow. Individual milk samples of 319 cows were collected from 24 commercial dairy herds (13±4 cows per farm). The animals were selected so as to have individuals homogeneously distributed along the entire period of lactation. Using a standard questionnaire, management and nutritional parameters were recorded. On the basis of questionnaire results, farms were grouped into 4 different feeding regimens: group1 (low level of forage and fat integration), group2 (medium level of forage and fat integration), group3 (high level of forage and fat integration), group4 (high level of forage and no fat integration). Methyl esters of FA were prepared by a direct extraction and alkali catalyzed transmethylation procedure, followed by gas-chromatographic analysis. A reference standard butter was used to estimate correction factors for short-chain FA. Data were analyzed by principal component analysis (PCA) to determine how the feeding regimen of the dairy cows and the composition of the milk were differentiated from each other. Data were analyzed by the GLM, with farm, days in milking, season of calving and diet group as main effects. Significance was declared at $P<0.05$. The mean value for the different variables was an average of all milk samples and was used directly in the PCA plot. In the present study, production data collected from 24 farms showed a large variation in feed intake between and within the 4 feeding regimens. The first (PC1) and second (PC2) principal component explained 28% and 12% of variation, respectively. The results showed that the different feeding regimens adopted by farmers, were significantly discriminated according to milk FA composition. In particular, the low level of forage is associated with a higher content of n-6 fatty acids and a high level of n-6/n-3 ratio. Fat integration is related to increasing amount of vaccenic acid and CLA (+25%).

C-028

Medical molecular farming: recombinant proteins from *Salmonella typhimurium* expressed in plant model

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In livestock, plant-based vaccines could represent an innovative strategy for oral vaccination, especially to prevent infections by enteric pathogens; furthermore, edible vaccines could be an efficient way to reduce antibiotic treatments, according to EC Regulation 1831/2003. *Salmonella* spp. infections are responsible for serious human and animal diseases. In particular, *Salmonella typhimurium* strains are recognized as the most frequent cause of foodborne outbreak and the swine specie is the main reservoir. The first aim of this study was to isolate flgK flagellin gene, a principal component of bacterial flagella, recognized as virulence factor by the innate immune system. The gene encoding for flgK was isolated by a polymerase chain reaction (PCR) from genomic DNA of a wild type *Salmonella typhimurium* strain, using specific oligonucleotides (including unique cloning sites for BamH I-5', Sac I-3'). The second aim of this study was to induce the seed-specific expression of flgK gene by the genomic transformation of tobacco plants. An overnight culture of EHA105 *Agrobacterium tumefaciens* strain, harboring the pBIpGLOB-flgK chimeric construct, was used for tobacco leaf disks infection. Regenerated plants and tobacco seeds were evaluated by PCR and immunoblotting techniques using established protocols. Obtained results showed that flgK gene can be incorporated into plant genome stably. Western blot analysis was carried out on all plants positive for flgK mRNA. flgK signals have been detected in all samples tested. By comparison with a positive control (flgK protein expressed by pET-system in BL21 *Escherichia coli* strain), the amount of flgK was estimated to be about 0.6 mg per gram of seeds. This amount corresponds to 0.3% of the total soluble proteins in tobacco seeds. In conclusion, flgK gene was specifically expressed, with the correct folding, in tobacco seeds. Tobacco plants represent an efficacious system for flgK flagellin expression and transformed tobacco seeds will be evaluated *in vivo* as a useful way for oral vaccination.

C-029

Effects of diets with different protein content and alternative protein sources on productive performances of Holstein young bulls

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One of the strategies to reduce the environmental impact on nitrogen excretion of ruminants is the lowering of protein in the diet. In this study, we analyzed the level of protein in the diet of Holstein young bulls and the lowering of protein content in the diet composed by alternative protein sources. The study was carried out on 35 bulls divided in 4 groups: P17 (n=8) fed, with a crude protein content of 17% on dry matter, NDF 34.17 and UFV (kg/DM) 0.90; P15 (n=7) with a crude protein content of 15% on dry matter, NDF 35.05 and UFV (kg/DM) 0.90; both these two groups were fed with a commercial protein dietary supplement based on soy bean. The other two groups were: F (n=10) fed with Field beans; C (n=10) fed with Chickpeas. Both these two groups were fed by changing the crude protein content from 15% to 11%, NDF 41.45 and UFV (kg/DM) 0.97. The study is started at an average weight of 336 kg. After 184 days bulls were slaughtered at commercial maturity an average weight of 544 kg. Significant differences was find on in vivo performances, P17 and P15 groups reached commercial maturity 47 days before groups F and C. Group F showed the highest carcass weights (308 kg *vs* 295 kg in average). The highest percentage content of Longissimus thoracis were in F and C groups (34.38% *vs* 21:34%), in these groups we found also the lowest content in lean meat (25.59% *vs* 41.21%) and in internal fat (6.50% *vs* 9.64%). The higher external fat was found in P15 group (4.15%) and in group C (4.03%). In conclusion the present study showed that P15 had the best results on *in vivo* performances, while groups fed with alternative protein sources, in particular group F, showed the best slaughter performances. F and C groups, fed with modulated protein level, showed the worst in vivo performances but the best ratio of tissue samples, for that reason we might use modulated level protein to breed animal with high genetic and commercial values.

C-030

Comparison of fatty acid profile in blood and in other tissues of piglets during the suckling time

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The aim of the study was to evaluate the changes of fatty acid composition of different body tissues and blood in piglets during the suckling time. Three sows of the same genotype artificially inseminated with the same pooled semen were used. Two days after delivery, four piglets per litter were chosen (for a total of 12 piglets) and tagged. Piglets were weighed daily until the 28th day and during this time suckling pig fed only milk. At 7, 14, 21 and 28 days of age one piglet per litter was sacrificed and the blood was sampled. Each piglet was dissected and brain, liver and longissimus muscle were collected. Lipids were extracted from tissues and plasma fraction of blood and the fatty acid (FA) profiles were determined by GC. The ANOVA was carried out for the fatty acid profile of each tissue using age of piglet as the main effect. During the experiment the weight of piglets increased linearly and the ADG was about 0.264 kg. The FA composition of plasma was significantly affected by piglet age. This is mainly due to the linear decrease of two relevant components like C18:0 and C18:1-c9. Along the experiment the most abundant FA in plasma was C16:0 that represents about 30% of total FAMEs. Brain was the tissue with the highest fat concentration (more than 30% of DM) and this value was not affected by the piglet age. In brain the very long chain fatty acids such as C20:4-n6, C22:4-n6, C24:0 and C22:6-n3 were largely represented (about 12, 6, 3.5 and 8% of FAMEs, respectively) and did not change along the experiment. In liver C18:0 was the main FA, relevant was also the concentration of C20:4-n6, both were not affected by age. In muscle of piglets 7 days old C18:1-c9 the main FA but its concentration decreased linearly, while an opposite trend was observed for C16:0 that become the main FA in piglets 28 days old. These results suggest to handle diet of lactating sow to meet the requirements in FA of suckling piglets.ating sow to meet the requirements in FA of suckling piglets.

C-031

Effect of slaughter weight on carcass traits and meat quality of *Nero d'Aspromonte* pig reared outdoors

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Nero d'Aspromonte is an autochthonous pig ecotype from the Calabria region, traditionally reared under extensive conditions. Information on carcass, meat and fat traits of this genetic type are still very few. Therefore, this study aims to assess the effect of slaughter weight on carcass traits and physico-chemical traits of subcutaneous fat and meat (pH, colour, cholesterol content and collagen properties) in *Nero d'Aspromonte* pigs. Sixteen barrows were reared outdoor and slaughtered at two live weights/ages: 8 animals at 164.3±5.9 kg/14 months and 8 animals at 206.3±12.0 kg/20 months. Feeding was based on natural pastures (woods, grasslands and Mediterranean bush) and fed *ad libitum* commercial mixtures, according to the growing period. Heavy pigs compared to the light ones had higher ($P<0.01$) dressing percentage (81.9±0.11 *vs* 81.0±0.16%, respectively) and backfat thickness (6.60±0.28 *vs* 5.59±0.79 cm, respectively). Slaughter weight did not significantly affect pH, colour and cholesterol content (ranging from 67.92±5.98 to 71.39±5.23 mg/100 g) in *Longissimus lumborum* (LL) muscle, as well as the total fatty acid composition of subcutaneous fat. Intramuscular collagen (IMC) amount (ranging from 27.51±5.92 to 31.66±6.28 µg/mg) and crosslinking (Hydroxylysylpyridinoline=HLP) concentration (ranging from 11.71±2.30 to 12.11±2.82 µg of HLP/mg) were not affected by slaughter weight ($P>0.05$). Differently, a significant effect on the stability of collagen fibers was observed; crosslinking were more stable in light pigs than in heavy ones (0.310±0.05 *vs* 0.259±0.01 moles of HLP/mole of collagen, respectively, $P<0.05$). In conclusion, the increase in slaughter weight of Nero d'Aspromonte" pigs is accompanied by an increase in dressing percentage and backfat thickness (can be exploited as a typical product). In addition, lighter pigs produced meat that could be tougher than that of the heavier, but more acceptable from the technological point of view.

C-032

Effect of genetic type and dietary crude protein level on carcass and ham traits of finishing heavy pigs

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This trial was supported by AGER, grant n. 2011-0280, and aimed to study the effect of genetic type and dietary crude protein (CP) level on carcass and ham traits of heavy pigs. Genetic types were selected according to their diffusion in commercial herds and involved Duroc x Large White ANAS (A), C21 Goland (G), Tempo x Topigs 40 (T) and DanBred (D) lines. A total of 184 pigs (gilts and barrows) progeny of at least 4 sires per line were raised in 2 subsequent trials from 90 to 165 kg BW and were fed conventional or low-protein diets providing on average 140 g CP and 6.45 g lysine/kg or 106 g CP and 4.85 g lysine/kg of diet, respectively. Pigs were slaughtered at 9 months of age (average BW: 165±12.4) in the same abattoir and on a single day per trial. Hot carcass weight, backfat thickness and typical cuts weight were recorded for all carcasses. After a 24-h chilling period, hams were trimmed and then weighed and evaluated for shape, fat coverage and marbling. Fat covering depth was recorded through ruler and ultrasound, and a sample of trimmed fat was taken from all hams to assess iodine number and linoleic acid content. All hams were cured according to the San Daniele procedure, and weight losses during ageing and number of hams discarded were recorded. All traits were analyzed through ANOVA. Genetic type highly affected most carcass traits, and D pigs provided carcasses with the highest incidence of lean and the lowest incidence of fat cuts. Also ham quality traits were mostly affected by genetic types, and hams from A and G had higher fat covering thickness, lower iodine number and a better overall evaluation than other genetic types. Incidence of hams not suitable for DPO production was nearly double in D and T with respect to A and G. Effects of diets were less important, but lowering dietary CP tended to improve ham subcutaneous fat covering and quality. No interaction effect between genetic type and diet was detected.

C-033

Slaughtering performance and meat quality of *Suino della Marca*: first results

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The *Suino della Marca*, officially recorded in Swine Hybrids Bulletin Board Registers (ANAS), was monitored to develop breeding and feeding techniques suitable for this Genetic Type. Fifty-two *Suino della Marca* pigs were reared outdoor in two farms and fed two different diets. In farm F pigs were fed *ad libitum* commercial feed for the whole growing period, whereas in farm V animals in autumn were fed acorns and chestnuts, while in winter diet was supplemented with a farm mixture (barley and field beans). After slaughter the carcass cuts were weighted, pH measured and steak sample from each pig was taken, sectioned and analyzed for chemical composition, drip loss and colour. The overall mean age at slaughter was 340±22 d and the daily carcass growth was 359±76 g. The carcass weight was 134±28.73 kg with a yield of 80.87%. The incidence of total lean and fat cuts on carcass weight was 63.8±4.42% and 26.6±4.22% respectively. Ham pH was 5.68±0.34 with a narrow coefficient of variability (5.98%). The average weight of steak was 293.78±88.6 g distributed in bone 79.34±21.13 g (27.13%), loin 67.78±26.44 g (23.58%), fat 38.77±11.88 g (14.11%) and other muscles 95.22±48.13 g (30.83%). The chemical composition showed a protein content of 20.46±1.29%, fat 6.37±1.90% and ash 1.13±0.07%; the drip loss was 1.38±0.58%. The colour, measured on the loin, showed L value of 58.02 with a coefficient of variation of 9.68%; a* value was 11.20±2.95 and b* value 5.67±2.18, indicating the perception of red colour but brighter than that recorded for meat of pigs reared indoor. Significant differences due to farm effect resulted for some meat and colour parameters. Carcass gain, slaughter age, slaughter performance and steak analytical composition data suggest a high genetic variability of "Suino della Marca" and warrant further investigation to identify genetic lines with the best features to meet the requests of local farmers and processors.

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C-034

Medium-heavy swine and quality of *Longissimus dorsi* muscle: preliminary results

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Medium-heavy swine represents an alternative for the Italian heavy pig farming. Medium-weight pigs are suitable for fresh meat consumption and for the production of cooked ham. In literature no data are available on meat quality parameters of *Longissimus dorsi* (LD) muscle of medium-heavy swine. The quality of LD muscle of two Italian heavy pig genetic type slaughtered at a live weight of 130 kg was investigated. Three hundred pigs balanced for body weight and sex, half PIC 1050 x TOP D (PT) and half ANAS F1 (Italian Large White x Italian Landrace) x Duroc (AD), of initial weight of 60±0.8 kg were selected from two different farms. Pigs were fed commercial diets on the basis of 9% of metabolic weight (LW0.75) and were slaughtered at a live weight of 135.4±0.6 kg. Twenty pigs per genetic type (10 castrated males and 10 females) were randomly selected and LD muscles were sampled. Physical, chemical and sensory parameters were evaluated. Data were analyzed by two way ANOVA with genetic type and sex as the main factors (SPSS Inc., Chicago, IL, USA). Sex affects some chemical and sensory parameters. Genetic type significantly affects physical and chemical meat quality parameters. These data are also evidenced by texture attribute of sensory evaluation. In particular the LD muscle from AD genetic type showed a higher ($P<0.05$) tenderness and juiciness and a lower ($P<0.05$) fibrousness and stringiness than PT genetic type. Also the global preference of the panellists resulted higher ($P<0.05$) for AD genetic type. Further studies are required to confirm the present data and to better characterize the medium heavy swine LD muscle.

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C-035

Influence of pH decline rate on meat quality of *Apulo-Calabrese* breed

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Apulo-Calabrese is an autochthonous black pig breed of Southern Italy reared to produce meat destined to be processed in four PDO Calabrian salami. Although the importance of raw meat quality for the salami production, there are very few information on meat quality traits of this breed. A research was conducted on 51 *Apulo-Calabrese* pigs reared indoor in one farm located in Cosenza province. All subjects were NN genotype for CRC gene and registered in the National Pedigree Register of the Italian Pig Breeders Association (ANAS). Pigs were slaughtered at about one year of age and 135 kg of live weight in a plant located about 60 km far from the farm. At 1, 3 and 6 hours post mortem, the pH of the *Longissimus thoracis* muscle at the level of the sixth thoracic vertebra was measured. On the basis of pH measured at 3 hours post mortem, the carcasses were classified into three groups with slow (n=11, pH3> 6.10), intermediate (n=20, pH3 between 5.91 and 6.10) and fast (n=20, pH3 ≤5.90) pH decline. A sample between 6th and 10th thoracic vertebra was collected and transported in a laboratory where pH and colour were measured at 1, 3 and 7 days post mortem. In addition, drip losses were assessed at 3 days post mortem. The pH values of groups with fast and slow pH decline were far from the thresholds for PSE (pH1<6.00) and DFD (pH24 >6.00) defects, respectively. The pH was significantly different between the three groups at 3 h and 24 h post-mortem. The L* colour-coordinate values were lower in groups with slow and intermediate pH decline with respect to fast pH decline group at all measurement time. Drip losses were higher in the group with fast rate of pH decline. The results show that pH decline rate have to be considered in producing meat of *Apulo-Calabrese* breed in order to guarantee the suitability of the raw material for the production of PDO Calabrian salami.

C-036

Effect of different lipid supplements on steer performance, meat quality and fatty acid composition of *Longissimus dorsi* muscle

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The aim of this study was to improve the nutritional properties of beef by identifying the most appropriate nutritional strategies to increase the concentration of ω-3 fatty acids and cis-9, trans-11 conjugated linoleic acid (CLA) in intramuscular fat. Sixty-three steers were divided in 7 homogenous groups that differed both in length of administration (trial period, 180 days) and for lipid source used: group T1 (control) received a basal diet composed of corn silage (8 kg), beet pulp silage (5 kg), corn meal (2.5 kg), commercial feed (2 kg) and wheat straw (0.8 kg); group T2 received the basal diet supplemented with 250 g/head/day of extruded flaxseed (EF) and 1 g/head/day of vitamin E and β-carotene (VE) for 180 days; group T3 received the basal diet supplemented with 500 g/head/day of EF and 2 g/head/day of VE for the last 90 days; group T4 received the basal diet supplemented with 25 g/head/day of rumen protected CLA (rpCLA) (Luta-CLA® 20 P, BASF) and 1 g/head/day of VE for 180 days; group T5 received the basal diet supplemented with 50 g/head/day of rpCLA and 2 g/head/day of VE for the last 90 days; group T6 received the basal diet supplemented with 250 g/head/day of EF, 25 g/head/day of rpCLA and 1 g/head/day of VE for 180 days and group T7 received the basal diet supplemented with 250 g/head/day of EF and 25 g/head/day of rpCLA, without vitamin supplementation, for 180 days. Slaughtering took place at the conventional weight of 700 kg; carcasses were chilled for 24 h before sampling. Samples of *Longissimus dorsi* from 56 steers were collected for analysis of meat quality (composition %, WBSF, color and cooking loss) and fatty acid content. Results were analyzed by one-way ANOVA using Student's t-test and Tukey's post-hoc HSD test. Small or no differences were observed in zootechnical parameters, slaughtering performances and quality; as regards the fatty acid profile of meat, no differences were observed in CLA content, linolenic acid and the ω-6/ω-3 ratio significantly improved in steers fed with EF (particularly for the last 90 days) compared to the rpCLA and control groups.

C-037

The caspase and calpain proteolytic systems in *Longissimus dorsi* and *Infraspinatus* muscles of Italian Simmental young bulls

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Calpains and capsases are two families of cytosolic proteases essential for a proper skeletal muscle function, which recognize specific target proteins for degradation. Moreover, μ-calpain is believed to play a major role in post mortem proteolysis and meat tenderization, which in turn depends on fibre type composition. Recent data also support the involvement of caspases in the development of tender meat. In fact, it is plausible that the hypoxic conditions occurring in post mortem may activate these proteases, which function as vital executioners of apoptosis in hypoxia/ischemia and degrade structural components of the cytoskeleton. The goal of this work was to evaluate the activities of μ-calpain and caspases in two different muscle types from bulls, immediately after slaughtering, by immunodetection of their specific proteolytic products and to compare such activities to their related mRNA expression level. In particular, caspases 9 and 3 have been analysed, which are an initiating and an executer caspase, respectively, to follow the entire cascade of events occurring during a hypoxic stress. Samples of *Longissimus* dorsi (white/type IIB muscle) and *Infraspinatus* (red/type I muscle) muscles were collected within 20 minutes from slaughtering of 16 Italian Simmental young bulls, and stored at -80° until the analysis. By SDS-PAGE the alpha II spectrin and its degradation products 145kDa (from μ-calpain degradation) and 150 kDda and 120 kDda (from caspases degradation) were separated and then quantified in relative terms. Total RNA was extracted from the two muscles and cDNA was produced; the DNA was amplified by q-PCR technique. The genes analyzed were CAPN1 (coding for μ-calpain), CASP3 (caspase 3), CASP9 (caspase 9). Genes coding for GAPDH, β-Actin, RPLP0 and Cyclophilin was used as reference genes. Both caspases and μ-calpain were found active, having been recognized their target degradation products. In particular no difference in the level of alpha II spectrin fragment, derived from the caspase-3 activity, was found between the *Longissimus dorsi* and *Infraspinatus* muscles, in agreement with gene expression results. On the other hand, the μ-calpain activity was influenced by the type of muscles.

C-038

Meat tenderness and proteolysis as affected by breed and ageing time

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A large number of genetically distinct cattle breeds are reared in Italy and this genetic diversity produce meat with many different qualities. The effects of breed and ageing time (1, 7, 14, 21d) were evaluated on meat tenderness and on proteolysis in *Longissimus dorsi* muscles from 24 young bulls. The breeds have been chosen for their different purposes: Friesian as dairy type, *Romagnola* x Podolian crossbreed as beef type and Podolian as dual purpose.

Warner Bratzler share force (WBS), myofibrillar fragmentation index (MFI) and changes in myofibrillar proteins were estimated. The extracted myofibrillar fractions were analyzed by SDS-PAGE (8-18%) and two dimensional gel electrophoresis (2DE). All data were subjected to an analysis of variance using the GLM procedure including fixed effect due to breed, ageing and breed x ageing. Shear force decreased with ageing in all breeds and showed the highest values at 1 and 7d in Podolian meat. Myofibrillar fragmentation index significantly increased in Podolian meat throughout ageing whereas in Friesian and in Crossbreed meat it increased only in the first week. Proteolysis was investigated by SDS-PAGE and 2-dimensional electrophoresis showing a different quantity and expression profile of myofibrillar proteins among breeds. In all breeds were observed a decrease of Troponin-T and an increase of Troponin-T derived polypeptides during ageing. The highest decrease of Troponin-T together with the presence of fragments of MHC in Podolian meat during ageing was an outcome of a more extensive proteolysis in this breed. Data suggest that tenderness and proteolytic changes during ageing are related to animal's breed.

C-039

Effect of ageing time on veal quality

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Tenderness is one of the most important meat quality traits and ageing is one of the main factors that could affect it. This study aimed to evaluate the effects of ageing on veal quality, a comparatively less investigated issue. Two days after slaughtering, four *Longissimus dorsi* (LD) and four *Biceps femoris* (BF) muscle were collected from Holstein male veal calves, coming from the same farm, fed with the same diet, slaughtered at the same day and homogeneous for age (227±18 days), cold carcass weight (165.25±14.84 kg), conformation (SEUROP classification: R), fatness score (from 1 to 5: 2) and serum lactate (54.05±1.52 mg/dL). Muscles were cut in four subsamples, vacuum packaged and randomly assigned to one of four ageing treatments: 4d, 8d, 12d, and 16d, ensuring that each portion was equally represented in each ageing time. At the end of ageing, samples were frozen and kept at -20°C, until the analysis of physical characteristics, as cooking loss, pH, WBSF and color parameters (L*, a* and b*), chemical composition and sensory traits (flavor, taste and texture; not evaluated for sample aged 12 days). All the collected data were analyzed by ANOVA. Ageing did not affect muscular pH and cooking loss but increased L*, a* and b* ($P<0.01$), in both muscles, between 4d and 8d. Similarly, there was a reduction of shear force in both muscles aged for 8d compared to those aged 4d ($P<0.01$), but an ageing longer than 8d did not further reduce WBSF. Results from the sensory evaluation showed that ageing did not affect taste and flavor of veal in both muscles. On the contrary, ageing improved texture characteristics, indeed perceived tenderness significantly increased from 4d to 16d in LD ($P=0.01$) and BF ($P<0.01$), and tended to increase comparing 4d to 8d ($P=0.10$). Furthermore, in BF there was an improvement of juiciness ($P<0.10$) and a reduction of fibrousness ($P=0.05$) from 4d to 16d of ageing. An ageing time longer than 4 days, lasting between 8 to 16 days, seems to improve texture quality of veal without affecting its flavor and taste characteristics.

C-040

The effects of maternal linseed supplementation on fatty acid composition of leg muscles of suckling lamb

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Nowadays, the increase of the content of polyunsaturated fatty acids (PUFA) n3 in meat is an important goal in applied animal science. This work investigated the effect of alpha-linolenic acid (ALA) supplementation to dairy ewes during late-pregnancy and early lactation on ALA conversion into longer-chain PUFA (C20:5, eicosapentaenoic acid, EPA; C22:5, docosapentaenoic acid, DPA; C22:6, docosahexaenoic acid, DHA) in the muscles of suckling lambs. Thirty-eight ewes were fed experimental diets from 8 weeks before lambing to 28 d of lactation. A control diet (CTL) and an ALA-enriched diet obtained by adding linseed (LIN) were used in a 2x2 factorial design. During gestation, ewes were divided into two groups: one fed the CTL diet and the other the LIN diet. After lambing, both CTL and LIN groups were divided into two subgroups, fed with CTL and LIN diets. Thirty-eight lambs (21 from single and 17 from twin lambing) born from the experimental ewes were slaughtered at 28 d of age, to determine the fatty acid composition of intramuscular fat of their leg muscles by gas-chromatography. Data were analyzed with a model that included fixed effects of ewe's gestation diet, ewe's lactation diet and their interaction. The interaction was not significant for any FA of interest. Feeding LIN to ewes during late-pregnancy increased significantly the C18:3 n-3 (+20%) and EPA (+52%) proportions and tended to increase the DHA proportion in leg muscles (+22%). Feeding LIN to ewes during lactation increased the ALA (+110%) and EPA (+35%) proportions, but did not affect DPA and DHA proportions in leg muscles. In conclusion, the linseed supplementation to ewes diet during late-pregnancy and lactation enhanced the total long chain PUFA n3 in leg muscles of lambs.

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C-041

Meat yield and carcass quality in Cornigliese sheep breed: preliminary data

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Aim of the study was to describe some traits related to meat production and carcass quality in Cornigliese sheep, a local breed (about 650 heads) reared in Parma province. On 72 subjects (47 females, 25 males) divided into two age-classes (43 aged >1 year, 29 aged between 6 months and 1 year), slaughtered and sectioned by the same staff, the liveweight was recorded, as well as the weight of carcass, skin, head and offal; on the left half-carcass the weight of shoulder, thigh, neck, loin, ribs and fat and lean trimmings was A sample cut (first 4 thoracic vertebrae) was collected to assess the incidence of lean, fat and bone. Data were analyzed by ANOVA with sex, age and interaction as fixed factors; for slaughter data, carcass weight, nested within age-class, was used as a covariate. Least squares means for liveweight (kg), carcass weight (kg) and slaughter yield (%) were affected by age-class (resp. 78.2, 33.1, 41.10 for adults; 53.3, 22.2, 47.4 for youngs; $P<0.05$); adults showed higher yield than youngs for skin (13.41 *vs* 11.39; $P=0.06$). The carcass compactness index (carcass weight/carcass length) was higher in youngs than in adults (0.353 *vs* 0.333; $P<0.05$). Yields of shoulder, thigh and lean trimmings were higher in males (16.73%, 30.98%, 9.32%) than in females (15.63%, 27.71%, 7.35%), as fat trimmings were higher in females (19.50%) than in males (12.31%) ($P<0.05$). The percentage composition of the sample cut showed an effect of sex on the content of muscle and fat tissues (males: 62.77 and 6.82; females 59.79 and 11.01, resp.; $P<0.05$) and an effect of age-class on the content of muscle and bone tissues (adults: 64.72 and 25.32; youngs: 57.84 and 31.48, resp.; $P<0.05$). The muscle/fat ratio was higher in males than in females (9.70 *vs* 5.47; $P<0.05$) and in adults than in youngs (9.71 *vs* 5.46; $P<0.05$). In conclusion the Cornigliese sheep, in the current conditions of use, can be considered as an extremely late breed, with high weights at slaughter, low carcass yields and high incidence of bone in the carcasses, particularly in those of animals slaughtered between 6 months and one year.

C-042

Meat quality in donkey foals

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A study based on 10 entire donkey foals was performed to evaluate meat quality and collagen content in *Semitendinosus* (ST) and *Semimembranosus* (SM) muscles taken from foals slaughtered at 10 months of age and a mean final body weight of 126 kg. The meat showed a significantly ($P<0.05$) higher (1.77 g/100 g) fat content in SM muscle compared to ST (1.61 g/100 g), a valid protein content in both the muscles (20.2 g/100 g in SM, 19.9 g/100 g in ST); cholesterol content was very similar in SM (57.6 mg/100 g) and ST (55.8 mg/100 g) muscles. Glycogen content was not statistically different between the two muscles examined. Palmitic acid (C16:0) was the most abundant fatty acid in both muscles; a significantly higher ($P<0.05$) content of polyunsaturated fatty acids was determined in SM muscle (22.11%) compared to ST muscle (20.61%). ST muscle had a collagen concentration (44.2 μg/mg) significantly higher ($P<0.01$) compared to SM muscle (32.1 μg/mg). The results obtained in the present study can represent a starting point for the promotion of donkey meat, that can be considered a suitable protein source for those seeking an alternative to other red meats.

C-043

Heavy metals in free-living wild boars harvested in Viterbo (Central Italy): are hunters at risk?

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The aim of this study was to measure cadmium, lead, and chromium in edible tissues and organs of wild boars to perform an exposure assessment study on hunters and their relatives in order to compare the results to that of other European countries. These studies are motivated by the increased availability of wild boar meat, mainly for hunters and their families, which is reported as an important dietary source of heavy metals. We determined the cadmium, lead and chromium content in the tissues of wild boar hunted in six areas within the Viterbo Province (Italy) by atomic absorption spectrophotometry, and gathered data on wild boar meat consumption through a specific diet survey in the same areas. On the basis of these data we performed an exposure assessment for hunters and members of their households, both adults and children, who consumed wild boar meat and offal. The game product based intake of cadmium, lead and chromium was simulated with Monte Carlo Simulation Models appropriately developed. Cadmium and lead content in the wild boar meat and offal (liver) hunted in the study area were similar to or below the values reported in European studies. Some samples showed metal content above the EU limits (*e.g.*, lead) although the bullet contamination was accurately avoided. Higher values of cadmium have been recorded in other studies probably due to intense environmental pollution. Since no regulatory limits are set for chromium in meat or liver we can only affirm that our results suggest a moderate concentration in these foods. The diet survey confirmed that hunter population show a consumption of wild boar meat and liver higher than the Italian population. The consumption of wild boar products amounted to (median values) 10.02 and 17.62 μg Cr week^{-1} person^{-1}; 10.24 and 27.58 μg Pb week^{-1} person^{-1}; 8.16 and 11.52 μg Cd week^{-1} person^{-1} for people eating only meat or meat and liver respectively. Liver consumption contributed to the total metals intake for members of the households of wild boar hunters, and the rate of liver consumption should be kept as low as possible for children living in these families.

C-044

Ostrich meat: physicochemical properties and fatty acid composition

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Knowledge on physicochemical composition of Italian ostrich meat is limited. Therefore aim of this research was to improve scientific information on its nutritional properties. Forty-two meat samples were collected after slaughtering 21 ostrich crossbreds (18.5 months-old) reared in a farm of Marche region; animals were fed dehydrated alfalfa meal (*ad libitum*) and a commercial feed (Protein 18%, Fiber 6.5%, Fat 3%, Ash 8.6%). Live and carcass weights and carcass yields have been evaluated. Moisture, protein, fat, ash, iron content, colour CIELab parameters (L*a*b), intramuscular acidic profile and saturated/unsaturated fatty acid ratio were determined on *Iliofemoralis* (fillet) and *Crural flexor* muscle (small steak). Average live weight, carcass weight and carcass yield were respectively 90.10 kg, 45.24 kg and 50.07%. Meat proximate composition was: water 74.58±1.78%; protein content 18.78±1.42% with significant difference between muscles (19.36±1.48 *vs* 18.19±1.09); average fat content was significantly lower (1.32±0.46) in iliofemoralis (fillet) than crural flexor muscle (2.57±0.75), confirming the exceptionally low fat content of ostrich meat compared to other edible meats from mammals and poultry. Iron level (3.54±0.65) confirmed literature data. Brightness (L* 36.85), red-green range (a* 24.50) and blue-yellow range (b* 11.69) confirmed the dark red colour of ostrich meat that partially should be also ascribed to high level of iron and heme-pigment. Palmitic acid was the most represented intramuscular fatty acid between SFA, oleic acid between MUFA and linoleic acid between PUFA. The low ratio SFA/PUFA (1.52) due to the increase in PUFA content, and the higher level of MUFA could be positive analytical parameters to promote ostrich meat as alternative to other red meats. However, the ratio of n-6/n-3 fatty acids greatly overcome the recommended value of 1, suggesting the opportunity to evaluate the inclusion of n-3 fatty acids in the ostrich diets to reduce human risks for atherosclerosis and heart diseases. Estimated Iodine value was lower (66.16±5.52) than values found in the literature, indicating good meat stability. Despite the lack of diet fatty acid composition, analysis of acidic profile suggests minor changes in feed composition to get better n-6/n-3 and MUFA/PUFA ratios further enhancing the nutritional value of ostrich meat.

C-046

Geographic information systems approaches to investigate the production environment descriptors of local breeds

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In accordance with the Global Plan of Action for Animal Genetic Resources (FAO, 2007), a set of production environment descriptors (PEDs) has been established to be used throughout the world as a common framework for describing the geographic and socio-economic features of the breeds' production environments. The aim of this research is to test geographic information systems (GIS) approaches for the description of production environment of local breeds at different spatial scales of analysis. We used as case study the Rendena cattle, a dual purpose breed of the Eastern Italian Alps. The breed originated from a small valley (Val Rendena) in the Alps, but in the last two centuries spread towards the lowlands. The PED approach implemented on DAD-IS overestimated the actual distribution of the breed, and produced a highly variable range in terms of elevation, climate and land use. A second approach followed what suggested by Alderson (2009) to estimate breeds risk status; in case of epidemiological outbreaks, a breed is considered at risk when the circle including 75% of population has a radius shorter than 25 km. For Rendena cattle this radius was 63 km, and the native area of the breed was excluded from the circle, suggesting that the expansion in other areas contributed to the viability of the breed. Finally, we analysed geographic features at the farm level on the 25 single-breed farms, with 849 cows and 3912 land parcels, of the Rendena Valley. Here, geographic features of the productive environment were typical of mountain regions. The average elevation and size of farms were 823 m asl (SD=197 m) and 14.4 ha (SD=9.3 ha). Utilizable agricultural area comprised mostly meadows, with arable crops almost absent; all the farms moved cows to highland pastures during summer. In conclusion, GIS approaches are helpful to characterize local breeds. The approach and scale of analysis and the data standardization must be tailored to the different descriptive targets.

C-047

Use of canonical discriminant analysis to study selection signatures in five cattle breeds farmed in Italy

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Intensive SNP genotyping using bead-chip at different densities is used to study genetic variation both within and between breeds of several livestock species. However the large amount of information to be processed requires computational procedures suitable for summarize results. Canonical Discriminant Analysis (CDA) is a multivariate dimension reduction techniques that could be of great help for the detection of genomic regions involved in the between breed variability. CDA extract from the original variables (SNPs) new orthogonal variables that maximize the differences between groups on the basis of variances within each groups. Canonical variables (CANi) could be interpreted on the basis of canonical loadings (*i.e.* correlations between CAN and the original variables). CDA was used to analyze a total of 4,095 bulls of Italian Holstein (IH) (2,093), Italian Brown (IB) (749), Italian Simmental (IS) (479), Piedmontese (P) (364) and *Marchigiana* (M) (410) genotyped with the 50K SNP panel. The analysis was carried out separately for the 29 autosomes. A total of 44,325 markers were considered after edits. CDA allowed for a clear separation of the breed for all of the chromosomes. The first CAN explained on average about 50% of total variability and it was able to distinguish HF from all the other breeds. CAN2 and CAN3 explained on average about the 25% and 15% of total variance, respectively. Usually they discriminate dairy breeds (IH and IB) from the P, while the IS tended to be located in an intermediate position. Finally, CAN4 explained on average the remaining 10% of the total variability and separates P from four other breeds. In order to identify putative candidate genes involved in the determination of productive traits of interests (milk and beef) canonical loadings were analysed. Considering only value exceeding the 0.99 quantile as conservative threshold, SNP located in genomic regions where genes found to be associated traits were identified. For example, CAN1 was associated to SNP located on BTA6 at around 72Mbp where Kit *locus* maps and for CAN2 near ABCG2 (37-38Mbp). Moreover, another significant signal was observed on BTA2 for CAN4 at 6.5 Mbp where myostatin gene is located.

C-048

Comparing inkage disequilibrium, linkage analysis and inkage disequilibrium-linkage analysis approaches for quantitative trait *loci* detection in a complex pedigree dairy sheep population

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Three different approaches were applied to detect QTL in an experimental population of sheep with a complex pedigree structure. The population consisted of around 900 backcross *Sarda* x Lacaune ewes sired by 10 F1 rams and two generations of their descendants sired by 33 pure *Sarda* rams. All animals were genotyped with the 50K-SNP OvineBeadChip and ewes measured for several functional and production traits. Different approaches were applied to detect QTLs segregating either just within few families or at population level and to distinguish QTLs segregating between or within breeds. All models were based on a multiple regression of pheno-

types on the probabilities of carrying male founders' haplotypes. A specific Fortran 90 software was developed to perform LD, LDLA and LA analyses to allow a combined interpretation of results. The LA approach was a within multi-generation family analysis (Casu *et al*., 2010), where the founders' haplotypes corresponded exactly to the sires' haplotypes (10 Lacaune and 10 *Sarda* from F1 and 66 from *Sarda* rams). The LDLA approach clustered the sires' haplotypes on the basis of their identity by descent matrix (Meuwissen & Goddard, 2001). In the LD approach, 5 SNP wide sires' haplotypes were clustered according to their identity by state. Before the regression analysis, males' haplotypes, transmission probabilities and allele breed origin were estimated. Significance thresholds were estimated by permutations. Considering milk yield and the 1% chromosome wise significance threshold, the three analyses gave as expected not completely coincident results. In fact, LA was able to detect just 2 peaks, one of which also identified by LDLA; LD identified 7 significant positions, 5 of which also detected by LDLA; LDLA identified 13 positions, 6 of which were not detected by the other analyses. As expected LDLA was the most powerful method in terms of significant associations. However in such a complex population structure, with heterogeneous family sizes and diverse breed origins of QTL alleles, LA and LD analysis allow to identify further significant associations respect to LDLA.

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C-049

Genomic regions affecting milk traits in *Sarda* dairy sheep breed

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A genome wide association analysis was performed on milk (MY), fat (FY) and protein (PY) yields and fat (FC) and protein (PC) contents, recorded from 2000 to 2012 in an experimental population of 2,370 ewes. 917 out of them were *Sarda* x Lacaune backcross ewes sired by 10 F1 rams. The remaining 1,453 ewes belonged to 2 generations of offspring sired by 33 *Sarda* rams. Genotypes of 44,936 SNP from the Ovine SNP50 BeadChip (Illumina) were available. Firstly, transmission probabilities of male's haplotypes to their descendant were computed using marker information. Then, identity by descent (IBD) probabilities between rams' haplotypes were estimated using 40 markers windows surrounding each SNP (Meuwissen and Goddard 2001). Finally, between 4 and 27 linear combinations of the 76 actual males' haplotypes were obtained through eigen vectors extracted from the IBD matrix. Linear combinations were considered as unrelated founders' haplotypes. LDLA analysis was performed by regressing yield deviations of ewes on the probability of carrying one of the unrelated founders' haplotypes. On the whole, 208 genomic regions associated with the analyzed trait and significant at the 1% chromosome-wise threshold were identified on 19 chromosomes. Fifty-two were 5% genome-wise significant. In several cases two or more significant peaks showed close locations, not allowing to conclude for a single or more linked mutations. The most significant regions for MY were identified on OAR5, 10 and 11; on OAR 11 and 20 for FY; on OAR10 and 20 for PY. The highest number of significant associations was found for PC (OAR1, 2, 6, 11, 13 and 16) and FC (OAR1, 6, 13, 14, 18, 19, 20). Thirty four close markers on OAR6 showed significant association with PC, the most significant one mapping (OAR6_94066660) the region of caseins genes. This analysis allowed detecting several genomic regions affecting milk traits in *Sarda* sheep breed. Researches are ongoing to precise locations and detect causal or LD mutations by using either HD beadchips or high resolution sequencing.

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C-050

Whole genome genetic diversity of buffalo

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The domestication of water buffalo likely took place between 4000 and 6000 years ago in the regions of Indus and Yangtze valleys. Domestic water buffalo comprises two subspecies that differ in a number of phenotypic traits, including morphology and behaviour, in geographic range and number of chromosomes. River buffalo has 50 chromosomes and is mainly reared in India, Southwest Asia and the Mediterranean region, including Italy, where the mozzarella di bufala campana cheese is produced from river buffalo milk contributing to the excellent reputation of the made in Italy brand worldwide. Swamp buffalo has 48 chromosomes and is mainly reared in Southeast Asia and China. The river buffalo genome has been recently sequenced and de-novo assembled, through the coordinated effort of an international Consortium led by a core group of Italian scientists. An associated HapMap project has sequenced 47 river buffaloes from 4 breeds at low coverage. As a result, more than 22M SNPs were discovered, permitting the development of the Axiom Buffalo Genotyping 90K (Affymetrix) marker panel. In this study, the SNP panel has been used to assess the main genetic parameters and genetic diversity of different water buffalo breeds sampled in Italy and elsewhere. Genotyping accuracy and main population genetic indexes have been compared between the 90K SNPs from the SNPchip and the 22M SNPs discovered by sequencing. Data were filtered for missing data, MAF ≥ 0.05 and deviation from HW equilibrium. Sequence data was filtered for quality score and SNP coverage. Preliminary analyses indicate that genotypes from SNP arrays are better suited to diversity studies than low pass sequence by correctly detecting genotypes at SNP *loci*. Both sequence and SNP data indicate that expected heterozygosity values are quite variable among breeds. Sequence and SNP array data produced similar estimates of the Fst index (5.2% and 5.6% respectively), revealing that a moderate proportion of the total genetic diversity is explained by the between breed component. Principal Component Analysis showed a clear differentiation among breeds corresponding to geographic origin.

C-051

Sequencing of the water buffalo genome: results and perspectives

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The water buffalo (Bubalus bubalis) is important for small-holder producers in developing countries and an important source of products for specialized markets. Knowledge of the buffalo genome will help to unravel the evolutionary relationships among different breeds and will contribute to understanding the genetic control of phenotypic variation. Resulting genomic tools will help the genetic improvement of animals for milk and meat production. The International Buffalo Genome Project has three main phases: 1) the complete sequencing and assembly of the buffalo genome and its annotation; 2) SNP and variation detection in river and swamp buffalo genomes; 3) analysis of genetic variation among buffalo populations. The project has produced sequence for a female Mediterranean buffalo genome using Illumina GAII and Roche 454 high throughput sequencing platforms. These sequences were assembled using the MaSuRCA assembler, obtaining a contig N50 of 21Kb and a scaffold N50 of 1.4Mb organized in 367K scaffolds, giving an estimated genome size of 2.7 Gbp. Eighty-six buffaloes from 8 breeds were sequenced to a depth between 5 and 12X by Illumina PE reads, yielding a total of 470X genome coverage. Four of these breeds (Mediterranean, Murrah, Jaffarabady and Nili Ravi) were analysed to yield a total of 22.2M SNPs. The within and across breeds allele frequencies for these SNPs were calculated and the minimum allele frequencies (MAF) were determined. These data were used to design an Axiom Buffalo Genotyping 90K array (Affymetrix). Preliminary comparative analyses of the buffalo genome assembly with the bovine sequence, highlights extensive conservation between genomes of cattle and buffalo, including a high degree of between species conservation of genomic and transcribed sequence. However, detailed analysis of the largest buffalo genome scaffolds suggests a complex history of genomic rearrangements since divergence. Finally, current estimates suggest that 13%-18% of the buffalo genomic sequence is unique.

C-052

Analysis of candidate genes for fertility and seasonality traits in *Bubalus bubalis*

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Water buffalo is an important livestock resource, which occupies a critical niche in many ecologically disadvantaged agricultural systems, providing milk, meat, and work power. In Italy, the most important production related to buffalo breeding is milk, traditionally processed into mozzarella cheese. Reproductive efficiency is the main factor affecting productivity in female buffalo, but is greatly penalized by late achievement of puberty, seasonality of calving, long postpartum anoestrus and subsequent calving interval. Buffaloes living under Mediterranean latitudes can be considered to have a tendency to be seasonal breeding animals and their reproductive efficiency is usually negatively affected by increasing day-length. The main environmental factor affecting the regulation of reproductive seasonality is photoperiod, which regulates changes in the daily melatonin secretion by the pineal gland. The melatonin effect is carried out at hypothalamic level, by regulating of GnRH secretion. Aim of this work has been the polymorphism analysis of different candidate genes for fertility and seasonality traits (STAT5A, SERPINA14 and TNFA for fertility and MTNR1A for seasonality) in buffalo species. Two sets made up of 12 samples were used for SNP discovery analysis, one for fertility traits, containing animals belonging to the tails of distribution for calving interval, and the other for seasonality, composed by 6 animals with calving occurred from August to September and 6 with calving from March to May. Seven single nucleotide polymorphisms were found, of which a C>T substitution in MTNR1A gene, already found in literature and associated to seasonality in different species, and 6 novel SNPs, of which 2 in exon 4 of TNFA gene and 4 in STAT5A gene (1 in exon 8 and 3 in introns). The results of SNP genotyping analysis on a preliminary sample of 100 buffaloes are presented.

C-053

Extensive study on fertility of bovine sexed semen in field conditions

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In this study a field trial was conducted to evaluate the main factors affecting the fertility in Holstein Friesian cows inseminated with sexed semen. Semen was collected from ten bulls housed in three semen production centers and the sex-sorting was carried out by two companies according to Belstville Sperm Sorting Technology. In Spallanzani labs the semen quality (concentration, total and forward motility, average path ve*locit*y, membrane integrity, mitochondrial membrane potential, membrane fluidity and acrosome status) and the percentage of spermatozoa bearing the X chromosome (purity) were estimated before the field trial. A total of 4797 cows (2960 heifers and 1837 pluriparous) distributed in 291 farms in Italy were inseminated in this study. Cows returning to estrus were classified as not pregnant, while cows that did not return to estrus were diagnosed to be pregnant after ultrasonography or rectal palpation. Chi-square test was used for evaluating statistical differences among pregnancy rates obtained in pluriparous and heifers. The effects of parity, bull, herd, semen production center, number of inseminations per herd and sex-sorting company were evaluated using the logistic regression. The purity of the sexed sperm was 90.6%±1.93 and was not affected by the sex-sorting company. A significantly lower pregnancy rate ($P<0.001$) was reported in pluriparous compared to heifers, respectively 419/1837 (22.8%) and 1,337/2,960 (45.2%). Pregnancies were affected by herd, semen production center and sex-sorting company, while they were not affected by the number of inseminations per herd. With regard to the sires, two bulls showed lowest and highest pregnancy rates in heifers or pluriparous (respectively, 16.6% in pluriparous, for the first bull and 50.9% in heifers, for the second bull). These results suggest a careful selection of bulls of proven fertility to be used for sexed semen production in order to avoid lowering of the fertility in the herds. The low percentage of pregnancies obtained in pluriparous demonstrates a reduction in fertility efficiency that negatively affects the use of sexed semen in this age class.

Acknowledgments
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C-054

Fertility traits of crossbred dairy cows obtained from Brown Swiss, Swedish Red and Montbéliarde sires compared to Holstein Friesian cows

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The objective of this study was to compare fertility traits of heifers and primiparous Swedish Red x Holstein (SR x HO) crossbred cows (n=643, n=572, respectively), Montbéliarde x Holstein (MO x HO) crossbred cows (n=129; n=114, respectively), Brown Swiss x Holstein (BS x HO) crossbred cows (n=61, n=50, respectively) and MO x (SR x HO) crossbred cows (n=245, n=121, respectively) with pure Holstein (HO) heifers and cows (n=3526, n=2381, respectively). Animals were born between 2007 and 2011 and belonged to 4 herds of northern Italy. Heifers were compared for age at first service (AFS) and age at first conception (AFC). Primiparous cows were compared for days from calving to first service (DFS) and days open (DO). Traits were analyzed under a proportional hazards cox model able to make use of censored and uncensored information. Hazard ratios (HR) were estimated for breed combination and herd. For traits related to the heifers, crossbreeds between SR x HO were significantly different than pure HO heifers for AFS and AFC (HR=1.40; HR=1.43, respectively), in the same way, MO x (SRxHO) crossbreed (HR=1.33; HR=1.32, respectively) had a higher risk of having the first service and conceiving than HO heifers used as a reference breed. For the primiparous cows all the crossbreed showed a significant difference for DFS and DO relative to pure HO, except for the crossbreed between BS x HO, that was not significantly different from pure HO for all traits. Crossbreed cows had more chances to be inseminated (HR=1.34 for MO x HO; HR=1.24 for SR x HO; HR=1.28 for MO x (SR x HO)) and became pregnant after the first calving (HR=1.52 for MO x HO; HR=1.43 for SR x HO; HR=1.64 for MO x (SR x HO)). Results indicate a higher reproductive potential for the crossbreed cows compared with the pure Holstein.

C-055

Association between COX4I2, CKM and MSTN gene polymorphisms and racing performances in Italian Trotter horses

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Studies in humans and animal models have revealed a large number of genes involved in athletic performances. However, few studies are available in horses for which racing performances are a breeding aim. In the current study we analysed polymorphisms in three candidate genes (cytochrome c oxidase, subunit 4, isoform 2, COX4I2; creatine kinase muscle, CKM; and myostatin, MSTN) with relevant roles related in exercise physiology and muscling and evaluated for the first time their associations with athletic performances in Italian Trotter (IT) horses. Two polymorphisms of the MSTN (the ins227bp within 5'-untraslated region and a polymorphism located in the first intron) gene and two single nucleotide polymorphisms (SNPs) in the COX4I2 and CKM genes were genotyped in 365 horses of the IT breed. The two investigated MSTN mutations did not segregate in the IT breed. This result does not confirm previous results obtained in the Thorougbred horses where the two polymorphisms of the MSTN gene were proposed as predictive of athletic performance. SNPs in COX4I2 and CKM genes showed minor allele frequency >0.41, and provided an opportunity to examine their effects on racing phenotypes in this breed. Horses of the IT breed, like other Trotter horse breeds, are evaluated using qualifying tests before entering the races. Horses registered to the Italian Studbook are then recorded for three phenotypes: speed in trot (best time/km in career), earnings (overall earning in career and top-race winning) and rank traits. Association analyses showed that the CKM SNP, located within a putative transcription factor binding site of intron four, was significantly associated with variation of racing performances confirming results already observed in Thorougbred horses.

C-056

A single nucleotide polymorphisms detected in the overlapping 3'-UTR of two genes mapping on SSC2 is associated with pig meat pH

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The pH is an important parameter influencing technological quality of pig meat, a trait affected by environmental and genetic factors. Several quantitative trait *loci* associated to meat pH are described on PigQTLdb but only two genes with influence on this parameter have been so far detected: Ryanodine receptor 1 (RYR1) and Protein kinase, AMP-activated, gamma 3 non-catalytic subunit (PRKAG3). To search for genes influencing meat pH we analyzed genomic regions with quantitative effect on this trait in order to detect gene-tagged SNPs to utilize for an association study. The expressed sequences located within regions associated to pork pH mapping on porcine chromosomes 1, 2, 3, and selected after a bibliography survey, were searched in silico to find SNPs. 356 out of 617 detected SNPs were used to genotype Italian Large White pigs and to perform an association analysis with meat pH values recorded in semimembranosus muscle at about 1 hour (pH1) and 24 hours (pH24) post mortem using PLINK software. The results of the analysis showed that 5 markers mapping on chromosomes 1 and 3 were associated with pH1 and 10 markers mapping on chromosomes 1 and 2 were associated with pH24. After Bonferroni correction only one SNP mapping on chromosome 2 was confirmed to be associated with pH24. This polymorphism was located in the 3'UTR of two partly overlapping genes, Deoxyhypusine synthase (DHPS) and WD repeat domain 83 (WDR83), coded on opposite DNA strands. The overlapping of the 3'UTRs allows the co-regulation of the stability of both mRNAs by a cis-natural antisense transcript method of gene regulation. WDR83 has an important role in the modulation of a cascade of genes involved in cellular hypoxia defense by intensifying the glycolytic pathway to produce ATP. It is possible that glycolisis in muscle cells during post mortem phase and meat pH phenotypic variability could be influenced by molecular and biological processes involving one or both genes and the SNP detected in the overlapping 3' region. If confirmed, this finding can be applied to identify new biomarkers to be used to improve pig meat quality.

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C-057

Identification of candidate genes for fat deposition in heavy pigs

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Fatness is a very important trait to produce tights and meat suitable for seasoned products, like San-Daniele ham, and represents one the main targets of the Italian pig breeding industry. Association studies between genotypes and fat deposition can help breeders to select boars and sows with favorable characteristics.
In the present study, 103 SNPs distributed on 52 candidate genes were analyzed in a population of 800 pigs, crosses of Italian Duroc X Large White (400 pigs) and Commercial hybrid X Large White (400 pigs). Pigs were reared in commercial farms, with similar feeding and environmental conditions. At slaughterhouse, carcass weight, tight weight, lean percentage calculated with FOM, mm of lean and mm of backfat were recorded and a muscle sample was collected for chemical composition and for DNA analyses for each animal. SNPs were chosen in promoter regions of genes already known for association with fat traits according to available researches. Regulatory regions represent a very interesting target for research due to their effect on gene expressions and, potentially, on phenotypes. On the basis of allele frequencies, 67 SNPs were segregating in both hybrids, while 6 SNPs segregated only in Duroc x Large White (ACACA, CTSK, FABP3, OPN, PLIN2, SCD). Association analysis with intramuscular fat (%) and backfat thickness (mm) was carried out with Pearson correlation test and Bonferroni correction and evidenced significant correlations (P<0.01) between fat traits and analyzed polymorphisms. Genes with significant SNPs for investigated traits were ACACA, ATP1A2, CAPNS1, CCKAR, CTSK, CTSL, GH, ID3, MYOD, OPN, PLIN2, PPARGC1A. Correlations with grow traits as carcass and ham weight were also found.

C-058

Single nucleotide polymorphisms profiling in the transcriptome of Friesian and *Chianina* cattle breeds using an RNAseq approach

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The advances in next generation sequencing (NGS) technologies are now enabling a cost effective way for large scale SNPs discovery. Furthermore, the use of a high throughput mRNA sequencing (RNAseq) approach, allows to focus the analysis to the changes in the coding sequences which are more likely to affect the biological function of a protein. In the present study, an RNA-seq approach has been used to make a wide SNPs survey in the muscle transcriptome of two cattle breeds. Libraries prepared from 4 Friesian and 4 *Chianina* young bulls were sequenced on Illumina Genome Analyzer IIx to produce 50 bp paired end reads. The quality-filtered reads have been aligned to the UMD3.1.66 genome reference sequences using the Tophat program. After reads alignment, variants have been called using SAMtools. After filtering a total of 98,186 SNPs were found. The identified SNPs were checked for presence in dbSNP database. The 75% of all the SNPs found matched with previously identified variants. The SNPs were classified on the bases of their position (3' and 5' UTR coding regions) and effect (amino acid change, start and stop codon gains, start and stop codon losses) on the annotated genes. In this study the usefulness of the RNAseq approach in the development of high utility SNPs markers and the pipeline used for SNPs discovery and their functional categorization is discussed.

C-059

Transcriptome analysis of backfat tissue in Italian Large White pigs

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In the frame of the AGER project we are applying genomic tools to introduce innovations in pig production chain for the improvement of dry cured hams quality, studying in particular the traits of adipose tissue. We sampled 950 Italian Large White (ILW) sib tested pigs obtained from ANAS (National association of pig breeders). Phenotyping and collection of biological samples (fat tissue and muscle) was carried out at slaughterhouse and tissues were stored at -80°C with the aim to constitute a biorepository. In addition to fat traits (skeletal muscle fat content and fatty acid composition of backfat) other meat and carcass quality traits were scored. Furthermore, EBVs for the traits under selection were calculated and provided by ANAS. We are completing a catalogue of transcripts, including mRNA, ncRNAs and microRNAs (miRNAs) of pig adipose tissue, by exploiting RNA-seq assays. In order to find qualitative and quantitative differences of the transcripts expression we will compare groups of the most lean and the fattest animals selected according to their EBVs for backfat thickness. At present we have performed a pilot experiment on two samples, which allowed us also to optimize the whole process of: RNA extraction, RNA sequencing by Illumina technology, and implementation of the bioinformatic pipeline for sequence data analyses. This first assay allowed the identification of more than 350 miRNA expressed in adipose tissue, some of them till now unknown in pigs. We observed that all mitochondrial genes are expressed in adipose tissue and, as far as nuclear genes are concerned, it was possible to identify more than 16,000 genes expressed. We computed that around 400 genes, approximately the 2% of those annotated in the pig genome, are responsible for half of the overall transcripts expression in fat tissue and could. The proteins coded by these genes could have the highest functional relevance on fat metabolism in pigs. In order to elucidate their role a functional classification of these genes is under way.

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C-060

Combined genomics and metabolomics approaches to identify markers associated with production traits in pigs

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The development of high throughput genomics (next generation sequencing and high throughput genotyping) and metabolomics platforms has opened new perspectives for the identification of the genetics factors affecting traits of biological relevance in all species, including production traits in farm animals. In pigs, benefits derived from the recent sequencing of the pig genome can be fully exploited by combining advanced genomics and metabolomics approaches. In this work we integrated several resources, experiments and data with the final aim to identify markers (DNA polymorphisms and metabolites) associated with production traits in Italian Large White pigs. High throughput genotyping was carried out using the Illumina Porcine60SNP BeadChip array and customized Golden Gate system on extreme and divergent pigs for back fat thickness (BFT) estimated breeding values (EBVs) (300-560 animals) and average daily gain (ADG) EBVs (360 pigs), chosen among a population of about 12,000 performance tested pigs. Next generation sequencing was carried out using the Ion Torrent PGM machine to identify single nucleotide polymorphisms (SNPs) from two reduced representation libraries developed from pooled genomic DNA constructed from 50 pigs with most positive and 50 pigs with most negative BFT EBVs, respectively. A total of 7,510,918 reads were produced and 447,031 SNPs were called, using stringent criteria. Genome wide association studies made it possible to identify a quite large number of significant SNPs affecting BFT, ADG and correlated traits. In addition, several genome regions containing significant SNPs for BFT were enriched of SNPs identified from the Next Generation Sequencing experiment. Metabolomics information was obtained from 800 performance tested pigs using a mass spectrometry (MS/MS) analytical pipeline to measure 180 blood plasma metabolites. Estimated heritability and correlation among all these parameters and production traits indicated that a few metabolites could be useful predictors of EBVs for production traits. All these data will be used to develop a first systems biology platform to understand the fine biological mechanisms affecting production traits in pigs.

C-061

Proteomics for milk proteins characterization in Girgentana goat breed

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Proteomics has been widely used to characterize milk protein content in livestock species and unravel protein biological functions. The aim of this work was to characterize the Girgentana goat breed whole milk and milk fraction proteins by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) and mass spectrometry. Bulk milk samples from two farms locate in Palermo and Agrigento (Sicily) areas were collected during the early stage of lactation. Initially, 2D-PAGE and protein detection protocols were set-up. In particular, 2D-PAGE analyses were carried out on whole milk using 18-cm IPG strips ranging from pH 3-10 and 4-7 and 12.5% polyacrylamide gel for first and second dimension separation, respectively. Then, staining methods (silver-, colloidal Coomassie Blue G-250- and fluorescent dye-based method) were tested to reveal protein spots in 2D-gels. In silico 2D-gel image analysis, performed using Image Master 2D Platinum software, revealed that silver and fluorescent (based on SyproTM Ruby) staining methods showed similar results in terms of sensitivity/resolution. Then, centrifuge-based protocols were set-up to obtain milk protein fractions. The protein profiles of whole milk, caseins, whey and milk fat globule protein fractions were qualitatively compared in silico. This analysis showed that casein content decreased in both whey and fat globule protein fraction respect to caseins fraction which, instead, missed specific protein spots revealing in whey and/or fat globule fraction. Finally, a global protein differential expression analysis of whole milk samples from Palermo and Agrigento, respectively, was performed by 2D-Differential Gel Electrophoresis (2D-DIGE) using pH range 3-10. In silico analysis of the 2D-DIGE gel images (at least three per sample) showed a total of 143 differentially abundant spots with more than 1.5 fold change in volume and a statistically significant ANOVA value ($P<0.05$) with 58 up-regulated and 84 down-regulated in Palermo farm milk respect to the Agrigento one. In addition, differential expression analysis will be also performed on milk fractions from Palermo and Agrigento, respectively, and the differentially abundant proteins and others showing invariant abundance will be identified by mass spectrometry to generate reference milk proteomic maps of Girgentana goat breed which could be useful to identify specific molecular marker assessing milk quality.

C-062

Quantification of genetic variants of caseins in milk of Girgentana goat breed

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In the milk of ruminants, more than 95% of proteins are synthesized by six structural genes, four caseins (alphas1, beta, alphas2 and κ-caseins) and two whey proteins (alpha-lactalbumin and beta-lactoglobulin). Goat casein genes have been found to show high polymorphism, which influences not only the quantity of casein in milk but also the structural and nutritional characteristics and technological properties of milk. The aim of this work was to separate and quantify the most common allelic variants of caseins in milk of Girgentana goat breed, a Sicilian autochthonous breed, and to evaluate the effect of casein polymorphisms on casein content. The alleles at different casein genes were detected using PCR, PCR-RFLP, AS-PCR and sequencing analysis. Milk samples were prepared following the method proposed by Bobe *et al.* (1998) and analyzed by RP-HPLC method. A reversed-phase analytical column C8 (Zorbax 300SB-C8 RP, 3.5μm, 300Å, 150×4.6 I.D.) was used and the detection was made at wavelength of 214 nm. The procedure was developed using individual raw milk samples of Girgentana goats. For calibration experiments, pure genetic variants were extracted from individual milk samples of animals with known genotypes, considering that commercial standards for goat allelic variants were not available. In particular, were used animals with AA, BB, FF and NN genotypes at alphas1-casein; CC and C'C' genotypes at beta-casein; AA and FF genotypes at alphas2-casein; and AA and BB genotypes at κ-casein. Method validation consisted in testing linearity, repeatability, reproducibility and accuracy. A linear relationship between the concentrations of proteins and peak areas was observed over the concentration range, with low detection limits. Repeatability and reproducibility were satisfactory for both retention times and peak areas. For Girgentana goat breed the following levels of caseins for allele were obtained: alphas1-casein A 3.2±0.4 g/L, B 5.4±0.5 g/L and F 0.7±0.1 g/L; beta-casein C 3.0±0.8 g/L and C' 2.0±0.7 g/L; alphas2-casein A 2.9±0.8 g/L and F 1.8±0.4 g/L; and κ-casein A 1.6±0.3 g/L and B 1.1±0.2 g/L.

C-063

Identification of two new alleles at κ-casein (CSN3) gene in Girgentana dairy goat breed

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In the milk of ruminants, more than 95% of proteins are synthesized by six structural genes, four caseins and two whey proteins. The κ-casein fraction plays an important role in the formation, stabilization and aggregation on casein micelles and thus affects technological and nutritional properties of milk. Goat κ-casein (CSN3) gene comprises five exons with the mRNA coding region for mature protein spanning from exon 3 to exon 4. The κ-casein *locus* has been widely investigated and several genetic variants have been found in different goat breeds. At protein level, AIEF and BIEF groups corresponding to isoelectric point 5.53 and 5.78, respectively, have been identified. In this study, exon 4 of CSN3 gene was sequenced and analyzed in 205 samples of Girgentana goat breed. Analyses of the obtained sequences showed the presence of A, B, D, and G known alleles and two new genetic variants, named D' and N. The new D' allele differing from D in one transition G284→A284 that did not cause amino acid change. The new N allele differing from A for five SNPs T245/C245, G284/A284, G309/A309, G471/A471, and T591/C591. Comparing the amino acid sequences of N and A alleles, the first two SNPs caused no amino acid change, whereas the other SNPs produced changes Val65/Ile65, Val119/Ile119, and Ser159/Pro159, respectively. The most frequent allele was A (0.480) followed by B (0.363), D (0.112), and N (0.034). The D' and G alleles were identified only in two animals and in heterozygous conditions with a very low frequency (0.005). The most common genotype was AB (39.5%) followed by AA (19.5%), AD (12.7%), and BB (11.7%). Homozygous D'D', GG, and NN individuals were not found. Among the CSN3 alleles found in our study, only the D and D' alleles are included in BIEF group, whereas A, B, G and N alleles belong to AIEF group, which represents the less favorable variants' group in terms of milk composition and technological properties. Several authors reported that some genotypes at CSN3 *locus* were associated with higher level of total casein and protein content than others. Therefore, further analysis will be performed in order to establish associations among genotypes and quantitative milk traits.

C-064

Effect of κ-CN glycosylation on milk coagulation properties of Simmental cows

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Contents of casein fractions are known to affect coagulation properties and cheese yield of milk, but the effects of κ-CN composition on variation of milk coagulation properties have been scarcely studied. The aim of this study was to estimate the effects exerted by the degree of κ-CN glycosylation on variation of coagulation properties. Such effects were estimated using more than 2000 individual milk samples of Simmental cows. Cows were reared in 47 dairy herds and were progeny of 213 sires. Rennet coagulation time and curd firmness were measured using a computerized renneting meter. The content of all major casein fractions and the content of glycosylated and unglycosylated κ-CN were measured by reversed phase HPLC. The degree of κ-CN glycosylation was measured as the percentage ratio of glycosylated κ-CN to total κ-CN. Genotypes at CSN2, CSN3 and BLG were also obtained by reversed-phase HPLC. Glycosylated κ-CN in the investigated sample was, on average, 46.4±7.9% and ranged from 21.9 to 75.7% of total κ-CN. Genotypes at CSN2, CSN3 and BLG affected the variation of the degree of κ-CN glycosylation. This study provides evidence that rennet coagulation time is affected by the variation of κ-CN composition attributable to the degree of glycosylation. In particular, the favorable effect of κ-CN content on rennet coagulation time is to be entirely ascribed to the content of glycosylated κ-CN in milk. Heritability of the content of total κ-CN, glycosylated κ-CN, unglycosylated κ-CN and degree of glycosylation, obtained with linear models not accounting for CSN and BLG genotype effects, was 0.51, 0.46, 0.56 and 0.49, respectively.

C-065

Allelic frequency and linkage disequilibrium of 53 candidate genes in Brown Swiss breed, analyzed by customized Oligo Pool Assay

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Milk quality and milk technological traits (*e.g.*, milk coagulation properties) have recently become relevant for the dairy industry. Although genetic variation for these traits has been reported, their inclusion as breeding goals in conventional selection programmes is hampered by phenotyping costs. A possible solution can be found in the identification of candidate genes, especially by the high throughput Single Nucleotide Polymorphisms (SNPs) genotyping techniques. Such a technique was applied in Brown Swiss cows in order to investigate the polymorphism of 96 SNPs by using a 96 GoldenGate VeraCode Oligo Pool Assay (OPA) for the Illumina BeadXpress Reader. A total of 53 candidate genes were selected on the basis of their biological role in protein and fat yield and composition, in sanitary status of mammary gland and animal, for each gene one or more SNPs were analyzed. To assess the better 96 SNPs combination based on a designability rank score (0-1.0) to a successful Golden Gate assay, 131 primers paired were submitted to Illumina. Peripheral blood samples were collected from 1224 BS cows and were genotyped following manufacturer's protocol. The automatic allele calling was done using Illumina Genome Studio software with a Gene Call (CG) threshold of 0.25. A total of 51 SNPs were successfully genotyped and they were polymorphic, 21 SNPs showed a single allele so they were monomorphic and 24 SNPs failed because cluster separation and low intensity.

C-065bis

Intestinal microbiota in monogastrics and interplay with nutritional factors

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The intestinal microbiota and the host organism are affected by a balanced intestinal microbiota in a positive way, a state called eubiosis. In recent years it has become obvious that feed composition, feeding methods and feed additives offer a potential for performance improvement and stabilization of porcine health. Gross composition of feed, defined as balanced content of proteins, carbohydrates, fat and dietary fibre affects the intestinal microbial composition and metabolic acitivity and may stabilize the intestinal function in piglets especially after weaning. Feeding methods can have a major impact on gastrointestinal function and the susceptibility against invading pathogens, for instance *Salmonella*. A good example for gut flora modifiers are probiotics, that are used frequently in European farming practice. Appropriate products are classified as a microbial feed additive and have to undergo comprehensive trials for demonstrating safety and efficacy in accordance with the Regulation (EC) 1831/2003. Probiotic microorganisms must have sufficient stability, first of all against technological processes and further on in the gastrointestinal tract. They can have a regulating effect on the composition and the metabolic reactions of the microbiota, defined as the community of the intestinal microorganisms. Probiotics should not only affect the balance of the intestinal flora, but also have the capability for positive health effects. The effects of the microorganisms in the intestinal lumen can be theoretically explained by a shift in the competitive situation for existing nutrients and a change in the intestinal milieu through production of a broad spectrum of metabolites and molecules including antimicrobials. The reported inhibitory effect against pathogenic intestinal bacteria seems to be based on the influence on the microenvironmental conditions and a competition for epithelial binding sites and receptors, possibly also immune mediated reactions to this microenvironment. An increased colonization protection against pathogenic bacteria and an improved resistance to infectious diseases could be an interesting option for the use of probiotics in livestock in addition to the performance-enhancing effects.

C-066

Dietary fish oil in quarter horses: effects on hemocromocitometric values and plasma fatty acid composition

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Fish oil inclusion in the diet of horses could be of interest due to the beneficial effects already established in other animals, but at present day few references are available in horse nutrition on this topic. The aim of the trial was to evaluate the inclusion of fish oil in the diet of horses on blood parameters and plasma fatty acids composition. Eight Quarter Horses (3 to 8 years of age; 479±14.04 kg) were balanced for age, sex, body weight (BW) and body condition score (BCS) in two experimental groups and fed (FO) or not (C) 40 g/d of fish oil in a two-periods crossover design. The basal diet, established on 2% BW following NRC requirements for light working horses, had a 70:30 forage to concentrate ratio (CP 10.2% DM, EE 3.24% DM, NDF 44.3% DM, ED 23.1 Mcal/d). For each period an adaptation phase of 30 days to the basal diet was provided followed by 30 days of fish oil supplementation with a 17 days wash-out before switching the groups. Diet components and fish oil were analyzed for fatty acids (FA) profile using a modified Folch lipid extraction procedure. Body weight and BCS were measured weekly from the adaptation period. Starting from dietary treatment, individual feed intake was recorded daily, and blood samples were collected every 15 days for plasma fatty acids composition and Complete Blood Count. Data were analyzed using a PROC MIXED of SAS with treatment, time, period, and animal included in the model statement. No influence of fish oil was observed on BW, feed intake and BCS, at the same time no effect was reported on number, rather ratio, of white blood cells. Fish oil increased arachidonic (1.50 *vs* 1.29 g/100 g total FA; $P \leq 0.05$), EPA (1.58 *vs* 0.05 g/100g total FA; $P \leq 0.01$) and DHA (0.64 *vs* 0.05 g/100 g total FA; $P \leq 0.01$), and decrease linoleic (45.69 *vs* 48.72 g/100 g total FA; $P \leq 0.01$) plasma content. The administration of fish oil increased beneficial PUFA fatty acid plasma concentration, with no negative effect on feed intake, but further investigations are needed to determine the possible effects on immune response.

C-067

Carbon footprint of heavy pig production in eight farms in Northern Italy

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A preliminary Life Cycle Assessment was performed for estimating the carbon footprint (CF) of the heavy pig production in eight fattening pig farms in Northern Italy. The average pig number in each farm was 1967 (±1379), the average number of pigs fattened every year was 4123 (±3349). Initial and final weigths were kg 38.2 (±11.0) and 167.1 (±5.25), respectively. System boundaries of CF study comprised the growing and fattening phase in the pork production chain; they did not include the sow-piglet phase, slaughtering and feed production. Environmental burden of purchased feeds and piglets was calculated with background database values and was comprised in CF estimation as indirect carbon dioxide equivalent (CO_2eq) emissions. Functional unit (FU) was 1 kg of body weight at the farm gate. The global warming potential (GWP) was expressed as kilograms of CO_2eq; greenhouse gases (GHG) considered in this analysis were CH_4, N_2O and CO_2 with a GWP of 25, 298 and 1 kg CO_{2eq} respectively. The average CF of one FU was 3.83 (±1.07) kg CO_2eq. The contribution of each emission source was: enteric CH_4 3.34%, manure CH_4 17.7%, manure N_2O 1.38%, direct soil N_2O 6.59%, indirect soil N_2O 6.39%, direct CO_2 1.00%, indirect CO_2 63.6% (comprising production of purchased feed and piglets, fossil fuel extraction and refining, electricity production, and transportations). The results are consistent with those obtained in other studies carried out in Northern Europe or USA.
In respect to other animal foods, such as milk, where the enteric fermentation is a main GHG source and the crop production is an important component of the system, this study showed that the main drivers of GHG production in growing and fattening phase of pork production chain are the off-farm feed production and transportation phase; on the contrary the activities within the system boundaries are of minor importance. Mitigation strategies should be addressed to feed production chains and manure management systems. This assessment should be considered preliminary because more differentiated data are expected from in-progress studies concerning feed production chains and piglet production phase.

C-068

Effects of genetic line and dietary crude protein level on growth traits of finishing heavy pigs

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This trial was aimed to evaluate the effect on growth performance of restricted diets using conventional (CONV) or low-CP and low-essential amino acid diets (LP) on pigs of 4 genetic lines (GL): Anas (A), DanBred (D), Goland (G) and Topigs (T). In two rounds, 184 pigs (gilts and barrows), born in the same week, were raised on a common feeding regime till 80 kg BW. From 80 kg BW, the pigs were housed in 8 pens (10 to 12 pigs/pen) equipped with feeding station recording individual daily feed intake and received CONV or LP diets. During the early (90 to 120 kg BW) and late (120 to 165 kg BW) finishing periods the CONV diets contained 147 and 132 g CP/kg feed and 50 and 41 g lysine/kg CP, respectively, while LP diets contained 112 and 100 g CP/kg feed and 50 and 41 g lysine/kg CP, respectively. Every three weeks pigs were individually weighted and backfat thickness was ultra-soundly measured at P2 point. Data were subjected to ANOVA and diet was tested on pen within round as line of error. All growth traits were influenced by GL ($P<0.001$): the D pigs had greatest final BW (172 kg), ADG (0.700 kg/d), gain:feed ratio (0.272) and the lowest final backfat depth (15.9 mm) and backfat gain (+7.5 mm); the A and G pigs exhibited similar final BW (165 kg), average daily gain (ADG, 0.686 kg/d), gain:feed ratio (0.266), final backfat depth (18.6 mm) and backfat depth gain (+8.9 mm); the T pigs had the lowest final BW (159 kg), ADG (0.637), gain:feed ratio (0.248) and final backfat depth (16.2 mm) and backfat depth gain (+7.7 mm). The dietary treatment did not influence final BW and ADG, but LP increased backfat depth gain from +8.0 to +8.6 mm ($P=0.013$) compared to CONV. The GL × feed interaction was never significant. Differences among GL were more relevant than those due to the reduction of the dietary CP. These results are useful for the Italian Protected Designation of Origin (PDO) dry-cured ham production circuit.

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C-069

Digestibility, metabolic utilization and effects on growth and slaughter traits of diets containing whole ear corn silage for heavy pigs

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The effect of dietary inclusion of whole ear corn silage (WECS) on digestibility, metabolic utilization (using respiration chambers trial 1) and growth and slaughter performances (trial 2) was studied in heavy pigs. Three experimental diets were fed to pigs in both trials: a control diet (C), based on cereal meals, soya bean meal and wheat bran and two diets containing WECS (15 or 30% on DM: 15WECS and 30WECS, respectively) in partial or complete replacement of corn meal and wheat bran. Diets had similar NDF contents (15.2%-15.8% on DM), while the highest WECS inclusion determined a slight reduction in CP content (from 14.0 to 13.6% on DM) and a decrease of P content (from 0.47 to 0.30% on DM). Digestibility (6 barrows/diet, 118 kg BW on average) of OM, CP and fat was similar among diets, while P digestibility was lower for 30WECS diet in comparison with C and 15WECS diets (33.5 *vs* 45.5 and 44.1%, $P<0.05$). Nitrogen balance was not different among diets, while P retained decreased significantly with the increase of WECS (5.4, 3.7 and 2.2 g/d for diet C, 15WECS and 30WECS, respectively, $P<0.001$). No significant difference among diets was registered for energy balance. WECS resulted to have 13477 kJ ME/kg DM. In trial 2 (14 barrows/diet, BW range 90-170 kg) DM intake (from 8.0 to 6.9% BW0.75) was not depressed by WECS inclusion and the daily gain in whole trial was not different among dietary treatments (from 737 to 774 g/d). The pH of faeces was lower for WECS diets than control (7.10 and 7.00 *vs* 7.40, $P<0.01$) and faecal pH measured at 150 kg BW was lower (6.96, $P<0.01$) than at 130 and 110 kg BW (7.29 and 7.24, respectively). Slaughter traits were similar among diets except for a higher backfat thickness (30.5 *vs* 28.1 mm, $P=0.05$) and a lower lean tissue percentage (46.8 *vs* 48.3-48.6, $P<0.05$) for 30WECS in comparison with the other diets. The data obtained indicate that the substitution of cereal meals with WECS does not hamper nutrient utilization and performances of fattening heavy pigs.

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C-070

Phosphorus excretion and apparent efficiency in heavy pigs

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Forty intensive farms producing heavy pigs, located in the north-west plain of Italy (Piemonte region), were studied for a fattening period (about 7 months) to evaluate the actual level of P excretion. Management data, feeding systems, feedstuff composition inclusive of additives and integrators, feed consumption, and animal weight gain were recorded. Collected data were at first analysed altogether and P balance (P excreted=P intake - P retained; Pe=Pi-Pr) and efficiency to animal level (Pr/Pi) were calculated. Data were then analysed by ANOVA comparing diets with or without phytase or amino acids addition, number of feeding phases, and wet or dry rations. Correlations between farms variables and Pe were also studied. The diet was dry in 17 farms, wet in 20, and both dry and wet in different phases in the last 3 farms. 93% of farms added essential amino acids, and 48% added phytase to the diet. Average live performances were 0.69±0.47 kg/d for average daily gain (ADG), 3.49±0.39 kg/kg for feed conversion rate (FCR), and 2.39±0.25 kg/d for feed consumption. P balance showed 5.06±0.58 g/kg DM for P feedstuff content, 2.35±0.52 kg/head for Pi, 0.69±0.87 kg/head for Pr, and 1.66±0.48 kg/head for Pe. No statistical differences were found between P balance factors, except for Pi and Pr in different phase feeding systems, but efficiency was not affected from such differences. P efficiency was 32.18±7.24 *vs* 29.02±4.54% in diets with or without phytase addition, 30.91±6.19 *vs* 25.75±0.90% in diets with or without amino acids integration, 30.34±5.87 *vs* 27.06±4.06 *vs* 31.18±6.10 *vs* 32.43±7.96% in feeding systems with 1, 2, 3 and 4 phases respectively, and 30.09±6.02 *vs* 30.91±6.31% in wet or dry rations. Pe was positively correlated to pig weight ($P<0.01$), FCR ($P<0.01$) and feed consumption ($P<0.01$), but did not show any correlation to ADG. In conclusion, these data showed only a potential effect of adoptable mitigation strategies, even if these ones did not affect significantly P efficiency in the analysed farms. Probably, the different farming systems and the high number of farm variables are reciprocally influenced, affecting P balance elements and hence P efficiency; nevertheless, the results could be useful to study P potential excretion in heavy pigs.

C-071

Natural complementary feeds in gestating and lactating sows: effects on performance and metabolic profile

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Pregnancy and lactation cause high metabolic effort and high cellular metabolism in sows; the contemporary presence of stressors may lead to ROS (Reactive Oxygen Species) production, which may overcome the action of the physiological anti-oxidants. The consequences may be DNA, protein and PUFA oxidation, peroxides production, ROS release in blood, and finally oxidative stress. Liver function, sow immune defense and fertility, immunity transfer to piglets and their vitality may be unbalanced. In this work, two natural complementary feeds (GOLD and GOLD EPS), aiming to reduce oxidative stress, were used at 0.3%

in the diet of lactating (trial L, 83 sows in total, 3 groups: control, GOLD, GOLD EPS) and gestating-lactating (trial GL, 105 sows, 2 groups; control, GOLD) sows. GOLD contains: brewer's yeast and organic selenium from *Saccharomyces cerevisiae*; betaine, phosphatidylcholine, RRR-α-tocopherol. GOLD EPS also contains *Silybum marianum* extract. Collected data were: sows' feed intake and post-weaning performance; number of newborn and weaned piglets, mortality, individual growing; liver metabolic profile, oxidative stress indicators; blood, colostrum and milk IgG. In trial L, higher weights were recorded for GOLD and GOLD EPS piglets (7352 and 7329 g at weaning) in comparison with control (7077 g); a trend to post-weaning fertility improvement for GOLD EPS sows were also observed (new successful farrowing: 62, 68 and 77% for control, GOLD and GOLD EPS). In trial GL, GOLD piglets were slightly heavier at birth and less affected by diarrhea. A positive increase of serum anti-oxidant power (OXY) was observed at half pregnancy in GOLD sows, followed by a decrease at farrowing. GOLD sows also showed a constant decrease of bilirubin. Serum IgG, after an increase at farrowing (control 14.4; GOLD 16.4 g/L), showed a decrease in GOLD sows at the end of lactation, when IgG level increased in piglets (control 4.6; GOLD 7.8 g/L): this might indicate a higher transfer from mother to piglets. Notwithstanding the difficulty of on-field conditions, these trials provide partially good results about the performance of sows fed the tested products.

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C-072

In vitro evaluation of the impact of zein proteins on starch digestion and dry matter digestibility for pigs

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Zeins are hydrophobic storage proteins constituting up to 80% of corn endosperm proteins. Zeins encapsulate starch granules into a protein matrix, which can act as a barrier to enzyme accessibility. Despite zeins have been negatively related to ruminal starch degradability, little information is available for pigs. Therefore, the effect of zeins was assessed on starch digestion potential (as predicted glycemic index; pGI), rapidly digestible, slowly digestible and resistant starch fractions (RDS, SDS and RS; respectively) and total tract dry matter (DM) digestibility (DMdv) by using different *in vitro* techniques consisting in sequential multi-enzymatic incubations simulating digestion processes occurring in the digestive tract of pigs. Twenty commercial-available corn grains were selected to represent a wide range of zein content. All samples were grounded through a 1-mm screen. Corns were classified into five distinct classes characterized by increasing zein content (calculated as g zein/100 g starch): <4.0 (low: L; n=4), 4.0-5.0 (medium-low: ML; n=5), 5.0-6.0 (medium: M; n=4), 6.0-8.0 (medium-high: MH; n=4), >8.0 (high: H; n=3). Corns belonging to classes with a zein/starch ratio > 6.0 (MH and H) had lower pGI, slower rates of starch digestion (k) and higher RS when compared to other classes (on average 15 *vs* 38, 0.007 *vs* 0.016 /min and 37.2 *vs* 21.5 g/100 g DM, respectively; P<0.01). The DMdv decreased from 0.92 to 0.72 (P<0.01) from L to H classes, respectively. The simple Pearson correlations revealed zeins were negatively related to pGI, k, RDS and SDS and DMdv and positively to RS (P<0.05). The correlation matrix was used to extract four principal components (PC) using PC analysis (eigenvalues >1.0). PCs were associated to *in vitro* degradability parameters and chemicals (PC1), particle size (PC2), starch and ash contents (PC3) and fiber content (PC4). Samples were then clustered on two-dimensional plots. Only PC1 usefully discriminated corns between high (L, ML and M classes) and low (MH and H classes) *in vitro* degradability potential. The negative effect of zeins on *in vitro* degradability parameters of interest supports the need for more accurate estimates of nutrient composition and energy content of corn entering in pig diet formulation. Findings are based on an *in vitro* approach and require further *in vivo* validations.

C-073

Evaluation of zinc additives in intestinal *in vitro* models

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Zinc is an essential trace mineral with a wide range of metabolic functions. Different sources of zinc are commonly added into diets for intensive pig production as alternative to in-feed antibiotics. In fact feeding high levels of zinc to weaned piglets could decrease the incidence of non-specific post-weaning diarrhea and improve growth performance. Zinc is involved in different mechanisms related to host cells and pathogen metabolism, nevertheless its specific role in promoting piglet health is not completely understood. The absorption of zinc occurs primarily in the small intestine where it contributes to maintain intestinal barrier function and integrity, influence immune system and inhibit bacterial growth. The aim of this study was to set-up intestinal *in vitro* models to analyze the potential benefit of zinc sources on intestinal epithelium. In particular the effect of ZnO and $ZnCl_2$ on intestinal porcine epithelial cells (IPI-2I) and human embryonic intestinal cells (INT-407) was evaluated. IPI-2I and INT 407 cell lines were cultured in specific growing media. After incubation for 3 and 24 hours with a wide range of concentrations of ZnO (from 50 μM up to 4000 μM) and $ZnCl_2$ (from 50 μM to 6680 μM) the effect on cell viability was evaluated by MTT test. Obtained results showed that IPI-2I and INT 407 cell lines are sensitive to both zinc sources and a dose-response effect was observed. Treatments of INT-407 cells with 50 µM ZnO and 50 µM $ZnCl_2$ enhanced cell viability, respectively to 127% and to 115% of the control. The human INT-407 cells demonstrated higher sensitivity to zinc treatments than the porcine model. IPI-2I viability was not significantly affected by zinc treatments. This disparity was most likely due to different host cell lines. In conclusion, intestinal porcine epithelial cells and human embryonic intestinal cells could be used as an efficacious *in vitro* model to investigate zinc additives.

C-074

Intestinal proinflammatory citokines could be modulated by dietary nucleotides in post weaning piglets

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Dietary nucleotides are reported to improve swine immune response and intestinal health status, decreasing diarrhoea events and increasing performance. Such effects are particularly appreciable in a stressful period as weaning, when gut pathogen contamination more likely occurs. The aim of the study was to evaluate the effects of dietary nucleotides supplementation on ileal proinflammatory interleukin gene expression of post weaning piglets. Thirty-six weanling piglets (28d of age, 7.85±0.32 kg) were divided in two experimental groups (C and N) for a 28 d study. The N group received the basal diet supplemented with 0.8 g/head/day nucleotides (UMP 88.1%, GMP 5.51%, AMP 3.82%, CMP 1.94% and IMP 0.68%). No supplementation was given to C group. On 14 d all piglets were orally challenged with 1×10^9 CFU/g *E. Coli* 0149:F4(K88). Growth performance and fecal score were evaluated weekly, immunological serum parameters, Fe and Vitamin B12 serum content were analysed on days 0, 13, 18 and 26 d. At slaughtering IL1a, IL1b, IL6, IL10, and TNF, TRL2 and TRL4 gene expression of ileal Peyer's patches were evaluated by RT-PCR. No differences were found on growth performance, while fecal score was ameliorated in N piglets ($P<0.05$). Lower haptoglobin serum content (0.56 mg/mL *vs* 0.48 mg/mL; $P<0.01$), higher vitamin B12 (239 ng/dL *vs* 225 ng/dL; $P<0.05$), and IgG (5.00 mg/dL *vs* 4.83 mg/dL; $P<0.05$) level were found in N group. Nucleotide-fed pigs showed decreased IL6, IL10, TNF, TRL2 and TRL4 gene expression at slaughtering ($P<0.01$). Dietary nucleotides supplementation in post weaning piglets can positively affects gut health status, ameliorate inflammatory response and nutrients absorption in microbial stress conditions.

C-075

A new feed block for gut health and welfare of the weaning pig

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Pig weaning combines social, nutritional and environmental stresses, that hesitate in transient reduction of feed intake and development of intestinal villi, animal growth and higher susceptibility to diffusive diseases. Providing feed with texture that stimulates porcine explorative interest could lighten weaning drawbacks and provide a tool to respect the law for animal welfare.
Various series of small feed block (around 0.8 kg weight) were produced in a small scale and 8 formula were preliminary tested for block intake in a pig farm joined in the MIPAAF-OIGA project PIGBLOCK. Base ingredients were wheat by-products, dried milk whey, calcium carbonate, oil and molasses, in different combinations. The 3 best formulations were then tested in the same farm for block and total feed intake and live weight gain in the 3 days after weaning. Normal weaning feed was always provided *ad libitum*. Following the farm practice, the experimental unit for ANOVA was the box formed by 4 litter after moving away the sows. Each formulation was tested 4 times and compared with the other ones and a control, obtained by wooden pieces of similar dimension. Growth was in general not affected, while block intake was relevant. The same 3 formulations and the control were tested in experimental farm on a total 72 weaned pigs (24 d of age), penned in box of 3. Welfare was quantified through surveys with cameras (7 parameters, statistically analyzed by SAS GLIMMIX). The trial ended after 4 days with the slaughtering of pigs and the collection of small intestine samples from all of them, for the morphology of intestinal mucosa (subject mean values for villus height, crypt depth, mucosal thickness). Block intake in general resulted additive on the intake of normal feed. Growth was not affected. One formulation (n.8) increased the mucosal surface area by 7.9% ($P<0.05$). The same formulation increased ($P<0.01$) the time spent by pigs sleeping. The results obtained indicate that proper formulation of the feed block can give it the characteristic to reduce the impact of weaning on intestinal villi growth and improve the welfare, and are encouraging the development of the product.

C-076

The role of the European Food Safety Authority in the evaluation/re-evaluation process of feed additives

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Feed additives are substances, micro-organisms or preparations, other than feed material and premixtures, which are intentionally added to feed or water in order to perform, in particular, one or more of the following functions: favourably affect the characteristics of feed (technological) or animal products, favourably affect the colour of ornamental fish and birds (sensory); satisfy the nutritional needs of animals (nutritional); favourably affect the environmental consequences of animal production, favourably affect animal production, performance or welfare (zootechnical); or have a coccidiostatic or histomonostatic effect (coccidiostats). In the European Union (EU), all feed additives undergo an authorisation procedure as established in the Regulation (EC) No 1831/2003 in order to be placed on the market. In this procedure, the European Food Safety Authority (EFSA), and in particular the Panel on additives and products or substances used in animal feed (FEEDAP), is the responsible to perform the risk assessment. Any person seeking authorisation of a given additive should submit an application to the European Commission and a technical dossier to EFSA. The dossier should be compiled following Commission Regulation (EC) No 429/2008 and the EFSA guidance documents aimed to help the applicants. In order to be authorised, feed additives should be safe, and therefore should not have (i) 'an adverse effect on the animal health, human health or the environment', and (ii) should not 'be presented in a manner which may mislead the user'; the additives should also be efficacious. The risk assessment of the FEEDAP Panel (a group of 21 experts in the areas of animal nutrition, physiology, toxicology, microbiology, veterinary medicine, environment) finishes with the adoption of a scientific opinion which is the basis for the European Commission to grant or deny the authorisation of the product in the EU market. For those additives that were already in the market before the entry into force of the Regulation (EC) No 1831/2003 the need for a re-evaluation process was established. The aim of the re-evaluation is to ensure that all feed additives in the market are assessed following the same up-to-date standards and taking into account the newest scientific developments. This presentation is given under the sole responsibility of the authors, and is not considered as an EFSA output. The positions and opinions presented are those of the authors alone and are not intended to represent the views of EFSA.

C-077

Oxidative stress induced by high dietary PUFA in young pigs: effects on growth performance and some haematological parameters

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Farm animals in intensive farming systems are frequently exposed to oxidative stress, which demands adequate antioxidant protection. The aim of this study was to evaluate the antioxidant potential of natural antioxidant mixture in case of n-6 PUFA induced oxidative stress in young pigs. Twenty four female piglets (Hypor) (BW 10 kg) were randomly assigned to three dietary treatments. Oxidative stress was induced in two of the three experimental groups with the inclusion of 9% of sunflower oil (SO) and the same inclusion of SO added with a mixture of natural antioxidant (SO-NA). In control diet (CON) oil was replaced with corn starch on the basis of net energy. Diets were isoenergetic by the adoption of appropriate quantity rationing differences. The animals were individually fed 2.7 times the maintenance requirements for metabolisable energy. From each piglets blood was collected by anterior vena cava puncture on days 0 (T0) and 30 (T1) of the experimental period for the activity of Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GP) enzymes and assessment of total antiradicalic capacity by mean KRL test. KRL test measures the whole blood resistance to free radical attack and results were expressed as the time (min) required to reach 50% of haemolysis. Body weight and feed consumption were recorded weekly. No effect on growth performance was detected. Oxidative stress measured as KRL test showed a significantly reduction in both SO groups ($P<0.001$) and over time ($P<0.001$). The activity of enzymes did not differ among groups. Orthogonal contrast showed a significant reduction ($P<0.001$) of GPx activity at T1 in groups treated with oil and a trend effect ($P=0.081$) of CAT enzyme. In conclusion dietary PUFA produced a reduction of antiradicalic capacity well highlighted by KRL test. The dosage of the enzyme activity of catalase, glutathione peroxidase and superoxide dismutase showed a pattern overlapping KRL test even if not always able to evidence significant difference among treatments. The natural mixture of antioxidants did not limit oxidative stress at level of the inclusion adopted.

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C-078

Evaluation of histological changes, diet related in gastrointestinal tract of European sea bass (*Dicentrarchus labrax*)

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The reduction of dependence on fishmeal (FM) and fish oil (FO) in feeds is compulsory for a sustainable growth of aquaculture. Concerning the protein component, feed companies turned toward the use of alternative protein sources as vegetable meal (VM) that today vary from 48% to 68% in commercial aquafeeds, without any apparent loss of growth performances. Nevertheless, elevate VM in the diet may not always be tolerated by the fish gut, resulting in inflammations and histological damages, with reduced digestive functionalities and intestine integrity. Moreover, the damaged fish gut may become an important infection routes for microbial pathogens in fish. Therefore, biomarkers that may early describe the progression of any intestine damages, are indispensable tools to tune an appropriate nutritional strategy. A new arbitrary scoring system has been approached for seabass gut histology, to evaluate the cellular and tissue changes in the gastrointestinal (GI) tract of seabass fed at different levels replacement of FM by plant protein sources. The applied scoring protocol takes in account four parameter, empirically rated:

- supranuclear vacuolization of the absorptive cells
- lamina propria of the single intestinal fold
- connective tissue between base of folds and stratum compactum
- number and or shortening of folds (simple and complex) heights of mucosal foldings

For each parameter, the score rate ranges from 1 to 5: a rates 1-2 representing normal morphology, while a rate up to 5 being assumed as a morphological descriptor of severe enteritis. The scored histological changes are not specific pathological lesions, nevertheless they should be considered as indicator of GI modifications diet related. Moreover, in our study, the histological score is tentatively compared to the transcriptomic activity of genes describing intestine walls functionality, earlier developed by our group. In future, following a round intercalibration test with other worldwide laboratories, we aim to better set up the scoring as a method for nutritional welfare assessment in seabass, as well as in other farmed species of great interest in Mediterranean and Italian aquaculture, like rainbow trout and gilthead sea bream.

C-079

Effects of dietary nucleotides supplementation on growth and immune response of common sole *solea solea*

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Previous studies in certain farmed fish species have shown that a dietary addition of exogenous nucleotides (NT) could improve growth, gut integrity, stress tolerance and enhances immune response. In this contest a feeding trial was carried out to evaluate if, and to what extent, a dietary NT supplementation could elicit similar beneficial responses in a poorly domesticated species like the common sole. A basal diet (ES, control) was compared to a test preparation (EN) obtained from the basal one by adding 0.4 g/kg dry diet of Vannagen®, a commercial source of nucleotides. One hundred seventy six fish (mean body weight 122.9±9.6g) were randomly distributed in 4 tanks (1 m^2) at 5.4±0.7kg/m^2 stocking density. Fish were fed at 1% body weight/d over 8 weeks according to a single-factor design with 2 replicates per diet. At the end of the trial specific growth rate (SGR), feed conversion ratio (FCR), and survival rate were calculated per group; furthermore, 8 fish per treatment were analysed for plasma lysozyme activity, levels of total proteins, immunoglobulins (IgM), respiratory burst activity of head-kidney leucocytes and for gut and liver histology. After 8 weeks of feeding, fish fed diet EN exhibited higher final weight and SGR resulting in a better FCR and survival rate when compared to the controls (146.2 *vs* 132.4g, 0.23 *vs* 0.16, 3 *vs* 4, and 87.8 *vs* 76.3%; respectively, $P<0.05$). Soles fed diet EN showed higher plasma levels of total proteins and IgM relative to the controls ($P<0.05$). No significant ($P>0.05$) dietary effect on respiratory burst and lysozyme activities were observed. Dietary NT supplementation didn't affect liver histology, while the mean fold height of distal intestine was significantly improved in fish given diet EN than in those fed diet ES. These results obtained so far suggest that nucleotides addition in sole diets improves growth performance, mostly through increased palatability and feed intake, and adaptive immunity as well, probably by regulating the intracellular nucleotide pool. The lack of NT effect on lysozyme and burst activity could be due either to the high among-individual response variability or to the ineffectiveness of the NT dose here tested.

C-080

The influence of mussel meal dietary inclusion on performance, fatty acid composition, gut and liver histology of common sole (*Solea solea*) juveniles

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Common sole, *Solea solea*, farming in Europe, although potentially very promising, is still a little reality owing to the poor performances of fish when fed fish meal (FM) based diets. Mussel meal (MM), characterized by high palatability and good nutritional value, may represent an innovative ingredient in the formulation of a commercial feed for this species. The present study was carried out in order to determine the effect of graded level of MM dietary inclusion on growth performance, fatty acid composition, gut and liver histology of common sole juveniles. Four isoproteic (53%) and isolipidic (11%) pelletized diets were formulated to contain graded levels of MM (0%, 25%, 50% and 75% named respectively MM0, MM25, MM50 and MM75) to replace FM. Seventy sole juveniles (initial body weight 13 g) per tank were randomly distributed in twelve flat bottom 280 L square tanks (temperature 20±1°C and dissolved oxygen above 7 ppm). Animals were hand-fed twice a day to apparent satiation, over 91 days. Performance, carcass indices, gut and liver histology were investigated. One-way ANOVA and Tukey's post hoc test was used to analyze data ($P \leq 0.05$). Diets containing MM gave significantly higher Specific Growth Rate (SGR) and lower Feed Conversion Rate (FCR) when compared to the control diet (MM0). The administration of MM50 and MM75 also resulted in a significantly higher voluntary feed intake (VFI). Carcass composition and carcass fatty acid composition were not influenced by dietary treatments with exception of the omega-3 docosahexaenoic acid. A significant decrease in VSI was observed in fish fed MM50 and MM75 in comparison with the control group. HSI of fish fed MM0 and MM25 was higher than that of fish fed MM75, even though in all the experimental groups the histological examination of liver parenchyma showed uniformly a moderate to intense accumulation of intracytoplasmic clear material (lipidic content) that was sometimes arranged in optically empty droplets. Histomorphologic evaluation of intestine did not show degenerative or inflammatory changes. According to those results, MM is an effective ingredient for enhancing growth and feed utilization in sole irrespectively to the inclusion levels used in this trial.

C-081

Effects of light intensity on performances, larval development and foraging activity in common sole (*Solea solea*) larvae

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Abiotic factors have shown to play a significant role in fish larval rearing. Setting environmental parameters specific for each species can improve performances and survival. Among them, light intensity seems to influence growth and survival during the early development of fish larvae since different studies have shown that most marine fish larvae are visual feeders. This study investigated the possible effect of light intensity on performances, development and foraging activity on *Solea solea* larvae from day 4 to 51 post-hatching (dph). Four treatments were tested in triplicate in order to applied different light intensity regimes (LRs): light intensity of 1000 lx (LR1000), light intensity of 500 lx (LR500), light intensity of 50 lx (LR50) and light intensity of 3 lx (LR3). Larvae were exposed to 16 h light : 8 h dark photoperiod and were kept under constant temperature (18°C). Feeding protocol for all treatments consisted in live feed (Artemia spp.) until 18 dph followed by weaning onto commercial microdiet. Sole larvae reared under high light intensity (L1000 and L500) showed a high weight gain during the first period, whereas after metamorphosis best performances were achieved under low light intensity (L50 and L3). Mortality rates were lower in L50 and L500. No abnormalities of development were detected in the different treatments. Organs development, with special regard to sensory organs as eyes, olfactory sensory cells, otic vesicles, neuromasts, taste buds and barbells was also evaluated by histological analysis all through the trial. Results suggest that light intensity for sole larvae should be high (500-1000 lux) during the first rearing period and lowered (50-3 lux) after metamorphosis completion. This could indicate that sole is a preferential visual feeder during the larval pelagic phase, while, after metamorphosis, other sensory organs are mainly involved in foraging activity.

C-082

Effect of the diet on the stress response to a simulated transportation experiment of fingerlings of *Salmo marmoratus*

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Under intensive culture conditions, fish are subjected to increased stress that has negative impacts on the fish overall performance. Though good management practices contributes to reduce stressor effects, the possible role of the diet quality in modulating acute stress response has been poorly investigated in fish. The objective of this study was to evaluate the modulation of acute stress response by different diets in marbled trout, Salmo marmoratus. Twelve groups of trout fingerlings (150 mg ABW) each consisting of 1000 specimens were randomly allotted in triplicate to four commercial trout diets (S, B, D, V) differing in ingredient and nutrient composition (12-20% lipid and 51-59% protein). Fish groups, kept under controlled rearing conditions (water temperature 8.5-10°C) in a flow-through rearing tank system, were fed over 148 days at 3% body weight/d-1 in two daily meals. At the end of the trial one group of fish per diet was subjected to a standardized protocol of disturbance simulating an acute transportation stress (1 hour at 4.5 fish/L) and sampled 1 h after exposure. The fish remaining in the tanks were used as controls. Modulatory effects of dietary treatments were evaluated by means of cortisol level measured by RIA in plasma, fins and skin mucus and associated to the growth performance and mortality observed in the feeding trial. Diet B resulted in the poorest survival (59.2 *vs* 70.0%, $P<0.05$) and in the lowest weight gain relative to all the other diets which gave rise to similar responses (703 *vs* 1100% IBW, $P<0.05$). Moreover, fish fed diet B showed the highest cortisol burst after transportation in all matrices (4-12 folds higher than controls), such an increment being an adverse response against the stressor. Cortisol significantly increased in all matrices after the transportation stress ($P<0.01$), but only in plasma a significant effect of the past diet ($P<0.05$) has be detected. The results showed that a poor diet, beyond reducing growth and survival, can also affect fish acute stress response and that cortisol levels measured in non-invasive matrices such fins or skin mucus proved reliable to study acute stress response in marbled trout.

C-083

Assessing freshness of European sea bass (*Dicentrarchus labrax*) from different farming systems

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This study aimed to assess the evolution of freshness in 90 European sea bass (*Dicentrarchus labrax*) analyzed after 3, 6, 10, 13, and 17 days of storage in ice at 2°C. Sea bass were caught in three farms characterized by different rearing systems (extensive coastal lagoons, intensive inland basins, intensive sea cages). At each storage time, sensory analysis for freshness was performed on intact fish according to the Quality Index Method (QIM); skin colour and body firmness were measured on raw fish with a Minolta spectrophotometer and a dynamometer with a 20-mm diameter cylindrical probe, respectively; pH was recorded on the fillets. Data were submitted to analysis of variance using the GLM procedure with rearing system, storage time and their interaction as the variability factors. From the 3rd to the 17th day of storage, red and yellow indexes measured on skin decreased ($P<0.01$) as well as body firmness (from 28.9 to 23.4 N; $P<0.01$). Fillet pH remained stable (about 6.30) until the 13th day of storage and then increased up to 6.45 ($P=0.10$) at the 17th day. The total QIM score significantly increased with the days of storage and reached the maximum value (14.7 over a maximum of 22.0) after 17 days; skin appearance, body firmness, and gill traits showed some degradation soon after 3 days of storage. In contrast, eye and abdomen traits showed some appreciable degradation only after 13 days of storage. All traits were affected by the rearing system ($P<0.01$). In particular, sea bass from the extensive system degraded to a slower extent compared with those from the intensive systems: total QIM scores (averages of all storage times) were 7.45 *vs* 8.83 and 9.33 ($P<0.001$) for sea bass caught in extensive coastal lagoons *vs* intensive inland basins and intensive sea cages. Moreover, in sea bass reared in sea cages, skin appearance, body firmness and eye traits degraded quicker and to a greater extent compared to fish from the other farming systems.

C-084

Different farming techniques for *Crassostrea gigas* (Thunberg,793) in a Sardinian coastal lagoon (Italy)

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The aim of this work was to test the performance of triploid hybrids Pacific oysters (*Crassostrea gigas*) in order to evaluate the growth rate in two different farming techniques. The experiment was carried out in Tortolì coastal lagoon (eastern Sardinia, Italy: Lat 39°56’854’’, Long 9°41’160’’) from February 2012 to July 2012. Hydrological variables (temperature, salinity, pH, dissolved oxygen and chlorophyll a) were monitored monthly by multiparameter probe. Oysters from French hatchery (average weight 1.41± 0.59 g SD), were grown in oyster bags (poches) made in plastic mesh which measured 100×50 cm (length × width), using two techniques: poches floating on the water and poches submerged (50 cm depth). A total of 1800 oysters (100 specimens × 9 poches × 2 different farming techniques) were reared. Samples of *Crassostrea gigas* (30 specimens x poche x each kind of farming) were collected monthly and their total weight was measured using 0.01 g electronic precision balances. Then growth rate per day were calculated (TW/d: total weight per day). Two-way ANOVA and T-test on least squares were used to asses the effect of ‘farming techniques’ by SAS software system. The oysters weight steadily increased in both types of farming systems. After six months the oysters showed an average weight of 63.31±12.70 g and 87.86±18.29 g in floating poches and in submerged poches, respectively. As regards daily increase of Crassostrea gigas in both farming techniques, it rose until June (594.3±72.80 mg/d in floating poches and 786.09±97.67 mg/d in submerged ones). Furthermore, in floating poches a TW/d reduction was evidenced in mid- July (530.17±52.40 mg/d) and subsequently an increase was registered at the end of the same month. Probably this can be consequent to a temperature rise at the end of June (28.14°C in June and 23.91°C in July). It was also scored an increase in weight more constant in submerged poches than in floating ones. Oysters grown in submerged poches reached the commercial size within a short time.

C-085

Mineral contents in ass milk

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The added value of ass milk is related to its characteristics such a high digestibility and nutritional value, as well as its physiological properties. Ass milk is similar to human milk and it is a good alternative for feeding infants affected by cow's milk protein allergy. To our knowledge, there are few information on the minerals of ass milk and no data about their repartition between the soluble and colloidal phases. In order to gain knowledge in these concerns, 64 milk samples were collected from 9 Ragusano asses, during a period of 8 months. Asses were reared in the same herd, under the same management practices. On each milk sample, the total and soluble contents of calcium (Ca), magnesium (Mg), sodium (Na) and potassium (K) were determined with AAS. The contents of total and soluble phosphorus (P) were assessed by colorimetric method. Soluble fractions of minerals were obtained by ultrafiltration (cut off 30,000 Da). Colloidal contents were calculated as difference between total content and soluble content. Average (±standard deviation) contents (mg/100g of milk) of total Ca, P, Mg, Na and K were 56.04 (±22.84), 44.41 (±11.04), 6.26 (±1.29), 44.02 (±14.49) and 112.01 (±20.06), respectively. The values of Na/K and Ca/P were 0.41 (±0.16) and 1.23 (±0.32), respectively. Colloidal contents (mg/100g of milk) of Ca (39.92±17.99), P (28.88±9.61), and Mg (1.93±0.72) showed a high variability and ranged from a minimum of 5.71, 7.81 and 0.05 to a maximum of 60.78, 77.86 and 3.99, respectively. This average values resulted low if compared to the same parameters of cow milk (47.98, 78.00 and 4.37 mg/100g, respectively). The soluble fraction of Ca, P and Mg, relative to their total contents, resulted 30.69%±9.63%, 36.17%±10.61% and 69.09%±10.78%, respectively and were similar, lower and higher if compared to the same values in cow milk (30.31%, 49.58%, and 64.73%, respectively).

C-086

Prediction of calcium, phosphorus and titratable acidity, and relationships with coagulation traits and chemical composition of bovine milk

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Calcium (Ca) and phosphorus (P) content of milk influences the cheese-making process as these minerals have a significant impact on the coagulation process, particularly on the final consistency of the coagulum. Titratable acidity (TA) is another crucial technological trait as it plays a key role in the aggregation rate of para-casein micelles and in the reactivity of rennet. Milk coagulation properties (MCP), which can be measured as rennet coagulation time (RCT, min), curd-firming time (k20, min) and curd firmness (a30, mm), are important to determine the aptitude of the raw material to be transformed in cheese. The present work aimed at investigating the effectiveness of mid-infrared (MIR) spectroscopy to predict Ca, P and TA, and to assess the relationships of these traits with MCP and milk chemical composition (fat, protein and casein contents). A total of 208 individual milk samples were collected from Holstein cows in 13 dairy herds between January and March 2011. Chemical composition, MCP, TA, and Ca and P contents were assessed by reference methods. Mid-infrared prediction models were developed through partial least squares regression analysis using untreated spectral information and validated by cross-validation and external validation procedures. Calcium, P and TA averaged 1156±131 mg/kg, 934±110 mg/kg and 3.41±0.34 SH°/50 mL. Models developed using MIR spectroscopy were satisfactory in predicting minerals and TA, with coefficients of correlation of cross-validation and external validation from 0.70 to 0.78, 0.82 to 0.89, and 0.84 to 0.90, for Ca, P and TA, respectively. Moderate and statistically significant ($P<0.05$) Pearson's correlation coefficients were estimated between minerals and fat (0.27), protein (0.53) and casein (0.47) contents. Calcium content was favorably related to RCT (-0.16), k20 (-0.35) and a30 (0.29). The correlation between P and TA was favorable and moderately high (0.54). Results pointed out the potential of MIR spectroscopy to predict TA and Ca and P contents of bovine milk, and highlighted moderate but favorable relationships of these traits with MCP and chemical parameters.

C-087

Salt equilibria and milk rennet coagulation aptitude

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The most important requisite for milk processed in most cheese typologies is its rennet coagulation aptitude. Milk rennet coagulation ability is commonly assessed by lactodynamography (LDG) method, from which rennet coagulation parameters (RCP) - clotting time, curd firming time and curd firmness - are obtained. Milk with proper RCP gives rise to a curd with better rheological properties (syneresis). Several genetic and environmental factors were found to influence RCP. However, the causes of abnormal coagulation of milk are not fully understood. The aim of this study is to assess an eventual relationships between salt equilibria and RCP, in herd milk samples. The research was carried out on 81 herd milk samples, collected at the end of morning milking, from 81 dairy herds that produce milk for Parmigiano-Reggiano cheese. Milk samples were classified as Optimal, Discrete, Poor and Non-coagulating (NC), according to their LDG profile; their somatic cell content was lower than 400,000 cells/mL. On each sample, total contents of calcium (Ca) and magnesium (Mg) were determined by AAS. Total phosphorus (P) was assessed by colorimetric method. Ca, P and Mg soluble fractions were obtained by ultrafiltration (cut off 30,000 Da). Colloidal fractions were obtained as the difference between total and soluble. Casein was determined by Kjeldahl method. Data were analysed with ANOVA univariate, considering as fixed factor the LDG class. Optimal milk showed the highest ($P<0.05$) values of colloidal Ca, colloidal P and colloidal Mg as mg/100 g of milk (92.30±9.43, 54.93±5.13 and 2.98±0.77) and relative % to total Ca, total P and total Mg (71.15±1.15, 51.56±1.69 and 28.19±0.77). No difference were observed among other milk classes for the same parameters, which resulted in the ranges 74.97÷75.82, 43.24÷44.79 and 2.13÷2.25 mg/100 g of milk and 66.55÷67.31, 48.19÷49.00 and 22.65÷23.63%. Interestingly, the % of colloidal P in NC milk (51.81) was not different ($P>0.05$) than in Optimal milk. The casein micelle of Optimal milk was the richest in Ca, P and Mg (3.75±0.38, 2.23±0.21 and 0.12±0.03 g/100 g of casein) and even for these parameters, no differences were observed in other milk classes, which values were in the ranges 3.06÷3.08, 1.76÷1.82 and 0.09, respectively.

C-088

Genetic analysis of milk clotting aptitude in the Italian Holstein-Friesian

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The coagulation of milk is based on a series of physicochemical changes at the level of the casein micelle resulting in a gel formation. Milk coagulation properties (MCP) are known to be relevant for cheese quality and yield, important factors for the economic outcome of the dairy industry. In some instances, the MCP of herd bulk milk used to produce Protected Designation of Origin cheeses are periodically analyzed and the data used to reward or penalize producers. The economic importance of improving milk rennet clotting aptitude fully justifies the wish of taking this trait into account in the selection process. Thus, a pilot study was carried out on the possibility of including coagulation traits in the selection schemes of a dairy cattle breed. Milk clotting aptitude was evaluated in the Italian Holstein-Friesian by means of Fourier Transform mid-Infrared (FT-IR) spectroscopy on 1044 individual milk samples collected in 16 herds of Brescia province from half-daughters of 52 sires. Milk proteins were genotyped by isoelectric focusing and casein haplotypes (αS1-CN, β-CN, αS2-CN, and κ-CN) of the daughters, sires and dams were reconstructed. Data were analysed by an animal model in order to estimate: i. the heritability of milk rennet coagulation time (RCT, min), curd firming rate (k20, min), and curd firmness (a30, mm); ii. the breeding values (EBV) for these clotting variables; iii. the effect estimated of milk protein genotypes and casein haplotypes on the clotting aptitude of milk. Heritability of RCT (0.28) and k20 (0.30) were close to the average heritability described in literature (0.26 and 0.36 respectively), whereas the heritability of a30 was found to be higher (0.46 against an average value 0.27). The animal model was used to calculate the EBV for the milk rennet coagulation traits. The effects of β-lactoglobulin genotype and casein haplotypes on the EBV were tested resulting statistically significant, as expected from the wide literature available on the subject. On the basis of the obtained results, the coagulation traits can be proposed as interesting phenotypes to be considered for addressing the selection strategies of the breed.

C-089

Effect of somatic cell count on coagulation properties, cheese yield and nutrients recovery of individual milk of Brown Swiss cows

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The somatic cell count (SCC) is generally considered an indicator of udder health and technological quality of milk. The aim of this research was to investigate the effect of SCC on some technological traits: milk coagulation properties (MCP), cheese yield (CY) and nutrient/energy recoveries (REC) in the curd. A total of 1264 Brown Swiss cows, reared in 85 herds, located in Trento province, were milk sampled once. Individual milk samples were collected during the evening milking and analyzed for MCP (rennet coagulation time, curd-firming time and curd firmness at 30 and 45 min). Individual model cheese-manufacturing (1500 mL per sample) process was developed to assess CYs (weight of curd-CURD, total solids or water/weight of milk-WATER) and RECs (weight of constituent in cheese/weight of constituent in milk). All traits were analyzed through ANOVA considering the effects of vat (or measuring unit of the coagulation meter for MCP), herd-test day, parity, days in milk, milk yield and somatic cell score (SCS). Overall, it was observed better technological traits in milk samples with lower values of SCS (less than 3.45 units; $P<0.05$). Considering the MCPs, higher SCS (more than 3.45 units) values highlighted worse coagulation ability showing late coagulation- and firming-time, and poor curd firmness at 30 min whereas for a45 the effect was negligible. Results from micro cheese-making exhibited lower CYCURD for group of cows with high SCS. High milk SCS values also caused higher protein loss in the whey producing lower CYWATER whereas the effect of SCS on fatREC was trivial. Results confirmed the tight relationship between traits related to udder health and milk technological traits.

C-090

Validation of RP-HPLC method for separation and quantification of whey protein in dairy industry by-products

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In recent years dairy industries increased their interest in functional and nutritional properties of whey proteins (WPs), whose usage is not limited to food area, but is also extended to pharmaceutical and cosmetic industry. WPs include α-lactoalbumin, β-lactoglobulin A and B, bovine serum albumin, lactoferrin, several caseinomacropeptides and many others. Membrane processing has been improved to obtain whey protein concentrate (WPC), limiting the problem of whey disposal. In particular, the denaturation of WPC, which generates microparticulated whey proteins (MWPs), improves the rheological properties of WP derived products. Therefore, there is an increasing need for a quick, cheap and easy method to verify and quantify the composition of WP, WPC and MWP extracts. This study aims at validating a Reverse Phase-HPLC (RP-HPLC) protocol for the characterisation and quantification of WPs, WPC and MWPs provided by a local dairy industry. WP solubilisation was performed by incubating the extract in different buffers for different incubation times. Guanidine•HCl (6 M) overnight was used for further investigations. RP-HPLC analysis were conducted on C8 column and measured with UVB detector, in a total time course of 9 minutes. Standards at different concentrations were used to calibrate the chromatographic system. Repeatability test has been performed by quantifying the same sample in ten technical replicates, while reproducibility across days was performed using ten samples, in two replicates, analysed for four sequential days. Peak areas and retention times were used to validate repeatability and reproducibility, calculating the relative standard deviation (RSD) within and across days, respectively. Calibration results were determined by estimating parameters of the linear regression of the peak area and the injected amount, and show an $R^2 > 0.99$ for all protein fractions. Retention times were stable, with RSD between 0.03 and 0.80 within days, and between 0.18 and 0.69 across days. Repeatability for area ranged from 0.54 to 7.90, while reproducibility from 0.25 to 8.48 depending on the considered protein and its relative abundance. This study shows a quick, simply, high-throughput and high-resolution method for the characterisation and quantification of WPs, WPC and MWPs.

C-091

Evaluation of the relationship between mastitis problems and subjective judgments of facilities and animal cleanliness in dairy farms

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Mastitis is the most common and costly disease in the dairy industry impairing cows' health and welfare and their milk production. It was aim of this study to evaluate whether subjective judgments that are performed to assess the facilities and dairy cows' cleanliness may be useful to identify predisposing factors for mastitis. Two assessments were carried out by a field veterinarian, one during winter and one during summer, on 27 dairy farms in the Veneto region, Italy. After gathering data regarding the farm (size, production, feed, litter renewal frequency, etc.) from an interview to the farmer, the veterinarian evaluated directly the facilities and the animals. Characteristics and cleanliness of the facilities, and farmers' care for them were subjectively scored on a 125 mm long line where 0 was poor and 125 was excellent. Cleanliness of udders and hind legs of each animal were evaluated as binary (clean/dirty) measures. Results showed that the judgments of the veterinarian could be predictive of the management quality of the farm and could identify problems in the herd that negatively affect productivity and milk quality. In particular, judgments of the quality of lying area, drinkers and milking conditions correlated positively with the milk production level ($r=0.62$, $r=0.48$, $r=0.50$ respectively; $P<0.05$) and negatively with the bacterial load of the milk ($r=-0.68$, $r=-0.58$, $r=-0.56$ respectively; $P<0.05$). The judgment of animal cleanliness correlated positively with the production levels ($r=0.68$; $P<0.05$) and the judgments of the lying area ($r=0.80$; $P<0.05$) and negatively with the milk bacterial load ($r=-0.54$; $P<0.05$). Results pointed out how this simple method could be helpful to field veterinarians and farmers to identify weak points and where on-farm improvements are needed, and to track whether actions taken have resulted in benefits. In the visited farms it indeed allowed to identify, among main risk factors for impaired udder health, poor cleanliness and maintenance levels of the passageways and lying areas, insufficient drinkers' availability, poor hygiene during milking and improper milking technique.

C-092

Milk yield and quality of dairy and dual purpose cows reared in single breed and mixed breeds farms in mountain

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Dairy farms of mountainous areas are characterized by low productivity, but generally the milk is used for the production of high quality and PDO cheeses. For this reason, the initial disadvantage can become an opportunity for the traditional dairy systems. This study was conducted on 473 dairy farms of the Trento province with the aim to verify the effect of farm management (environment) on the variability of the milk yield and quality due to the breed effect (genotype). In particular, the differences between breeds were estimated in mixed farms with different management and feeding strategies. For each farm we collected the following data: number of cow per breed, housing condition (free *vs* tie stalls), diet administration (Total Mixed Rations *vs* separate feeding) and quality (percentage of starch, NDF and Crude Protein). Milk production and quality data were collected from test day recording and Consortium of Cooperative Dairies of the Trento Province (CONCAST), for a total of 110,711 milk analysis. A multiple regression analysis was performed to estimate the genetic contribution corrected for the structural and nutritional sources of variation. At national level, Brown Swiss (BS), Italian Simmental (IS) and two northeastern autochthonous breeds (Rendena – Re – and Alpine Grey – AG) produce much less milk than Italian Friesian (IF): -23.4%, -28.5%, -45.1% and -45.3%, respectively. These differences reflect both genetic and environmental effects on milk production. The estimates obtained in mixed breed farms of Trento Province, after having taken into account the main structural (herd size, housing condition) and nutritional (TMR, use of silages, NDF%, CP%) sources of variation, shows further lower differences among breeds (BS -13.0%, IS -13.4%, Re -30.6% and AG -30.7%, respect to IF), that reflect more the genetic contribution to phenotype. Taking into account the milk composition, the differences among breeds in terms of daily fat + protein yield (BS -6.7%, IS -10.7%, Re -33.4%, and AG -29.9%, respect to IF) are even smaller than for milk yield. In conclusion, farm management significantly affects milk yield and quality in mountainous dairy farms and it can strongly reduce the estimates of the differences among the breed of cows.

C-093

Milking technique in jenny: effects on milk production

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Jennies bred for milk production require foal suckling during lactation period in order to maintain the milk let down reflex. Foals were raised with the mother and separated some hours before milking. Aim of this study was to verify the effect of the length of foal separation time (FST) on donkey milk production. Eight lactating jennies, four of Martina Franca and four of Ragusana breeds, were milked once weekly from day 20 to day 220 of lactation; milking was performed by a machine milking and residual milk was dripped by hand. Foals were separated from the mothers early in the morning and milking was performed at different FSTs (from1.4h to 9.5h). Milk yield was recorded and milk samples were collected every milking from each jenny to assess its chemical composition. Multiple regression analysis was used to quantify the relationships among FST, milk yield, dripping milk yield, milk quality and days in milk. Anova with mixed model was used to analyse differences between productive groups (high *vs* low) and FST groups (Group 1=4.0±0.5 h *vs* Group 2=6.0±0.9 h). Regression equations were different according to different productive levels, however a linear increase of milk was found in both groups. After a mean value of 4±0.5 hours of FST, milk production was similar in both productive groups while significant differences in milk yield were found at 6±0.9h. The mean milk yield increase was about 50% of the milk produced at 4.0±0.5 h. A progressive decrease of dripping milk expressed as percentage of the total milk produced/milking was found for longer FST. Milk fat was negatively affected by FST and milk production, while protein did not vary. The lactation curve showed a reduction of milk yield at 220 days in milk of only 26% of the initial milk production. The farmer choice of one or more milking per day could be regarded in light of the milk yield level of the jennies, taking into consideration milking costs and milk price.

C-094

Effects of s1-casein and κ-casein genotypes on nutrient intake and milk production in Girgentana goats: a further investigation

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The influence of polymorphism at αs1-casein *locus* (CSN1S1) on goat milk properties is adequately known, whereas the knowledge is limited on the effects of κ-casein gene variants (CSN3), and the association between variants of CSN1S1 and CSN3 genes. This study aimed to investigate the effect of some CSN1S1-CSN3 composite genotypes (CG) on feed intake and milk production of 95 Girgentana goats from 6 experiments (551 observations). Goats, genotyped using specific PCR protocols at DNA level and IEF technique at milk protein level, were equal at αs2-casein and β-casein *loci*, and belonged to one of 6 CG derived from the association of strong (A) and weak (F) alleles for αs1-casein with A-IEF and B-IEF for κ-casein: AA-A (23 goats), AA-AB (7goats), AA-B (8 goats), AF-A (31 goats), AF-AB (18 goats), FF-A (8 goats). Data were analysed using a mixed model for repeated observations. No CG effect emerged for dry matter, crude protein, NDF and net energy intake, and milk yield of goats. However, CG affected milk casein content ($P<0.001$), which was the highest in AA-B genotype (3.25%), and the lowest in FF-A genotype (2.64%). Thus, FF-A goats showed the lowest efficiency of dietary N utilization for milk casein synthesis (118 g casein N/kg N intake), which was lower by more 50 g/kg than those of other genotypes (P>0.001). In goats with A-IEF for κ-casein, the strong A allele at CSN1S1 *loci* was associated to an increasing (P>0.05) in milk clotting parameters (clotting time, r: 11.0, 11.9, 13.5 min; curd firming time, k20: 1.69, 1.93, 2.38 min; curd firmness, a30: 34.7, 42.2, 44.0 mm, for FF-A, AF-A, AA-A). Moreover, in AA goats for αs1-casein, κ-casein alleles referred to B-IEF were responsible of a decreasing trend (P>0.05) in clotting time and curd firming time, and a further increase (P>0.01) in curd firmness (r: 13.5, 11.8, 10.3 min; k20: 2.38, 1.53, 1.50 min; a30: 44.0, 48.6, 51.6 mm, for AA-A, AA-AB, AA-B). The strong alleles (A) at CSN1S1 and B-IEF at CSN3 seem to improve synergistically casein synthesis and curd consistency (a30), whereas they show an opposite effect on curd formation rate (r and k20).

C-095

Comparison among different FT-MIR spectra treatments for the prediction of coagulation properties of individual milk of Brown Swiss cows

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Individual milk samples of Brown Swiss cows (n=1267), were collected in 85 herds and analysed for milk coagulation properties (MCP). Two mid-infrared spectra (MIR, 925-5011 cm^{-1}) for each sample were collected and then averaged and stored as log(1/Transmittance). The aim of this study was to investigate the effect of different spectra treatments on MCP prediction reliability (rennet coagulation time-RCT, min; curd firming time-k20, min; curd firmness at 30 and 45 minutes-a30, a45, mm). Models were developed using partial least squares regression (PLS) and modified PLS (MPLS) on untreated and pretreated spectra. Treatments of spectra involved: first and second derivative (D1 and D2 respectively), standard normal variate and detrend (SNV), multiplicative scatter correction (MSC) and combined spectral ranges (CW) of 925 to 1582 cm^{-1}, 1701 to 3048 cm^{-1} and 3673 to 5011 cm^{-1}. The best calibrations, using the coefficient of determination of cross-validation (1-VR) as indicator of effectiveness, were obtained with MPLS and some combinations of CW and D1, CW and MSC and CW only for RCT, k20 and a30 respectively. The best results in predicting a45 were obtained with PLS and CW. The lowest values of standard error of cross-validation (SECV) were obtained with some combinations of PLS and CW for RCT, MPLS and MSC for k20, MPLS and CW for a30 and MPLS only for a45. It is concluded that different traits are better predicted by different models of calibration and that, among MCP, RCT is better predicted (1-VR around 70%) than the others parameters (1-VR range of 40 to 54%), suggesting that this kind of methodology needs further study for a routine analysis application.

C-096

Prediction of coagulating and noncoagulating milk samples using mid-infrared spectroscopy

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Over the last years, a general deterioration of milk coagulation properties (MCP) has been observed in Italy and thus the prediction of noncoagulating (NC) milk, defined as milk not forming a curd within 30 min from rennet addition, is of immediate interest in Italian cheese industry. Aim of this study was to investigate the ability of mid-infrared (MIR) spectroscopy to predict NC milk using individual and bulk samples from Holstein cows. Samples were selected according to MIR analysis to cover the range of coagulation time between 5 and 60 min. The MIR prediction models were used for routine analysis of MCP on samples collected during monthly test-day milk in the laboratory of the Breeders Association of Veneto region (ARAV, Padova, Italy). Milks were then analyzed for MCP through the reference instrument (Formagraph) over an extended testing-period of 60 min to identify coagulating and NC samples. Measured traits were rennet coagulation time (RCT), curd-firming time (k20), and curd firmness 30 (a30) and 60 (a60) min after rennet addition. Statistical analysis was carried out using principal component analysis and partial least squares regression analysis on untreated spectra. Results showed that there is no specific spectral information that distinguishes NC from coagulating samples. The most accurate prediction model was developed for RCT, followed by k20 and a30. Coefficient of determination of cross-validation and standard error of cross-validation were 0.76 and 7.05 min, 0.72 and 3.54 min, and 0.70 and 7.68 mm for RCT, k20, and a30, respectively. Prediction models for a60 showed unsatisfactory coefficient of determination of cross-validation (0.42) and standard error of cross-validation (7.26 mm). Mid-infrared spectroscopy might be proposed in payment systems to reward or penalize milk according to MCP. The ability of MIR spectroscopy to predict MCP of samples that form a curd beyond 30 min from enzyme addition may be of interest for genetic improvement of coagulation traits in dairy breeds as until now most studies have excluded NC information from genetic analysis, leading to possible biases in the results and in the prediction of sires' breeding values.

C-097

Effect of the extent of digestion of dry matter and fiber on fecal particle distribution in cows feces

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Particle size reduction or comminution is a process generated as an effect of chewing, rumination, fermentation, digestion and friction between particles, and is continuous untill fecal excretion. Dry matter (DM) and fiber (NDF) intake have been demonstrated to affect both time dedicated to rumination and passage rate. Together with the specific digestibility of the substrates, passage rate deeply affects the extent of digestion of the substrates themselves potentially affecting fecal particle size distribution. The aim of the present work was to study the effect of the extent of dry matter and fiber digestion on fecal particle distribution in cow feces. A Latin Square design trial was carried out using 4 dry cows over a period of 1 month. Animals were fed 4 different levels (6, 8, 10 or 12 kg) of mixed hay only, to obtain variations in *in vivo* apparent Dry Matter Digestibility (DMD) and NDF Digestibility (NDFD) of the diet which were determined for each animal during the whole period using the total fecal collection method. Representative samples of daily fecal output were collected and wet sieved under water flow on a battery of 5 sieves (pores of S1:4.6, S2:2.36, S3:1.18, S4:0.6 and S5:0.15 mm) to measure particle distribution. Regressions of the proportion of residue content of each sieve on DMD and NDFD were conducted. Apparent *in vivo* DMD was 61.37, 65.26, 71.02 and 75.91% of DM while NDFD resulted of 51.84, 57.14, 63.71 and 69.90% of NDF respectively at 6, 8, 10 and 12 kg of hay intake. Significative trends for a reduction of S2 NDF residue ($r=-0.160$; $P<0.05$) and for an increase of S3 NDF residue ($r=0.188$; $P<0.05$) were observed. No significative effect of apparent *in vivo* DMD was observed on DM residues proportion in the sieves. Based on these results, it appears that the increase of *in vivo* apparent NDFD is associated with a slight increase in the presence of particles having size greater than 1.18 mm and smaller than 2.36 mm.

C-098

Evaluation of the neutral detergent fibre indigestible fraction in forages

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Several factors are known to influence forage chemical composition such latitude, climatic conditions, temperature and rainfall. In particular, above all, lignin is probably the most important from a nutritional point of view. Biologically, lignin is built up to both increase the plant structure, and defensive level against external pathogens or dangerous factors. For feed scientists is common to consider lignin as expression of the indigestible part of fiber (iNDF). Ability of this compound is to embed the other cell wall components, such as cellulose or hemicellulose, avoiding bacterial access to their fermentation. In CNCPS based models, the iNDF fraction is calculated as ADL * 2.4 for all feedstuff. Such equation is also used in kD calculation. This work wanted to evaluate the iNDF as the result of long time fermentations (240h), considered as the maximum extend of fiber digestibility, to then determine the multiplication factor. Moreover, an estimation was made among different forage families: alfalfa hay, grass hay and corn silage. Briefly, each 240h fermentation was carried out following the Tilley and Terry modified technique, with inoculation of buffer and rumen fluid every 72h. A total of 418 samples was processed. At the end of the 240h each sample was washed with ND solution to determine the residue. iNDF values obtained were divided by the ADL content to evaluate the multiplication factor, and statistically, by a one way Anova, paired with the 2.4 common value; averages of the observed results were: 2.36 for alfalfa hay; 3.19 for corn silages and 3.34 for grass hay, statistically different ($P<0.05$) than the 2.4 fixed value. In conclusion, value of 2.4 could be useful for alfalfa samples, but must be changed for other forage families, such grass hay and silages.

C-099

Reactivity of growing rabbits under different housing systems

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To evaluate the effect of housing system (bicellular cages *vs* collective pens) and, within collective systems, of pen size and stocking density on animal reactivity, a total of 456 rabbits was kept in pairs in bicellular cages or in large groups (20 to 54 animals) in collective cages of different sizes (small *vs* large) with wooden slatted floor and at two stocking densities (12 *vs* 16 rabbits/m2). The effects of the rearing system were assessed on rabbit reactivity to humans (tonic immobility test at 55 and 72 days of age) and against a new environment (open field test at 56 and 75 days of age). The reactivity of rabbits at the tonic immobility test was not affected by the age of the animals at the test, whereas at the open field test the younger rabbits spent less time in biting walls and edges of the enclosure (2.58 *vs* 6.33 sec; P<0.001) and showed a higher number of jumps (0.25 *vs* 0.01; P<0.01) and alerts (0.29 *vs* 0.05; P<0.01) than the older rabbits. With reference to the effect of the housing system, the percentage of rabbits that did not fall in immobility tended to be lower (6.2 *vs* 23.5%; P=0.09) and the amount of rabbits that rested in tonic immobility from 1 to 179 second tended to be higher (90 *vs* 73%; P=0.09) in the rabbits kept in bicellular cages than in those housed in collective cages. At the open field test, the rabbits reared in bicellular cages crossed more squares (47.5 *vs* 31.2, P<0.01) and moved more (58.3 *vs* 40.4 sec, P<0.01) than the rabbits in collective cages, whereas they stand in a vigilance position for a shorter time (38.7 *vs* 84.6 sec, P=0.04). Neither the cage size nor the stocking density in the collective pens significantly affected rabbit reactivity. In conclusion, the rabbits housed in bicellular cages showed a higher fear level against man but were more prone to exploration in a new environment in comparison with group-housed rabbits.

C-100

Effect of a differentiated access to water along 4 weeks post weaning on rabbit caecal fermentation

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A total of 1388 weaned rabbits (35 days old) were homogeneously divided into 2 groups based on body weight and sex. The groups were fed *ad libitum* the same diets both in the post-weaning (35-60 days) and fattening (61-84 days) periods. In the post-weaning period, *ad libitum* group (AL) also received drinking water *ad libitum*, while the water restricted group (WR) had restricted access to drinking water according to the following program: from 35 to 41 days of age two hours (9:00 – 11:00 a.m.) per day; from 42 to 48 days 2.5 h/day (9:00 – 11.30 a.m.); from 49 to 55 days 3 h/day (9:00 – 12 a.m.); from 56 to 60 days 4 h/day (9:00 a.m. – 01:00 p.m.). During the fattening period both groups had free access to drinking water. At 62 days of age, 15 rabbits per group were slaughtered and volatile fatty acids and ammonia levels of caecal contents were measured. WR group showed a higher (P<0.01) total volatile fatty acid production (tVFA) than AL group (118.5 *vs* 101.7 mmol/l) and also a higher proportion of acetate (73.7 *vs* 71.3% tVFA, P<0.05) and a lower production of butyrate (14.6 *vs* 16.5% tVFA, P<0.05), indicating a different fermentative activity of caecal microflora between groups: caecal content from WR rabbits had a more intense fermentative activity, in particular on structural carbohydrates, while AL group seem to have a higher fermentation of non structural carbohydrates. The branched chain proportion (the sum of isobutiric and isovaleric acids divided by tVFA), a useful index to evaluate the protein degradation activity of caecal bacteria, was higher (P<0.01) in WR than in AL group (0.027 *vs* 0.025), while ammonia content was higher (P<0.05) in AL than in WR group (13.76 *vs* 10.59 mmol/l). This indicates a more intense activity of caecal microflora from WR rabbits on protein degradation, and also a better synchronism between carbohydrate and protein fermentation than AL group. The body weight at slaughter was higher (P<0.05) for AL than WR group (2636 *vs* 2568 g); however, also considering the lower (P<0.01) mortality rate in the WR group in the period 35-60 days (5.2 *vs* 10.1%), water restriction can be considered as a useful way to improve the sanitary status of the rabbit digestive tract.

C-101

Effect of increasing soluble fibre and starch and reducing acid detergent fibre in diets at different protein level for growing rabbits

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From weaning (at 36 d of age) until slaughter (at 79 d), 282 commercial crossbred rabbits (951±31 g) were assigned to six experimental groups and fed *ad libitum* six diets formulated according to a bi-factorial arrangement with three (soluble fibre+starch)-to-ADF ratios (0.6, 1.2 and 2.0) and two protein levels (15.0% and 17.0%). The dietary soluble fibre (5.0%, 7.0% to 9.0%) and starch (10.0%, 14.0% to 18.0%) levels jointly increased at the expense of acid detergent fibre (ADF) (22.0%, 18.0% to 14.0%). The growth performance, and the *in vivo* nutritive value of diets were recorded. The caecal fermentation traits were measured on 36 rabbits slaughtered at 45 d of age. When the (soluble fibre+starch)-to-

ADF ratio raised, daily growth linearly increased (P=0.05) and feed intake decreased (P<0.001) and, thus, feed conversion improved (P<0.001); the digestibility of dry matter (50.8 to 71.5%), gross energy (50.3 to 70.7%) and fibre fractions linearly increased (P<0.001); at caecum, the concentration of total volatile fatty acids (VFA) raised (63.0 to 78.5 mmol/L; P=0.02), whereas the proportion of propionate decreased (4.0 to 3.5 mol/100 mol VFA; P=0.04). When the dietary protein increased, feed conversion improved (3.47 to 3.37; P<0.001) and the digestibility of dry matter increased; (60.3% to 61.6%; P<0.001) at caecum, the N ammonia level tended to decrease (P=0.07), whereas total VFA production and VFA molar proportions were not affected. The increase of the (soluble fibre+starch)-to-ADF ratio reduced the mortality rate (15.8%, 4.5% and 4.9%; P=0.02), whereas the dietary protein level did not affect rabbit health. In conclusion, the contemporary increase of dietary soluble fibre and starch at the expense of ADF increased digestive feed utilization and growth performance, and enhanced fermentation activity at caecum and health status. The increase of dietary protein moderately improved productive results and did not affect health status.

C-102

Effects of two different concentration of dimethylsulfoxide on the quality of turkey semen cryopreserved in straws over liquid nitrogen vapor

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The poultry semen cryopreservation is important both for the value of this technique as fundamental to the practice of artificial insemination, and as an essential tool for the conservation of genetic resources in cryobanks. The choice of the cryoprotectant (CPA) and its concentration is certainly among the most important factor involved in the cryopreservation process. The packaging of frozen semen in straws would optimize the cryopreservation process by a better identification and safety of frozen semen doses useful particularly in cryobanking. Therefore the aim of this study was to evaluate the effect of two different concentration of dimethylsulfoxide (DMSO) as CPA on the post-thaw quality of turkey semen cryopreserved in straws above the liquid nitrogen vapor. Seven pools of semen (9-12 ejaculates/pool) were collected from Hybrid Large White toms, an aliquot from each pool was taken for the analysis of fresh semen, the remaining part of pooled semen was cooled at 5°C for 25 minutes. Each pool was divided into 2 semen samples that were diluted 1:1 (v:v) with the freezing medium composed by Tselutin extender containing DMSO (final concentration of 4% or 10% of DMSO). Thus the semen diluted was aspirated into 0,25 mL plastic straws, equilibrated at 5°C for 20 min, frozen by exposure to liquid nitrogen vapour and then plunging into liquid nitrogen for storage (-196°C). The samples were thawed at 50°C for 10 seconds. Sperm mobility (phase contrast microscopy), viability and osmotic-resistance (SyBr-PI staining) were examined on fresh and post-thawed spermatozoa. The results obtained showed that the cryopreservation impaired the post-thaw quality of turkey spermatozoa respect to fresh semen. However the quality the frozen semen was affected differently in relation to the DMSO concentration. In fact, the cryopreserved semen quality was better with the concentration of 10% of DMSO, compared to the 4%, however only the motility and viability resulted significantly higher (36.92±1.69 *vs* 21.92±2.10; 42.09±1.50 *vs* 33.48±2.41; P≤0.05) respectively. In conclusion, these data shows clearly that the higher concentration of penetrating CPAs protect better from cryopreservation damages.

C-103

Magnitude of emerging meat quality defects in breast fillets of different commercial meat-type chicken hybrids

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Selection for fast-growing and increasing breast meat yield may negatively impact muscle structure and meat quality traits. In the last years, two emerging meat defects have been arisen in breast fillets of fast-growing chicken hybrids: white striping characterized by white parallel striations in the direction of the muscle fibers and poor cohesion of muscle structure with fibers bundles which can be easily pulled away by fingers. A trial was conducted to compare productive performances, cut up yields and incidence of white striping and poor cohesion (absence=normal, presence classified in 2 levels as moderate and severe) in four of the most representative commercial chicken hybrids which were classified as either standard (SBY1 and SBY2) or high (HBY1 and HBY2) breast-yield. A total of 2,160 1-day-old male chicks were equally divided into four experimental groups of 9 replications each, raised in the same experimental conditions and fed the same commercial diets. Birds were slaughtered at about 3.6 kg live weight reached at 48 (SBY1 and HBY2) and 51 d of age (SBY2 and HBY1), respectively. Both HBY1 and HBY2 had a lower daily feed intake if compared with SBY1 and SBY2 (120 and 124 *vs* 131 and 130 g/day, P<0.01). On the other hand, feed conversion rate was not significantly modified. As expected, HBY1 and HBY2 had higher breast yields than both SBY birds (30.9 and 31.5 *vs* 28.6 and 28.5%). The total occurrence (moderate and severe) of white striping in all the genotypes was remarkably high (>87%). However, HBY birds had an incidence of about 96% white striped fillets, while its occurrence in SBY groups did not exceed 90%. The overall incidence (moderate and severe) of muscle structure with poor cohesion also resulted extremely high (>90%). Indeed, only 10% of breast fillets from SBY1 birds resulted unaffected by this defect, while 40% of breast fillets from HBY2 showed poor cohesion with severe degree. In conclusion, from this study emerged that all the modern chicken hybrids here tested are affected by a high incidence of breast muscle defects which are particularly pronounced in high breast-yield birds.

C-104

Effect of white striping on quality traits of raw and marinated chicken breast meat

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The appearance of white striping or striations parallel to muscle fibres on the surface of chicken breast fillets (pectoralis major muscles) is a new emerging poultry meat quality issue. It has been already demonstrated that white striping noticeably decreased consumer acceptance of pre-packaged breast meat, however few information are still available on its impact on technological traits of the meat. The purpose of this study was to characterize raw and marinated meat quality properties of fillets showing different degrees of white striping. To pursue this aim, 153 fillets were selected based on white striping degree (normal, moderate and severe) after the breast-deboning area in a commercial processing plant. Samples were used to evaluate ultimate pH, colour, drip loss, cook loss and AK-shear force on non-marinated meat as well to determine marinade uptake, purge loss, cook loss, total yield and AK-shear force after marination with a solution containing sodium tripolyphosphate and sodium chloride. The ultimate pH of severe white striped breast fillets was significantly higher than in normal and moderate groups (5.95 *vs* 5.86 and 5.88; $P<0.01$). There were no differences in the lightness (L*) of meat, but moderate and severe samples showed a significant ($P<0.01$) increase in redness (a*) and yellowness (b*). As for non-marinated meat, cook losses increased as the degree of white striping increased from normal to severe groups (21.27 *vs* 23.20 *vs* 26.74%; $P<0.01$). Moreover, the severe white striping resulted in significantly ($P<0.01$) lower shear values if compared with moderate and normal groups. With regard to marinated meat, there was a decrease in the marinade uptake as the degree of white striping increased from normal to severe (12.67 *vs* 10.97 *vs* 7.92%; $P<0.01$). In addition, severe group had higher ($P<0.01$) purge loss, cook loss and lower total yield if compared with moderate and normal groups. Finally, severe white striped fillets had lower AK-shear force values. In conclusion, this study evidenced that white striping had a remarkable detrimental effect on breast meat quality attributes by mainly reducing ability of the meat to hold and bind liquids during processing and storage.

C-105

Effect of dietary thymol supplementation on lipid oxidation of chicken legs as related to storage conditions

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The aim of this research was to evaluate the effect of dietary thymol supplementation on lipid oxidation of chicken leg meat during refrigerated shelf-life. Chickens belonging to Ross 308 hybrid were raised under experimental conditions up to 3 kg of live weight, using three dietary treatments: control (without supplementation, C), treatment 1 (C+0.1% w/w thymol supplementation, T1) and treatment 2 (C+0.2% w/w thymol supplementation, T2). After slaughtering, the chicken legs with skin were stored under conventional (CON) and modified atmosphere (MAP) at temperature of 2-4°C for 14 days. Lipid oxidation was monitored by the determination of primary (peroxide value, PV) and secondary (thiobarbituric acid reactive substances, TBARs) products at 3, 7, 10 and 14 days of storage under both CON and MAP conditions and compared with values found on fresh meat. The three different dietary treatments did not significantly affect the lipid oxidation parameters. PV ranged between 0.5-13.0, 0.7-13.0 and 1.0-11.0 meq O_2/kg of lipid in poultry meat obtained with C, T1 and T2 diets, respectively. TBARs varied between 0.1-0.7, 0.1-0.6 and 0.2-0.5 mg MDA/kg of meat in poultry meat obtained with C, T1 and T2 diets, respectively. On the other hand, interaction effect of diets and storage conditions were significant ($P\leq0.05$) in PV formation, as it was delayed under MAP (maximum PV level after 2 and 5 days of storage in C and thymol-containing diets, respectively) with respect to conventional storage (PV apex after 2 days of storage). However, not significant differences ($P\geq0.05$) were found on TBARs level as related to storage conditions. In conclusion, this study demonstrated that dietary thymol supplementation coupled to MAP storage conditions delay lipid oxidation of chicken legs with skin, thus improving their shelf-life.

C-106

Effects of genotype and sex on carcass traits and meat quality in two Japanese quail generations

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Current research was conducted to compare carcass and meat quality traits (cholesterol content and collagen properties) of different lines and generations of adult Japanese quail. Forty-four quails (generation F0), 22 meat type males (F-33) and 22 laying type females (S-22), were reciprocally crossed to produce the F1 hybrids generation (24 males and 24 females). The birds (F0 and F1) were raised to 24 weeks of age (F0: 6 birds in each 6 cages and 4 birds in each 2 cages; F1: 6 birds in each 8 cages), and fed *ad libitum* the same commercial diet, according to their age, with free access to water. In the F0 generation, F-33 had higher ($P<0.01$) final body and carcass weights, carcass yield, and abdominal fat than S-22 quails. The muscle cholesterol content was found to be similar between the two lines. Compared with S-22 birds, those of F-33 had similar intramuscular collagen (IMC) amount (14.14 *vs* 13.22 μg/mg, respectively; SEM=0.61) but slightly higher degree of collagen maturation (0.121 *vs* 0.098 mol of HLP/mol of collagen, respectively; SEM=0.008; $P<0.07$) and hydroxylysylpyridinoline (HLP) concentration ($P<0.01$). In the F1 generation, females showed higher ($P<0.01$) live weight and slaughter performance than males; while, carcass yield and abdominal fat were higher ($P<0.01$) in males. Cholesterol content and IMC concentration were not significantly influenced by sex. Otherwise, males had higher ($P<0.01$) muscle HLP concentration (μg/mg) and collagen maturation (0.144 *vs* 0.119 mol of HLP/mol of collagen; SEM=0.008) than females. Compared to female of F0 generation, hybrid females showed better slaughter performance and a notably reduction of muscle cholesterol content (80.26 *vs* 67.05 mg/100g, respectively; SEM=1.66; $P< 0.01$), as well as a lower ($P<0.01$) IMC concentration (11.58 *vs* 13.22 μg/mg, respectively; SEM=0.28) and higher ($P<0.01$) degree of collagen maturation (+21%, HLP/collagen). The same trend was found in hybrid males compared to F0 males. Results suggest that carcass and meat quality traits of the two generations were influenced by gender; meat from descendants of Japanese quail lines' parents (F1) had lower cholesterol and higher degree of collagen maturation than those of F0 generation.

C-107

Soil degradation caused by animal grazing

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Grazing can influence the functionality of the ecosystem, the stability of environment and landscape. Unbalanced grazing activities (over-grazing) can produce the degradation of the vegetation as well as that of the soil with a negative impact on hydrogeologic setting. This study tested the variation of some soil quality indicators in function of animal density and species. Six experimental sites, with three different animal densities (high, medium and low) for each selected animal species (cattle and sheep), were chosen near the plain of Pisa. An undisturbed nearby site (never subjected to grazing activity) was also chosen as control soil. In each site, top soil (0-15 cm) samples were taken randomly from three homogeneous areas of 10 m^2 each. Five subsamples were collected in each area and thoroughly mixed to obtain a composite sample. On the soil samples, chemical (total organic carbon TOC and total nitrogen TN), biochemical (dehydrogenase and beta-glucosidase activities) and physical analyses (bulk density BD and cracking), were carried out. A clear worsening of physical characteristics of the soil, showed by the increase in BD ($P<0.05$; 30% in high-density disturbed soils) and decrease in total cracks ($P<0.05$; 38%), was found as a result of the high animal density, regardless of animal species. Among livestock species, cattle at medium and high grazing density caused a deterioration of soil with respect to the control, highlighted by the decrease ($P<0.05$) in TOC (50%), TN (50%), dehydrogenase (55%) and beta-glucosidase (55%)activities. On the other hand, in sheep grazing site, a great worsening of soil properties was observed only in high density site, ($P<0.05$; 50% decrease for all parameters), while a less marked variation of soil characteristics was detected at low and medium density ($P<0,05$; 20% decrease for all parameters). In conclusion, a clear livestock management plan, according to eating habits and behavior, is necessary in order to preserve soil quality and functionality.

C-108

Animal welfare assessment in dairy sheep farms in Tuscany

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The Regulation (EC) n. 1698/2005 on support for rural development by the European Agricultural Fund for Rural Development includes a specific measure for payments for animal welfare. This measure requires the evaluation of the initial well-being condition of the participating farms. The Tuscany Region included this measure in its rural development program for the period 2007-2013, supporting 219 cattle and dairy sheep farms. This study aims at evaluating animal welfare on a sample of 42 dairy sheep farms. Farm surveys were performed to collect information on farm management and to measure structure characteristics. A software was developed, that grades farms after processing data. The latter were recorded through a checklist, built upon objective and easily measurable parameters. Each parameter was scored according to its importance. The check-list embraced five focal areas: management; farming system; environment control; feeding and water supply; hygiene, health and behavioural aspects. The major parameters considered for evaluation were: mutilation; number of animals per hectare of pasture; space allowance; building ventilation; milking waiting area; watering system and sanitary control. An Excel spreadsheet was created to input the data and automatically assign both a partial and a total score to each area. Depending on the score, areas as well as farms were evaluated according to five classes of increasing level of animal welfare. The study displays an initial situation characterized by a satisfactory level of well-being, with only 10% of holdings showing deficiencies that place them at the minimum level. However, no farm in the sample reached the highest rating, corresponding to an excellent condition. The survey also revealed farmer's awareness of the importance of the issue, and his/her ability to use the measure 215 as a tool for improving animal welfare on farm.

C-109

Welfare of lactating ewes under heat stress: effect of shade and flaxseed supplementation

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The objectives of this study were to evaluate the effects of protection from solar radiation and whole flaxseed supplementation on the welfare of ewes under high ambient temperatures. The experiment was carried out during the summer (July and August): 40 *Sarda* ewes were divided into four groups of ten each, and either exposed (EXP; not offered shade) or protected from solar radiation (PRO; offered shade). For each solar radiation treatment, ewes were supplemented with whole flaxseed

(EXP-F and PRO-F,210 g/ewe/d of whole flaxseed) or not (EXP-C and PRO-C). At the beginning of the experiment and at d 21 and 44 of the trial, the body weight and the body condition score of the ewes were recorded. Respiration rate and rectal temperature were measured twice weekly. At d 29 of the experiment, blood cortisol concentrations were measured after an injection of ACTH. Cellular immune response was evaluated by intradermic injection of phytohemagglutinin at the beginning of the experiment, and at d 14, 29, 44 of the trial. Humoral response to ovalbumin was measured at 8, 14, 28, and 44 d of the study period. At d 1, 14, 28, and 44 of the experiment, blood samples were collected from each ewe for the determination of the blood metabolites and enzymes. Behavioral observations of ewes were recorded by trained observers weekly. Data were processed using ANOVA for repeated measures (SAS, 1999) having exposureto solar radiation (non repeated factor), diet (non repeated factor), time of sampling, and their interactions as repeated factors. Results demonstrated that whole flaxseed supplementation enhanced humoral immune and thermoregulatory responses during hot season. As expected, the provision of shaded areas reduced the mobilization of body lipid resources. Flaxseed supplementation resulted in significantly lower values of respiration rate both in shaded and non-shaded ewes. Small but significant differences were found in ewe rectal temperatures, which were lower in protected than in exposed ewes, irrespective of flaxseed supplementation. Solar radiation affected eating and ruminating activities: greater proportions of ewes of the exposed groups were observed eating than ewes in the shaded groups, and lower proportaions of ewes of the exposed groups were observed ruminating than ewes in the shaded groups.

C-110

Herd behaviour of the Roman Horse of Latial Maremma (*Cavallo Romano della Maremma Laziale*)

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The Roman Horse of Latial Maremma (RHLM) derives from the equine populations that lived in the Tuscan-Latial Maremma in Etruscan period; after Oriental, North European, and Thoroughbred blood was introduced to this breed. The RHLM is usually reared extensively, and still transhumance is practiced for this breed; traditionally the herd spends the Summer and the early Fall months in Central Appennino mountains and returns in lower plains in Winter. In this work the herd behaviour of RHLM deriving from Ponzano Romano (RM) was descript, through ethological observations. This herd spent the period from July to October in Terminillo Mount. 130 mares and 30 foals were observed at three different moments of the day: in the morning, noon, and in the late afternoon; herd behaviour data were recorded during the trial period, from July to October, on four consecutive days each month. The number of observed equines, and previous arcsine transformation, the percentage frequencies of animal activities, were submitted to two way ANOVA, considering as fixed factors month and time of day. The herd behaviour was observed during four subsequent days. The largest number of mares were visible in July, August, and September (113.5, 117.1, 111.7 respectively), and at noon (146.2 at noon *vs* 88.7 at morning, and 82.0 at afternoon). The grazing activity occurred in particular at noon (99.6%). Also the sniffing activity, strictly associated with food intake, predominantly occurred at noon (1.7% *vs* 0.1 and 0.4 at morning and at afternoon respectively). The dynamic activities occurred in the late afternoon, often with following foals (2.6% *vs* 0.8 and 0.2 at morning and at afternoon respectively). Mares were at rest in the morning (4.3%) and in the late afternoon (4.6%). The foals behaviour was similar to that of mares; the higher percentage of foals grazed at noon (91.8%), and the higher dynamic activities occurred in the late afternoon (8.3%), when the foals nursing was more prevalent.

C-111

Effects of feed and water on the welfare of fattening pigs

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The effects of water and feed provision on the risk of disease, abnormal behaviours and pig production are reviewed. Pigs obtain water from drinking, wet-feeds and, at a lesser extent, from moisture in feedstuffs and oxidative processes. Water deprivation can be accidental (system failures), from management errors (insufficient/inefficient drinkers) or voluntary (water restriction of wet-fed pigs). Water availability influences dry matter intake and, consequently, animal growth. Water deprivation and/or inadequate water provision can lead to serious health problems such as salt poisoning and contribute to urolithiasis. Although pigs are believed to be fairly adaptable to water quality, they clearly prefer clean water. In relation to water quality, total dissolved solids, pollutants and the colonisation by toxic algae should be considered. Liquid feed improves feed intake and efficiency, growth performance, and has behavioural benefits. Under good hygiene conditions, fermented liquid feeds reduce enterobacteria levels. Feed deficiencies may be due to accidental or deliberate causes. Accidental deficiencies are mainly attributable to system failures, sedimentation of liquid feeds and ingredient weighing errors. Adequate access to feed for all group individuals is a prerequisite for good welfare and feeder space availability influences aggressions. Feed restrictions are imposed on fattening pigs to improve feed efficiency, limiting carcass adiposity and to obtain meat with specific characteristics. Pigs submitted to partial or complete feed withdrawal display a series of negative behavioural changes mainly represented by a reduction of resting time and increased oral activities, which can be lowered by supplying fibrous rootable materials. Nutrient deficiencies are rare and, in the case of trace elements and vitamins, problems are mainly related to accidental excesses. Carcass quality can be impaired in case of imbalances in the energy/protein ratio (higher adiposity). The provision of good quality water and of adequate quantity of a balanced diet should therefore be regarded as essential elements not only to obtain high production parameters but also in terms of pig welfare. This presentation is given under the sole responsibility of the authors, and is not considered as an EFSA output. The positions and opinions presented are those of the authors alone and are not intended to represent the views of EFSA.

C-112

Effect of different infrared lamps on performances of piglets after weaning

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A preliminary study was conducted in the winter of 2012 to assess the effect of two different types of heating lamps on micro-environmental conditions and on performance of post-weaning piglets. Two adjacent rooms (m 8.00 x 3.00 x 2.50) with 12 slatted cages (m 1.00 x 1.20 x 0.60) arranged in two rows were selected. One room was equipped with 6 conventional heat lamps of 150 W suspended at 60 cm above the cage's floor, while the other was provided of 4 rectangular infrared lamps of 1000 W fixed on the ceiling above the corridor between the cage's rows. In both rooms, the lamps switched off when a temperature of 26°C measured at 1 m from the ceiling was reached. Temperature and relative humidity were continuously recorded with a data-logger positioned at the level of cage floor. Electricity used by one lamp in each room was measured continuously with kWh meter. After two days of heating, 96 piglets of 26-28 days old were randomly allocated in each room. Twenty-four ear-tagged subjects for each room were examined. Body weight was measured four times at interval of 10 days. During the weighing, rectal temperature and ventral and dorsal thermal infrared images were recorded. The daily averages of temperature and relative humidity at the level of the cage's floor were significantly different between rooms. Higher temperature and lower relative humidity were recorded in the room heated with the four lamps. Moreover, the ventral and dorsal thermal images recorded on piglets from this room showed a skin temperature higher from 0.5C° to 1.0°C. Daily gain and rectal temperature were not influenced by the different type of heating. Taking in account a retail price of 0.13 cents per kW/h, the daily expenses of electricity in the room equipped with the four lamps were higher of 0.23 Euro with respect those recorder in the other room. This preliminary study showed that the lamps fixed on ceiling lead to a better distribution of heat on the cages reducing the relative humidity but require a little increase of the heating cost.

C-113

Grass intake and use of outdoor pen in different organic poultry systems

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In organic and free-range poultry productions, animal welfare and product characteristics largely depend on the adaptation of the genetic strain to extensive conditions. For these reasons, it is crucial to optimize the grass intake through different strategies, keeping the chickens at densities that allow available pasture for the entire cycle and using genetic strains capable of moving and grazing. In many poultry farms, the runs are not used well, suggesting that the animals do not feel safe in and that they should be more attractive by offering different kinds of shelter, as protection against elements and raptors. The aim of this study was to analyse (in summer and winter), the effect of trees as shelters on performance, behaviour, grass intake and carcass characteristics of a commercial strain of chicken, reared under the organic method. This trial was carried out in two similar farms:

- the experimental section of University of Perugia, where the chicks were reared in two straw-bedded indoor pens with free access to forage paddock (10 m^2/bird); one where livestock sorghum was sown and the other one without bushes;
- Bachetoni farm (Spoleto, Italy), where the chickens were reared in the same above mentioned conditions, but the pasture area consisted in a mature olive grove.

Two hundred fifty male Naked neck provided by a commercial poultry farm (Avicola Berlanda, Italy), were used in both system and seasons. Chicken exploitation of the outdoor areas strongly varied with the season, time of the day, weather conditions and habitat. Chickens reared under olive trees showed a higher locomotory activity (25.0 and 20.1% of budget time, in summer and winter) respect to those reared in the other conditions (14.1, 20.6 and 10.2, 15.8%, respectively for no bushes and sorghum systems). Consequently, all the behavioural parameters were modified. Concerning grass intake, the presence of trees allowed the consumption of grass up to almost 50 meters from the huts, while in other systems the chickens away at most 20 meters. Also carcass damages were significantly affected by the presence of vegetation and the best results were obtained in olive trees group.

C-114

The use of homeopathy in beekeeping to increase the resistance of the bee families against Varroasis

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The aim of this work was to test the efficiency of homeopathy to improve the resistance against Varroasis of honeybee families treated also with block brood and Api-Bioxal® (based on oxalic acid), drug allowed in organic beekeeping. The trial was repeated for two years in two different apiaries placed in the same area. In both trials 20 honeybee colonies, homogeneous for consistency and mite infestation level, were selected. In both trials all queens were young, born in same year, sister and marked. The 20 families were divided in two groups: 10 were treated approximately every 2 months with Calcarea Sulphurica 200 CH sprayed on the combs (C), whereas the others 10 only with water (W). Both groups were treated in summer with block brood and Api-Bioxal®, and in autumn only with Api-Bioxal®. We recorded the incidences of Varroa destructor by recording the natural mite fall, in different sampling periods, every 3 days for 7 sampling times. All data were statistically analyzed by ANOVA, considering the group as fixed factor, the trial, the sampling period and the sampling time as at random factors, and the natural mite fall every 3 days as variable factor. In both trials, after the brood block and Api-Bioxal® treatments, the natural mite fall resulted lower in C group, but only after summer Api-Bioxal® treatment this difference became relevant (1458.2 A *vs* 822.4 C first trial; 484.2 A *vs* 290.5 C second trial). The mite fall count resulted significantly different between groups in the different sampling periods, and, on both trials, it resulted also significantly different inside every sampling time. Considering that block brood followed by Api-Bioxal® treatment has an efficiency of 89,4% on Varroa destructor elimination, as reported in the literature, the smaller mite fall in group C, in both experimental trials, seemed to demonstrate a good efficiency of homeopathic remedy on increasing the resistance of families, probably due to a reached balance of honeybees with their parasites.

C-116

Italian Goat Consortium: a first overview of genetic variability using a medium density single nucleotide polymorphism array

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The Italian Goat Consortium was established from the Innovagen Project to join local efforts and resources for the genomic characterisation of Italian goat breeds. Thirteen Italian goat breeds (Valdostana, Saanen, Orobica, Bionda dell'Adamello, Val Passiria, Grigia Ciociara, Teramana, Nicastrese, Aspromontana, Girgentana, Argentata dell'Etna, *Sarda* and Maltese – the latter sampled in Sardinia and Sicily) for a total of 350 animals (15-32 per breed) were analysed using a medium density SNP array. Data editing was based on the following thresholds: MAF <1%; Missing SNP >5%; Missing animal >5%; HW within breed FDR >20%. After filtering, a total of 51,136 SNPs were retained, showing that the adopted SNP chip is highly informative for the characterization of Italian goat breeds. Population structure and breed distinctiveness were investigated with the software Admixture, that revealed the best fitting solution at K=11 partitions. The software also highlighted variable levels of admixture and an early differentiation of Val Passiria, Orobica, Valdostana, Teramana, Girgentana and *Sarda* breeds. Within MDS plot, some breeds are clearly appreciable as distinct: Girgentana, Teramana, Orobica and Maltese. PCA analysis revealed a gradient of diversity North (from alpine breeds) – South (to Sicilian breeds) and East (from Val Passiria breed) - West (to Valdostana breed) in the Alps. The calculation of Fis index indicated little or no inbreeding, while LD analysis in Chromosome 6 showed levels of disequilibrium much lower than cattle at short physical distance.

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C-117

Introgression of European *Bos taurus* genome in Ugandan taurine and zebuine cattle breeds

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Among the major aims of the project NextGen stands the investigation of disease resistance in indigenous Ugandan cattle. A total of 915 animals from 9 populations belonging to Ankole (crossbred between *B. indicus* and *B. taurus*), Zebu and Ankole-Zebu crosses have been sampled in 52 grid cells throughout the country. Data on animal health status, GPS coordinates of individual sampling sites, disease vector distribution and disease prevalence were also recorded to allow the identification of selection signatures from whole genome sequencing and SNP data delivered by the project. Peculiar phenotypic traits recorded during the sampling suggested a possible introgression from European breeds into Ugandan local cattle. To test this hypothesis, we compared the genotypes of Ugandan and Italian Holstein Friesian cattle characterized with the BovineSNP50 Beadchip. After filtering (M.A.F.<0.01, genotype call rate - SNPs<0.95, genotype call rate - Animals<0.95), the working dataset included a total of 1188 animals and about 44K markers. The genetic structure was investigated by a Bayesian clustering approach with the ADMIXTURE software, which highlighted a remarkable level of admixture and identified four ancestral genomic components. Three of them likely represent indicine, African taurine and European taurine gene pools, while the origin of the fourth is still to be identified. Overall, about 20% of the genome of Zebu individuals derives from African taurine cattle, thus confirming previous data on the origin of African Zebu populations. The probable European taurine contribution is a minor component, rare in Zebu and evenly spread in Ankole and Ankole-Zebu crosses. Indicine and taurine components, in particular, show a clear geographical structure: the former is prevalent in north-eastern Uganda, while the latter predominates in the south-western area. Holstein Fresian introgression is present mostly in south-western Uganda, while the fourth component is restricted to a narrow geographical area in the East. To shed light on the origin of this fourth genomic pool, we have already planned further data analyses that will provide a comparison to a larger set of European cattle breeds, including Jersey.

C-118

Across breed comparison of genetic parameters for linear type traits in Valdostana cattle

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The Valdostana are indigenous dual purpose breeds, raised mainly in Valle d'Aosta region (north-west of Italy), and include two different strains. The Aosta Red Pied (ARP), has a unique herd book and is mainly selected for milk and meat production. The Aosta Black Pied and Chestnut (ABP-Chest) belonging to the same herd book is selected also for fighting ability. The Valdostana cattle are rustic and characterized by good grazing ability and high fertility. The aim of this study was to estimate heritability and genetic correlations of type traits in the Valdostana breed. Four composite type traits (body size, fleshiness, body shape and udder) and 22 individual type traits were recorded on 47,841 and 30,018 ARP and ABP-Chest cows, respectively. After editing and sampling, a representative dataset of 11,309 and 19,551 for first and second parity ARP and ABP-Chest, respectively, were available. All available pedigree information (32,003 for ARB and 32,397 ABP-Chest, respectively) were used to set up the relationship matrix among animals. Data were analyzed using a multiple-trait REML animal model, accounting for the following effects: herd-year-classifier, classes of days in milk and age at calving, and an additive genetic cow effect. For all strains, heritability estimates were medium to high for body size (0.30 for ARP, 0.26 for ABP-Chest) and fleshiness (0.28 and 0.17 for ARP and ABP-Chest, respectively) and medium to low for body shape (0.09 in both ARP and ABP-Chest) and udder (0.15 in both breeds). Heritability estimates for the individual type traits varied from 0.04 (thinness) to 0.33 (stature) for ARP and from 0.03 (thinness) to 0.29 (stature) for ABP-Chest. Genetic correlations between body shape and fleshiness were similar in both strains (*e.g.*, 0.55 for ARP and 0.52 for ABP-Chest), but correlations between body shape and udder (0.13 for ARP and -0.25 for ABP-Chest) and between body size and udder (-0.25 for ARP and null for ABP-Chest) differed by strain. More detailed knowledge of these parameters can aid the development of specific breeding goals of Valdostana cattle.

C-119

Cytogenetic investigations in endangered animal breeds raised in Campania region: an update

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In Campania region (Southern-Italy)is active a project trying to characterize, valorize and save some endangered animal breeds of cattle (*Agerolese*), sheep (*Laticauda* and *Bagnolese*), pig (*Casertana*), horse (*Napoletano, Persano* and *Salernitano*) and goat (*Cilentana*). One of aim of this project concerns the cytogenetic screening of representative groups of animals for each breed, especially the male reproducers. At present, 196 animals were investigated of which 54 cattle, 83 sheep (47 *Laticauda* and 36 *Bagnolese*), 31 horses (8 *Napoletano*, 17 *Salernitano* and 5 *Persano*), and 28 of *Casertana* pig. Peripheral blood samples were cultured to obtain both normal and R-banded chromosome preparations. Slides were treated for CBA-banding and RBA-banding techniques. Some specific case was also investigated by using FISH-mapping techniques with both bovine BAC-clones and bovine chromosome painting probes. All investigated Agerolese cattle had normal R-banded karyotype, exception of two cows found heterozygous carriers (2n=59, XX) of rob(1;29). These two carriers were also used to provide a genomic characterization of this famous translocation: a chromosome region of 5.4 Mb moved from proximal p-arms to proximal q-arms of translocated chromosome by chromosome transposition and inversion. In sheep all investigated animals showed normal R-banded karyotype, exception of two females of *Laticauda* breed which were found carriers of two new reciprocal translocations involving chromosomes 4;12 and 8;23, as revealed by both banding and FISH-mapping techniques. Both horses and pigs had normal karyotypes.

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C-120

A phylogenetic overview of five Italian local chicken breeds from a mitochondrial DNA standpoint

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Domestic chickens have long been important livestock species for food, social and ornamental aspects. Furthermore, chickens are not a migratory species, have a small home range, do not fly well over long distances, and are not equipped for swimming. As results, their current global distribution can be largely attributed to human mediated dispersal. The objective of this study was to investigate the maternal lineages of five Italian chicken breeds (*Ancona, Livornese Bianca, Modenese, Romagnola* and *Valdarnese Bianca*) and their evolutionary relationship in order to enhance current knowledge of breeds history. To achieve these targets, a 506 bp fragment of the mitochondrial DNA control region was sequenced in 50 chickens of the five breeds (10 samples for each breed). Eighteen variable sites were observed which defined 12 haplotypes. The resulting median-joining network assigned them in one major and two minor haplogroups, derived from three different lineages (A, B and E). Results suggest that the Italian chicken breeds mainly originate from the Indian subcontinent, at least from the maternal lineage standpoint, since most individuals are included in the E lineage (90%). However, the small proportion of birds belonging to lineages A and B could arise from South China and Japan. In fact, the arrival of these haplotypes to Europe as a result of Romans and Phoenicians trades is well documented at least by archaeological finding.

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C-121

Eight years of monitoring genetic variability in local chicken breeds, implications and perspectives

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The aim of this study was to monitor the genetic variation in six Italian local chicken breeds over a eight year period of conservation. The local breeds were Ermellinata di Rovigo, Padovana, Pèpoi, Robusta Lionata, Robusta Maculata and Polverara. A total of 571 samples were analyzed among the birds hatched in the years 2002 to 2010. Genetic variation over 30 microsatellite *loci* was analyzed. All 30 microsatellites were polymorphic, with a total number of alleles of 258 and a mean (±SD) of 9.56±4.06 across populations. A loss of alleles was observed during the first four years for the majority of microsatellites and for all the breeds. The mean number of alleles (MNA), considering the whole population, decreased from 7.52±3.00 in 2002 to 4.96±2.39 in 2006. In 2010 the MNA increased significantly ($P<0.05$) to 6.41±3.26 in all breeds compared to values observed in 2006. The mean observed heterozygosity (Ho) of the entire population was 0.35±0.20 and it constantly decreased during the period of observation in all breeds with the exception of Padovana where a slightly increase of Ho was observed in 2010 compared to 2006. The mean expected heterozygosity (He) was 0.50±0.20 and He estimates showed a pattern similar to that of MNA where, in all breeds, after a decrease in 2006, they reached values comparable, or even higher, to those detected in 2002. A significant increase ($P<0.001$) in the inbreeding coefficient (Fis) was evidenced in 2010 in all the breeds with the exception of Robusta Maculata whereas molecular coancestry estimates (fij) reached the maximum values in all the breeds in 2006. All populations showed evidence of a significant deviation from Hardy-Weinberg equilibrium caused by an excess of homozygotes. No evident genetic structures were detectable within breed, for 2002, 2006 and 2010 individuals even though a slight increase in the proportion of membership in each breed has occurred during the time. Molecular markers analysis proved to be effective tools to monitor the genetic variability of local breeds involved in a conservation scheme.

C-122

Genetic diversity of five Italian local chicken breeds by microsatellite markers

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The loss of livestock biodiversity in the face of increasing pressures from modern farming is a cause for global concern. In a more recent time, the family poultry farms were largely responsible for the local production of eggs and meat but now this role has steadily dwindled in Italy; in fact local production is entirely replaced by intensively reared poultry. The autochthonous breeds are an important resource of genes for future breeding and research purposes. The objective of this work was to evaluate the levels of genetic variability, the genetic structure and the level of admixture of the five Italian chicken breeds, using 27 microsatellite markers FAO suggested. A total of 137 blood samples were randomly collected from five Italian local chicken breeds: Ancona (n=30), Livornese bianca (n=30), Modenese (n=23), *Romagnola* (n=24), Valdarnese bianca (n=30) of both sexes. A total of 147 alleles were detected. The Italian breeds showed a slightly higher inbreeding index (Fis=0.08) when compared to six commercial populations used as reference. These populations consisted of a broiler dam (BRD) and a sire (BRS) lines, two brown-egg layers (BLA and BLC) and two white-egg layers (LSS and WLA). The other populations were commercial lines. VA displayed the highest value of the observed and expected heterozygosity (0.53 for both of them) while AN and MO the lowest (0.39). The average inbreeding value at the total sample level (Fit) was 0.349±0.017 (P<0.01) and higher in commercial lines than in Italian breeds. The genetic differentiation (Fst) of Italian breeds was lower (0.225±0.019) than the corresponding value of the commercial lines (0.354±0.025), indicating a lower variability than between commercial lines but still substantial sub-structuring of the Italian breeds. Structure analysis showed a separation of the Italian breeds from the reference populations; a further sub-clustering allowed to discriminate the five Italian breeds and it confirms their genetic differences. This research provides insight into population structure, relatedness and variability of the Italian breeds.

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C-123

Genetics footprints of Canary Hair sheep in South American sheep breeds

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Canary Hair sheep is an autochthonous sheep breed reared in Canary Islands characterized by the absence of wool. This breed is widely used as genetics resources in tropical environment where shown a greater resistance to parasitism and adaptability to poor alimentation. Probably it participated to American Continent colonization starting from Caribbean after the Spanish conquest. There is no consensus on whether this import into the Caribbean occurred directly from West Africa or via the Canary Islands and several MtDNA studies has not proved its origin (African or European). Phonotypical data show the participation in several South American breed formation. In this study we analyzed with a 27 STR markers panel a sample of Canary hair sheep (N=50) and then we perform a comparative study with African and Saharawi camps (Morocco) breeds (UDA sheep=48; Sidaun=21; Keran=20), Spanish Merino (40) and Churra (83) sheep and two wool breeds from canary Arcipelago (Canaria=50 and Palmera sheep=50) and South American breeds (Mexico Pelibuey=42; Chiapas Creole sheep=128; Cuban hair sheep=27; Brazilian Yaguaribe=20 and Morada Nova=20; Carribean barriga Negra=20) . Our results show that Canary Hair sheep has a high genetics influences on South American sheep breeds (Creole sheep). An interesting evidence is the detection using Structure software of both influences of continental Spanish breeds (merino and long wool type) and Canary Hair sheep (no wool type) on wool sheep breeds like Chiapas Borrego: this result could indicate a widely diffusion of this genotype on the American continent.

C-123bis

Quantifying physiological stress in livestock transport: understanding the implications for welfare and meat quality

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The latest figures indicate that in the EU (27) 28 million cattle, 61 million sheep, 260 million pigs and 6.4 billion broiler chickens are slaughtered per annum. Each animal is transported from its site of production to slaughterhouses or processing plants on journeys of varying distances and durations (sometimes in excess of 24 hours) and under a range of external meteorological conditions. The nature of the journeys and associated procedures, the external climatic conditions and the design and operation of transport vehicles and containers combine to impose a range of potential stressors upon the animals which will impact upon their welfare and affect meat quality and losses in transit. A major source of transport stress which may increase mortality in transit, induce poor welfare and result in reduced meat quality is the thermal environment. Both heat stress and cold stress may have profound effects upon an animals' well being during transportation. The capacity for an animal to thermo-regulate under transport conditions may be compromised and the resulting physiological stress may result in the induction of specific pathologies, tissue dysfunction and changes in metabolism. In turn, such dysregulation may precipitate meat quality problems such as PSE and DFD like conditions. It is proposed that in order to optimise transport conditions and vehicle design and operation it is essential to develop predictive, integrative, physiological (outcome based) models for each species to define acceptable ranges and limits for stressors in transit. Quantifying the physiological stress responses of animals to quantified stressors facilitates definition of thermal comfort ranges for each species and thus optimal transport micro-environments in addition to characterising the interactive effects of other potential challenges or sources of stress. Through this approach it is possible to develop strategies to minimise physiological stress during transportation and to ensure that the welfare of the animals is maintained in transit and the incidence of meat quality problems is minimised.

C-124

Biometric characteristics of a red deer population in the Central *Areale Cervo Appennino Tosco Emiliano Romagnolo*

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The red deer (*Cervus elaphus*) population of the Central Apennine has been monitored since 1999, when Toscana and Emilia Romagna regions adopted a single protocol for the management of this ungulate. Selective hunting, an important management tool, allows to collect a large number of biometric data, whose analysis is used to describe morphometric characteristics of wildlife populations. In this work, we report the results of a study aimed at describing the morphometric features of a red deer population living in Prato province, near an historical reproductive area. Body measures of 905 animals were collected during 12 hunting seasons (2000-2012), in detail for males: 76 adult stags, 107 subadults, 77 yearling, 148 calves; for females: 290 hinds, 73 yearling, 134 calves. For each animal were recorded: live weight, eviscerated weight, body length, chest circumference, height at withers, hind foot length, tail length, jaw length and other skull measurements; some antlers measurements were also collected and analyzed. The Buchli index, adopted on alpine hinds to evaluate the body constitution and the quality of the population, was also calculated. ANOVA was used to test the differences between sexes and age classes for morphological measurements. The relationship between live and eviscerated weight was tested with linear regression. Data were analyzed with SPSS software version 16.0. The results provide a pattern of the morphological traits of the population for each sex and age class, *e.g.* the average of eviscerated body weight resulted 130.7±17.1 kg for stags and 71.7±10.5 kg for hinds. Significant differences were observed between sexes and between age classes for many parameters. The relationship between live (LW) and eviscerated weight (EW) was significant in every case, both considering the whole animal sample ($LW=1.228*EW +8.907$, $R^2=0.97$) and for different sexes and classes of age. The biometric features observed in this sample are similar to the ones reported for the red deer of the eastern *Areale Cervo Appennino Tosco Emiliano Romagnolo*, confirming the greater body size of the Apennine populations compared to Alpine deer.

C-125

Impact of the main dairy cattle technopathies

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High-production dairy cow's intensive farming can seriously lead animals to run into several diseases compromising health, welfare and farm profitability due to direct (treatments and low milk yield) and hidden costs (fertility reduction, higher probability of death or early culling). The knowledge of such cost items is essential for the correct farm management. Therefore, was performed a study to monitor incidence and costs of the main technopathies in an Italian high-production dairy farm. Data collected from 108 entire lactations were: date of calving, average milk production during a 250 days normalized lactation, monthly milk production, somatic cell count, milk fat and protein content, number of artificial insemination to conception and calving to conception rate. Animals were divided into 6 groups according to their health status during early lactation (H: healthy; K: animals incurred in subclinical ketosis; AD: right abomasal displacement; M: severe clinical mastitis; PR: placenta retention; L: lameness). For economic evaluation were considered the cost of each single treatment, the value of milk not sold in accordance with drugs withdrawal period, the value of the difference of milk produced compared to the animals of the group H. According to literature, to each disease was assigned the probability of death or early culling. The main technopathies detected were L (29.6%) and PR (29.6%) with incidence higher than considered alarming by literature. Instead, low incidence was detected regarding AD (1.9%), M (5.6%) and K (7.4%). Compared to similar studies, in this case were found higher costs due to the high incidence of lameness (921.53 €/case of L) with tendency to chronicity and placental retentions (718.42 €/case of PR) that in more than 70% of cases evolved in endometritis or metritis. These two technopathies negatively affected the average productivity, increased milk somatic cells content causing the payment of quality penalties, and increased the calving to conception rate. The economic evaluation covering all the cost items is a useful tool to identify the main critical points of the herd health management, helping farmer and vet to assign different priority degrees to managerial and structural interventions to be taken.

C-126

Effect of floor surface on claw health, horn growth and locomotion score of dairy cows

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Concrete slatted floor is common in free stall houses for dairy cows because it reduces labour in comparison with other solutions. However, concrete slatted floor has been associated with welfare problems. Cows prefer to walk and stand on soft surfaces. Several studies showed that covering a solid concrete floor with rubber produce benefits to claw health and locomotion; studies on the effect of using rubber on slatted floors are few and inconsistent. In one of the two sides of a free stall barn, the existing slatted concrete floor (CONCR) of the feeding alley was covered with rubber mats (RUBB). Fifty-six cows (38 pluriparous and 18 primiparous) were blocked according to parity, days in milking (246±179) and locomotion score(LS); LS1 indicated normal gait, LS2 indicated moderately lame cow and LS3 indicated lame cow. Cows in blocks were randomly assigned to one of the side of the barn. Claws health was assessed during three hoof trimmings: T0 (32.5±4.9 days before the installation of RUBB); T1 (142.5±10.5 days after T0) and T2 (97±9 days after T1). Hooves of all feet of each cow were examined for the presence of claw horn lesions (sole haemorrhage, sole ulcer, white line disease, fissure, abscess) and infectious lesions (digital dermatitis, phlegmon). At T0 a burn mark was imprinted on the surface of the right rear and left front hoof, below the upper edge of walls; the distance from the upper edge to the mark measured at a successive trimming, was assumed as hoof growth. At T1 claws health was improved in both groups but the prevalence of sole haemorrhages was tendentially (P=0.091) lower in RUBB than in CONCR; horn growth of right rear hoof resulted higher (P=0.053) in RUBB (0.20 mm/d) than in CONCR (0.18 mm/d). Frequency of LS1 over the 5 months following the rubber installation was higher in RUBB than in CONCR (P=0.024). Results from this experiment indicating moderate short-term effects of RUBB will be input to an economical model to assess pro and cons of different types of floor.

C-127

Comparison among different dairy cattle welfare monitoring schemes

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The study aimed to investigate the degree of agreement among three different dairy cattle welfare monitoring schemes. Animal welfare was evaluated in 17 dairy cattle farms using the following monitoring systems: 1) the Animal Needs Index 35L/2000 developed in Austria (ANI); 2) a welfare index developed in Italy by the Lombardy Extension Service (IBS); 3) the Welfare Quality® (WQ) assessment protocol for dairy cows. The ANI and IBS mainly use resource-based measures, whereas the WQ system rely on animal-based measures. As IBS is exclusively devoted to cubicle housing all the selected farms were loose housed with cubicles in the resting area to make comparisons possible. Data were collected by one trained assessor. Each farm was assessed by the three systems in the same day. The mean herd size was 93 cows (range 40÷180). Spearman rank correlation coefficients (rs) were used to determine the correlation between pairs of schemes. For each farm the time taken for the assessment was approximately 1, 1 and 5 h for ANI, IBS and WQ schemes, respectively. Total scores are reported in Table 1. Only 4 farms presented an IBS score (scores can range from 0 to 100) lower than 50. All farms presented an ANI score (scores can range from -9 to 46) greater than 21, which is considered as a fairly suitable level of welfare, with 8 farms showing a score greater than 28, which is considered as a very suitable level of welfare. According to the WQ scoring system a given farm can be assigned to four welfare categories (excellent, enhanced, acceptable and not classified). In our sample 7 farms were classified as acceptable and the remaining 10 as enhanced. A positive correlation was found between ANI and IBS total scores (rs=0.568; P=0.0174), thus ranking the farms in the same order possibly because they both rely on resource-based measures. Conversely, no correlations were found between WQ *vs* ANI (rs =-0.002) and *vs* IBS (rs =0.173) as WQ mostly relies on animal-based measures. We conclude that for a valid assessment of animal welfare a combination of resource and animal based measure is recommended.

Table 1. Animal Needs Index (ANI), Lombardy Extension Service Index (IBS) and Welfare Quality® (WQ) scores obtained in 17 dairy cattle farms.

Farm	IBS	ANI	WQ
1	61	33,0	Acceptable
2	56	28,0	Enhanced
3	52	23,5	Enhanced
4	49	22,0	Acceptable
5	55	32,0	Acceptable
6	59	29,0	Enhanced
7	72	34,0	Enhanced
8	63	26,0	Acceptable
9	67	30,5	Acceptable
10	31	28,0	Enhanced
11	69	29,0	Enhanced
12	33	25,5	Enhanced
13	56	28,5	Acceptable
14	48	22,5	Enhanced
15	62	31,0	Enhanced
16	75	31,5	Enhanced
17	51	32,5	Acceptable

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Effects of grazing on welfare and production traits of organic dairy cows

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In organic livestock systems, grazing is the preferred feeding source, especially for animal welfare and health implications, but it is not an obliged practice. In order to contribute on this topic, in an organic dairy farm, grazing was compared with permanent free-stall housing using two homogeneous groups of eight Bruna cows allocated to the following regimes: (G) grazing on barley grass for 5 h/d, and then kept in a free-stall structure and fed with unifeed, composed of hay and concentrate; (S) kept in a free-stall building for 24 h/d and fed with a diet based on the same unifeed. Comparison was based on milk production, behaviour, immune responses, metabolic status and oxidative stress of animals, observed 4 times during a 64-day period. Grazing barley pasture did not affect milk yield, whereas led to an improvement in milk quality in terms of fat increase and reduction in urea, somatic cells count and total microbial count. Regarding behaviour, S cows spent more time inactive, whereas G cows better interacted socially, devoting more time to sniff, lick or rub between them in non-aggressive forms, thus giving indications of their higher level of comfort and relax. The skin test, performed with PHA at the end of trial, resulted in a higher skin thickness in cows G, index of a better immune response, than S cows. The regime had an effect on Oxidative Stress (OS), since G group exhibited higher ROMs and lower BAP concentration than S group, indicating a moderate OS in G group as result of grazing activity, which can increase free radical production and decrease antioxidant capacity. However, in the first period of grazing, G group showed a higher content of polyphenols in plasma than S group. In both regimes, low NEFA and BHBA concentration indicates that cows did not display a state of metabolic stress. In conclusion, grazing of dairy cows in an organic farming system did not reduce milk yield and contributed to improve milk quality. Moreover, grazing would seem to induce a better welfare status and a more intense immune response in the cows.

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Administration of an homogenate of *Aloe arborescens* to periparturient dairy cows: effects on energy metabolism and inflammatory status

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The peripartum of the high genetic merit dairy cows is characterized by reduced immune competence, inflammatory-like conditions and severe lipomobilization, which lead to higher risk of diseases and to a worsening of performance and welfare. The nutraceutical approach seems a very promising tool to improve these aspects. In this study we investigated the effect of a homogenate of *Aloe arborescens* (AA), administered in the 4 weeks around calving, on energy metabolism and inflammatory response. The experiment involved 11 multiparous dairy cows: 5 received 150 g/d of AA and 6 were not supplemented (CTR). Health status, body condition score (BCS), rectal temperature, milk yield and composition, metabolic-inflammatory profile were frequently monitored from 4 weeks before calving to 4 after it. AA reduced the rectal temperature in the first week of lactation, the incidence of clinical diseases and the somatic cell count in the milk during the first month of lactation. AA *vs* CTR showed a slower reduction of glycaemia in the first week of lactation (4.09 *vs* 3.46 mmol/l respectively; $P<0.05$) and had a lower post-partum raise of NEFA and BHB (0.50 *vs* 0.88 mmol/l respectively; $P<0.01$), suggesting a less severe post-calving lipomobilization, as supported by the smaller BCS drop in the first month of lactation, and reduced risk of ketosis. The raise of the positive acute phase proteins was similar between groups, suggesting that inflammation occurred as usual in this phase. On the contrary, the levels of negative acute phase proteins (*e.g.* albumin, paraoxonase) and related indices (*e.g.* cholesterol, index of lipoproteins and vitamin A, index of retinol binding protein) during the first month of lactation are often statistically higher in AA *vs* CTR cows. Indeed, the Liver Functionality Index, which measures the consequences of the inflammation, is higher in AA (+3.1 *vs* -0.4 points of CTR; $P<0.05$). Overall, these differences suggest less severe consequences of inflammation around calving in AA group. The effects of AA during the transition period seem favorable: it cuts down lipomobilization and subclinical inflammation, improves the energy balance, and ultimately leads to a better performance.

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Evaluation of productive efficiency of dairy cows divergent for genetic merit

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Productive efficiency of mid lactating cows was evaluated in 75 Italian Friesian cows, from 2 commercial herds, and 80 Simmental cows, from 3 commercial herds. The animals were chosen based on their genetic merit for kg of milk protein production and clustered in positive (Class 0), null (Class 1) or negative (Class 2) according to their Estimated Breeding Values (EBVs). One sampling of blood and milk was performed for each cow at the morning after milking and before meal distribution. On the same day, BCS was evaluated by the same expert. Blood samples were analysed for total proteins, albumin, globulin, urea, creatinine, glucose, FFA, beta hydroxybutyrate (bOHB), aspartate aminotransferase (AST), Zn, total antioxidant status (TAS), Hb and glutathione peroxidase (GPx). Milk samples were analysed for cortisol, fat and protein percentage and somatic cell count (SCC). All these parameters were strongly influenced by the breed (P values ranged from<0.05 to<0.01), probably reflecting also the effect of the farms, that in any case was considered in the statistical model used for the analysis. Friesian cows showed higher levels of albumin, globulin, bOHB, AST, Zn, TAS, Hb, GPx and milk cortisol, whilst Simmental cows showed higher values of BCS, urea, creatinine and FFA. The effect of EBV class was evident for the BCS, that resulted higher ($P<0.001$) in Class 0, confirming that cows with higher protein and milk production are more prone to mobilize body reserves and can restore them with a delay in comparison to the animals with a lower EBV for kg of milk protein. Albumin ($P<0.01$) and TAS ($P<0.01$) were higher in Class 2, whilst urea ($P<0.001$), creatinine ($P<0.001$), bOHB ($P<0.001$) and SCC ($P<0.05$) showed higher values in Class 0. Milk cortisol was not influenced by EBV class.

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Preliminary results on a sequential approach to qualitative behaviour assessment in buffaloes

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We aimed to verify whether the qualitative behaviour assessment (QBA) is able to detect the behavioural fluctuations occurring in animals during the observation period. An 8-member panel with a varied experience in animal behaviour was used. The panel was briefly trained on the temporal dominance of sensations (TDS) procedure and subsequently observed the behaviour of 4 buffalo heifers in 4 videos lasting 2 min each following this procedure. Each video was obtained by assembling two clips portraying the same animal in two different conditions: home indoor pen (1 min) and novel outdoor paddock (1 min). Two videos started with the animal in the outdoor environment and two others in the opposite order. Six behavioural descriptors were chosen from a previous work conducted on the same animals: calm, active, curious, nervous, shy and apathetic. TDS consists in presenting to the panellist the list of behavioural descriptors on a computer screen along with each clip. Each assessor was asked to select the dominant descriptor, which was considered as dominant when it gained most of the attention of the observer. Each time the observer felt the behaviour changed, he/she scored the new dominant descriptor until the behaviour ended. Each clip was observed 4 times by each observer (4 replications) in a randomised order at 24-h intervals. For each point of time, the proportion of runs (subject x replication) for which a given descriptor was assessed as dominant (dominance rate >30%) was computed. Results showed a satisfactory agreement among observers and replications. The observers clearly discriminated the first from the second half of each video. Calm and apathetic were dominant indoors, nervous and active were dominant outdoors, whereas curious was used in both conditions (Figure 1). In addition, the combination QBA-TDS showed how the behaviour changed in time (*e.g.* habituation to the novel environment) in outdoor conditions (the dominance of nervous and active at the start switched to calm at the end in 3 out of 4 videos), whereas indoor animals showed reduced behavioural variations, possibly because they were already habituated to those conditions, thus they did not have to change their way to interact with the environment.

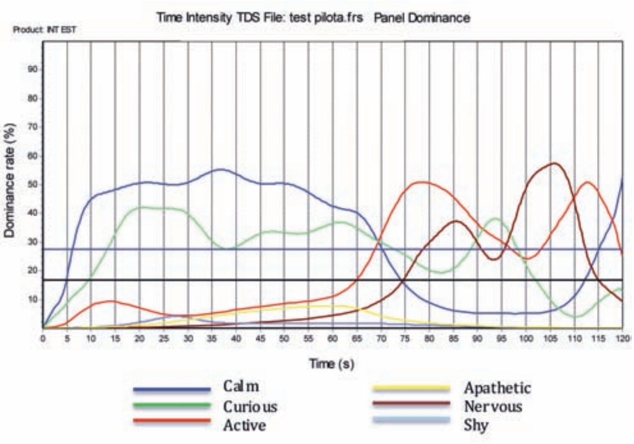

Figure 1. Qualitative assessment of the behaviour of heifer n. 1 through TDS procedure (temporal dominance of sensations).

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The content of cla and alpha-linolenic acid in Sn-2 of ovine milk triglycerides as affected by dietary lipid supplementation

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In the human intestine, lipids are adsorbed as Sn-2 monoglycerides (Sn-2), as effect of the pancreatic lipase action, which hydrolyzes the triglyceride on alpha-position (Sn1-3). Hence, the fatty acids esterified in Sn-2 are preferentially adsorbed. In a previous research we obtained a significant enrichment in Conjugated Linoleic Acid (CLA) and alpha-linolenic acid (ALA) of milk and cheese from sheep fed a diet supplemented with linseed. The aim of the present work was to study the triglyceride (TG) composition of the milk of the mentioned study, in order to evaluate the selectivity of CLA and ALA for the Sn-2 position on TG. Sn-2 monoglycerides were obtained by reacting the milk total lipids (TL) from 24 sheep fed with control diet (C) and 24 sheep fed with a diet containing 200 g/die of extruded linseed (L) with swine pancreatic lipase, separated by thin layer chromatography (TLC). The selectivity of individual fatty acids for the Sn-2 position was evaluated by means of a selectivity index (SI) calculated as FA(Sn-2)/FA(TG); where SI<1 indicated a preferential esterification in alpha-position and SI>1 in Sn-2. As regard the fatty acid composition of triglycerides, L compared to C, showed a significant higher content of both ALA and CLA: +45% and +75% respectively (P<0.001). Regarding to the beta-position of TG, ALA was 1.1 g/100g of TL in C and 2.2 g/100 of TL in L (P<0.001), and CLA was 1.2 g/100g of TL in C and 3.5 g/100g of TL in L (P<0.001). As a consequence, in the milk from L group, the content of ALA and CLA increased by 100% and 191%, respectively, than in the milk from C group. These results seemed to indicate a preferential esterification of ALA and CLA in the beta-position of milk triglycerides, probably as a consequence of changes in selectivity of acyl-transferase enzyme (LPAAT). In particular, as regard CLA content, the affinity of LPAAT was inverted: in fact, in C milk, CLA was preferentially esterified in the alpha-position (SI=0.56), while in L milk the SI was higher than 2, suggesting a preferential esterification in the beta position.

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Use of extruded linseed in cow diet to improve cheese nutritional quality

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Nutritional and nutraceutical quality are considered more and more strategic for the differentiation of animal products. One of the factors able to influence the cheese quality is the animal diet. Aim of this trial, carried out within the NOVOROD project (PSR Campania 2007-2013 Misura 124 H), was to evaluate the capability of a diet enriched with extruded linseed to enhance the quality of cheese made from milk of dairy cows, reared into ordinary farms. With this aim, in 3 different farms, two homogeneous groups of 5 dairy cows were compared: T (traditional feeding) and L (extruded linseed supplemented). The amount of extruded linseed in the diet was calculated replacing part of the concentrate supplementation on a DM basis by 10% on average. After two weeks of adaptation to the diet, animals were fed with the experimental diet for other 15 days. During the experimental period, the milk was collected and cumulatively processed in Caciotta cheese (a soft cheese, 25 days ripened) for three times for each group in each farm. On cheese samples, the fatty acid profile was evaluated in duplicate. The fatty acid extraction was performed following the method by Blygh and Dyer (% FAME), using a gas-chromatograph Varian CP-800 for the separation of methyl esters. Moreover, the Thrombogenic Index (TI) and the Health Promoting Index (HPI) were calculated. The results showed a significant effect of linseed supplementation in L groups of all farms in comparison to T group, in particular on total omega-3 content, higher by 29% (0.98% *vs* 0.70%) and the ALA, alpha-linolenic acid, higher by 39% (0.71% *vs* 0.42%). Also the conjugated linoleic acid (CLA) showed a significant increase on average by 21.8% (0.98% *vs* 0.77%) in L groups. Saturated fatty acids meanly decreased by 5.5% (64.18 in L *vs* 67.92% in T), while unsaturated, monounsaturated and polyunsaturated fatty acids increased from 32.08 to 35.82%, from 27.15 to 30.07% and from 4.93 to 5.74% respectively. The lowest mean value for TI was found in L group cheeses (2.98 *vs* 3.49 in T), together with the highest HPI value (meanly 0.51 *vs* 0.41 in T). The results showed that it was possible to improve the fatty acid profile of cow cheese by enriching the diet with a concentrate source naturally rich in polyunsaturated fatty acid.

pagepress

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Milk aptitude for cheese-making as affected by substitution of maize silage with barley silage in dairy cow diet

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Aim of the study was to evaluate if substitution of maize silage with barley silage in dairy cow diet affects milk yield and chemical composition, milk coagulation parameters, cheese yield and sensory properties. Eighty Italian Friesian cows were divided in two groups of 40 animals. Treatments were: maize silage-based diet (MS) and barley silage-based diet (BS). Experimental design was a cross–over 2x2 with two periods of four weeks. The sampling period was on the last two weeks in each treatment period. Experimental controls were made on ten cows per group. These cows were homogeneous for milk characteristics, parity, distance from calve and milk yield. Milk aptitude for cheese-making was evaluated as rennet coagulation time (RCT, min), curd firming (k20, min) and curd firmness (a30, mm). About 8000 kg of milk were processed to Grana Padano DOP cheese in 4 days (1000 kg of milk/group/day) for a total production of 16 cheese wheels (2 cheeses/group/day). All cheese wheels were weighted at 3, 6 and 12 months of rippening. Data were subjected to statistical analysis with the MIXED procedure of SAS. Quantitative Descriptive Analysis was made on four 16-month cheese wheels (2 cheeses/treatment) by a trained panel of 10 judges. Generalised Procustes Analysis was performed on sensorial data. No differences were observed in milk yield and quality traits while the milk urea content was higher in BS than in MS ($P< 0.001$). No difference was found in cheese yield between treatments, although the cheese yield was always higher in BS than in MS, with a cheese weight loss of 6.3% (BS) and 7.2% (MS) during a seasoning period ranging from the 3rd to the 12th months. No differences among treatments were detected in cheese sensory profile. The experiment evidenced that barley silage can substitute maize silage in dairy cow diet without affecting cheese characteristics.

C-135

Carbon footprint of cow milk production in different farming systems

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There is an increasing concern about the role of livestock production on natural resources depletion and climate change. Dairy production is a recognized source of greenhouse gas (GHG) emissions and the expected doubling of world milk demand by 2050 creates additional alarm. The aim of this study was to estimate Carbon Footprint (CF) of cow milk production at the farm gate through Life Cycle approach and to identify production strategies that can help to mitigate GHG emissions. Data were collected in 102 dairy farms from Northern Italy, characterized by various farming systems and different location (plain and mountains). On-farm GHG emissions were estimated using IPCC (2007) models while off-farm emissions were quantified mainly using data from Ecoinvent (2007) database. CF (expressed as kg CO_2-eq.) of 1 kg of Fat Protein Corrected Milk was assessed using the Simapro software. Farming systems were characterized by wide variability in terms of size, milk yield, stocking rate, feed self-sufficiency and composition of cow ration. CF was on average 1.47±0.29 kg CO_2-eq. per kg FPCM and it also showed a large variation range. The main contributors to CF were emissions from enteric fermentations and manure storages (50.5%±5.87%) followed by commercial feed production (19.8%±5.82%). Farms were divided into groups on the basis of stocking rate, feed self-sufficiency and nitrogen balance and a GLM analysis was performed. No significant relation was observed between stocking rate and CF; feed self-sufficiency strongly affected the contribution to GHG emission from production of concentrate feed, but the relation with the total GHG production was not significant. Nitrogen balance showed a significant relationship with CF: farms with higher N surplus (> 600 kg/ha) had lower emissions (1.38±0.07 kg CO_2-eq./kg FPCM) than farms with lower N surplus (<200 kg/ha; 1.69±0.06 kg CO_2-eq./kg FPCM). Farms with higher N surplus had also significantly higher milk production level and higher feed conversion efficiency, characteristics that are recognized to have a mitigation effect on CF of milk production. The variation identified in milk CF indicates that it is possible to reduce GHG emissions from milk production at farm level through changes in management.

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Carbon footprint of dairy cattle farms in Southern Italy

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Accurate estimates of carbon footprint at local level are needed, to assess inventories from regions and countries and to detect emission sources to which mitigation strategies might be applied. This work aimed to study the sources of greenhouse gas emissions (GHG) in dairy cattle farms in Southern Italy. A total of 120 farms from 4 different cooperative milk processing plants of Sardegna, Puglia, Basilicata, Calabria, and Sicilia were considered. Farms were selected to obtain a wide range of number of present cows, milk yield per cow, and animal housing type. Data were collected with a direct survey asking information about: farm characteristics, number of heads and diets of each animal category, land use and management (ha, cultural plan, fertilizers, yield), facilities and equipment, and fuel and energy consumption. Life cycle assessment was used to estimate GHG, based on the 2006 IPCC Guidelines for National Greenhouse Gas Inventories. In particular, the Tier 3 method was used for livestock and for energetic emission, and the Tier 2 method was used for the soil management emissions. The results showed that the studied farms raised a total of 8836 mature cows and produced 60.1 kiloton of fat and protein corrected milk (FCPM; 4% fat). On average, farms had 73.6±90.2 cows, 39.9±46.3 ha, 2.3±0.67 animals per mature cow and 21.9±37.9 ton of milk produced per ha. Average milk yield was 6073±1810 (range: 1876-10,341) kg/year per present cow. Farm nutritional efficiency was, on average, 0.68±0.2 (range: 0.12-1.27) kg of milk per kg of consumed DM. Total GHG were 82.5 kilotons of CO_2 equivalent (CO_2-eq), being 95% attributed to milk and 5% to meat. On average, farms emitted 1.57±0.7 kg of CO_2-eq/kg of FPCM. The GHG weighted mean based on farm milk sold was 1.30 kg of CO_2-eq/kg of FPCM. Values of CO-$_2$eq per kg of FPCM were <1 in 5%, between 1 and 1.5 in 57%, between 1.5 and 2.75 in 27% and >2.75 in 10% of the farms. Contribution of emissions sources was estimated at 0.69±0.23, 0.37±0.29, 0.15±0.17, 0.13±0.10 and 0.123±0.13 CO_2-eq/kg of FPCM for enteric production, manure management, energy, soil management, and purchased feeds, respectively.

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Emission of greenhouses gas in Italy: an inventory for the sheep sector using the Tier 3 of IPCC guidelines

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Greenhouses gas emissions (GHG) inventories are often performed by using the TIER1/TIER2 of the 2006 IPCC Guidelines for National Greenhouse Gas Inventories (IPCC) that applies standard references to perform estimates for all livestock species. The IPCC procedures are not largely detailed for breed aptitude, livestock categories and management systems, especially in the sheep sector. Objective of this work was to assess the GHG emission of the Italian sheep sector by accounting for CH_4 from enteric fermentation and, CH_4 and N_2O from manure management, both expressed as CO_2 equivalents (CO_2-eq). Calculations were performed in a spreadsheet file (Excel®). Information about ovine population was obtained by the official national database of livestock population (www.statistiche.isz.it). The number of raised mature ewe for milk and meat production in each region (n=20) was obtained by gathering the average data of the year 2012. Regional diffusion, population structure and livestock system of the most representative milk breeds (Sarda, Massese, Comisana) and meat breeds (Bergamasca and Biellese; Appenninica, *Laticauda*, and Fabrianese; Sopravvissana and Gentile di Puglia) were derived from published literature. A meta-modeling approach was applied, within each animal category, to estimate: diets and metabolizable energy requirements for maintenance, activity, cold stress and production (Small-Ruminant-Nutrition-System software, www.nutritionalmodels.tamu.edu); emitted methane as a percentage of metabolizable energy intake (INRA, Prod.Anim., 2008, 22:403-418); nitrogen excretion (Option Mediterraneennes, 2011;59:201-209); emission factors for CH_4 and N_2O from manure (IPPC 2006). Total sheep population accounted for 8.03 million heads of which nearly 60% and 20% represented mature dairy and meat ewes respectively. Sheep were distributed in the whole national territory, but more than 70% in Tuscany, Lazio, Sardinia and Sicily. Total GHG emissions in Italy were 2463, 1792, 671 kiloton of CO-$_2$eq for total, dairy and meat sheep, respectively, representing the 15% of total GHG emission from the Italian livestock sector. In both dairy and meat productive systems, 90% of total emissions derived from enteric fermentation and 10% from manure management. Enteric fermentation was on average 10.96 and 11.28 kg/head/year of methane emitted from milk and meat sheep, respectively. Those values are higher than that proposed by the TIER1 of IPCC (8 kg/head/year).

P-001

Analyses of genes affecting ovulation rate in the native sheep breeds of Campania Region *Bagnolese* and *Laticauda*: preliminary results

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Bagnolese and *Laticauda* sheep are two native breeds reared in Campania Region for meat and milk production. Both breeds have an high fertility rate (93% and 97% respectively in *Bagnolese* and *Laticauda*), a high prolificacy rate (170% and 180% respectively) and a high fecundity rate (169% and 175% respectively). Aim of this work was to characterize for the first time the genes influencing ovulation rate BMPR1B, BMP15 and GDF9 in *Bagnolese* and *Laticauda* sheeps in order to verify the presence of polymorphisms that as in other breeds could be related to high prolificacy rate. Genomic DNA of 20 (6 males and 14 females) *Laticauda* and 30 (8 males and 22 females) *Bagnolese* sheep, coming from different farms located in the provinces of Avellino and Salerno, enrolled into breed register, have been analyzed with RFLP technique for the detection of FecB (BMPR1B) and of the polymorphism in GDF9 exon 1 G>A at nt 260 of the coding sequence. Among these animals BMP15 exon 2 and GDF9 exon 2 were sequenced in 10 *Laticauda* and 10 *Bagnolese* sheep, 2 males and 8 female in both cases. The allele FecB, notoriously associated with high ovulation rate in sheep, was absent in both breeds as well as the polymorphism G>A in GDF9 exon 1. In BMP15 exon 2 no one of the polymorphisms FecXH, FecXI, FecXG, FecXB associated in other breeds (Hanna, Iverdale, Cambridge and Belclared) with high ovulation rates has been found. In the GDF9 exon 2 new polymorphisms have been found either in *Laticauda* and *Bagnolese* sheep breeds. In *Laticauda* it has been found a G>A transition at nt 1358 of the coding sequence; in *Bagnolese* sheep a G>C transvertion at nt 412 and C>T transition at nt 531 of the coding sequence have been found. Moreover in both breeds other polymorphisms of GDF9 exon 2 already seen in other sheep breeds like Cambridge and Belclare have been found.

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P-002

Unofficial results of the first international genetic evaluation for body condition score in Italian Holstein Friesian cattle

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Italy participates in the official international evaluation for body condition score (BCS) since 2009 using angularity as best predictor. For the test-run of October 2012, Italy sent to Interbull BCS as direct trait. The aim of this study was to summarize the results of the test-run and compare them with the official-run of December 2012. The available information was: trait definitions, changes in national and international procedure, across-countries genetic correlations, and international breeding values of bulls. The change in trait used by Italy has led to an increase of average genetic correlations between Italy and other countries from 0.71 (test-run) to 0.87 (official-run). Regarding countries that used BCS as direct trait, the most visible changes in genetic correlations occurred with Swiss black and white Holstein (from 0.66 to 0.91), Germany (from 0.69 to 0.94), Czech Republic (from 0.71 to 0.94), and France black and white Holstein (from 0.75 to 0.96). Among countries that used a best predictor, the genetic correlation with France red Holstein increased from 0.51 to 0.81 and the relationship with USA decreased from 0.91 to 0.77 (USA uses angularity as best predictor). When Italy used BCS as direct trait, the number of Italian top-bulls increased in all ranks, particularly the number of top-1% bulls in ranks of Ireland (from 11 to 31), Switzerland (from 10 to 29), and USA (from 1 to 19). At the same time, in the Italian rank, the number of top-bulls of countries that used BCS as direct trait increased when Italy also used BCS (*e.g.*, in the Italian rank of October 2012 there were 113 top-1% bulls from the Netherlands, while in the official-run they were only 36). The number of bulls from countries that used a best predictor was lower, for example, bulls from USA doubled in the Italian rank from 224 (test-run) to 445 (official-run). Due to the unfavourable genetic correlation between BCS and production, the genetic trend of bulls for BCS was unfavourable for all countries. In order to slowdown such a trend, BCS should be considered as a selection criteria.

P-003

Polymorphisms in HSP70A1A, HSP90AB1 and HSF1 genes associated with heat tolerance in Italian Holstein cows

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Heat shock proteins (HSPs) and transcription factors (HSFs) play an important role in regulating heat stress response. Nucleotide changes in the flanking regions [5'- and 3'-untranslated region (UTR) or intron] of gene might affect inducibility, degree of expression, or stability of HSPs and HSFs mRNA. The aim of the present study was to investigate the presence of single nucleotide polymorphisms (SNPs) in HSP70, HSP90 and HSF1 genes associated with heat tolerance in Italian Holstein cattle. Three-hundred four Italian Holstein cows were genotyped for five SNPs in HSP70A1A gene, g115 C/- and g348 G/T in 5'UTR, g1524G/A in coding region and g3494T/C, g6600C/T, g6601G/A in 3'UTR; one SNP in HSP90AB1 gene g4338T/C within intron 3; two SNPs in HSF1 gene g909T/C in intron 3 and g4693G/T in 3'UTR. This study on genetic polymorphisms of inducible bovine HSP70A1A gene in Italian Holstein population showed monomorphic alleles for transition from guanine to adenosine at base 1524 in coding region. Distribution of alleles of the nucleotide sequence polymorphism within the 5'UTR of the bovine HSP70A1A gene were 70.6% and 29.4% for C and -, respectively, and 69.9% and 30.1% for G and T, respectively. In the 3'UTR of the bovine HSP70A1A gene, alleles distribution of SNPs were 65.8% and 34.6% for T and C, respectively, and 68.2% and 31.8% for C and T, respectively. The results on SNPs of bovine HSP90AB1 gene revealed an allele frequency of 58.6% and 41.3% for C and T, respectively, in intron 3. The distribution of allelic frequencies of g909T/C in intron 3 and g4693G/T in 3'UTR of HSF1 gene were 72.6% and 27.4% for T and C, respectively, and 73.4% and 26.6% for G and T, respectively. The significant novel aspect of these studies is that we have ascertained the presence of variants in the bovine HSP70A1A, HSP90AB1 and HSF1 genes in Italian Holstein dairy cows. These SNPs were identified in the same breed but in different population and were associated with thermo tolerance. We propose that these mutations should be studied in depth to ascertain the association with heat-tolerance in Italian Holstein cattle population.

P-004

Application of the Ion Torrent technology to identify single nucleotide polymorphisms in the rabbit genome

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Next generation sequencing (NGS) is changing the way to analyse and extract genetic information from all species. One of the most promising NGS platforms is represented by the Ion Torrent PGM (Personal Genome Machine) technology. The sequencing process of this machine is based on the detection of pH variation which occurs when H^+ is released during a nucleotide incorporation in the sequencing reaction. This chemical signal is then converted directly to a digital information. In this study we applied the Ion Torrent PGM technology to identify single nucleotide polymorphisms (SNPs) in the rabbit genome, a species for which massive SNP information is not available yet. Two reduced representation libraries (RRLs) were prepared. Genomic DNA pools were constructed with equimolar DNA of 10 rabbits from 4 breeds (Burgundy Fawn, Champagne d'Argent, Checkered Giant and Rhinelander) and from a commercial line. Pooled DNA was digested separately with two different restriction enzymes (HaeIII) and (RsaI). Digested DNA was electrophoresed on agarose gels from which a slice containing DNA fragments of about 500-600 bp was cut out and used to extract DNA. The isolated DNA was then prepared for the sequencing on 318 Ion Torrent chips following manufacturer instructions. From the two RRLs a total of 697.79 Mb (609.38 Mb with base quality ≥ 20), derived from 6,964,750 reads (with a mean length of 100 bp) were sequenced. Of these reads, 6,312,660 were mapped on the reference rabbit genome sequence (oryCun2.0) and then used for variant calling analysis. Retaining only single nucleotide variation with a mapping quality ≥10 and detected in at least 4 bases, 65,695 SNPs were identified, with a mean distribution of 1 SNP every 287 bp. Annotation of these SNPs was based on the oryCun2.0 genome version. Some of the putative SNPs were validated by visual inspection using IGV (Integrative Genomics Viewer) and by Sanger sequencing. These SNPs could be useful to design a commercial SNP genotyping platform for the rabbit.

P-005

Analysis of Illumina BovineSNP50 BeadChip data in different Italian cattle breeds to identify useful markers for breed authentication of dairy and beef products

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The identification of the breed of origin of cattle derived products has recently assumed particular relevance since the increasing interest in marketing mono-breed labelled cheese and beef. These products are usually sold at a higher price creating financial incentives to substituting them with lower value products coming from undifferentiated or common breeds or crossbred animals. Frauds can damage these valuable niche markets that, indirectly, may help to preserve genetic diversity through the economic incentive on raising local breeds, derived by the high value products, that are usually less productive. DNA analysis can help to identify fraudulent behaviour as we already demonstrated for *Parmigiano Reggiano* cheese produced from only Reggiana cattle milk. In this study we used Illumina BovineSNP50 BeadChip genotyping data from different cattle breeds to identify single nucleotide polymorphisms (SNPs) that could be useful for breed authentication of dairy and beef products. The dataset was made of genotyping data obtained from the chip v.1 or v.2 analysed on 749 Italian Brown, 2093 Italian Friesian, 479 Italian Simmental, 410 *Marchigiana*, 71 Cinisara and 72 Modicana cattle. About 51,000 SNPs were retained for further analyses after filtering according to a few parameters (Hardy-Weinberg equilibrium ≥0.0001; call rate ≥0.90). A quite high number of SNPs presented fixed alleles in one breed that had minor allele frequency (MAF) >0.30-0.40 in the other breeds. This was true for all breed combinations. These SNPs were indicated as highly informative for authentication purposes. Using the selected panel of SNPs, Principal Component Analysis (PCA) was able to discriminate investigated breeds. These results may represent a starting point to develop targeted DNA based tests for authentication of mono-breed cattle products.

P-006

Selection signatures in five cattle breeds detected by a 50K single nucleotide polymorphism panel

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The widespread use of single nucleotide polymorphism (SNP) chip has enabled, among others, the investigation of selection signatures at genome-scale level within and across breeds. Indeed, genome evolution studies are a prerequisite for present and future selection strategies for cattle breeding allowing the detection of regions under recent or past selective pressure. In our study, a total of 3220 animals from dairy (Italian Holstein=1088, Italian Brown=775), dual purpose (Italian Simmental=493) and beef (*Marchigiana*=485, Piedmontese=379) breeds, were genotyped with the Illumina 50K SNP v.1. BeadChip, in the frame of the Italian livestock genomic project SelMol. After standard cleaning procedures, genotypes were phased with FASTphase software. Then, core haplotypes were identified, and the age of each core haplotype were assessed measuring the Linkage Disequilibrium (LD) decay at various distances from that *locus* with the extended haplotype homozygosity (EHH). To correct for the lack of good estimates for local recombination rates, the relative EHH (rEHH) was calculated for each core haplotype. rEHH is the factor by which EHH decays compared with the decay of EHH on all core haplotypes combined. Therefore, in order to identify genomic regions under recent positive selection, we searched for core haplotypes that have significant rEHH values, obtained using Sweep 1.1 software. Significant independent core haplotypes were aligned across breeds to identify those specific for dairy (Italian Brown, Italian Holstein and Italian Simmental) and beef (*Marchigiana*, Piedmontese and Italian Simmental) production. Overall, a total number of 82 and 87 genomic regions appear to be under selection for dairy and beef cattle, respectively; bioinformatics analysis identified respectively 244 and 232 genes mapping in these genomic regions. These common signals of selection, detected independently in each breed, increase the power of the analysis and allow to better pinpoint the genomic regions of interest in dairy and beef production.

P-007

Deep mRNA-sequencing reveals alternative spliced isoforms differentially expressed in nervous tissue of cattle affected by spastic paresis

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Bovine Spastic Paresis (BSP) is a neuromuscular disorder which affects both male and female cattle, characterized by spastic contraction and overextension of the gastrocnemious muscle of one or both limbs and related to a scarce increase in body weight. It has been proposed that the disease is caused by an autosomal and recessive gene with incomplete penetration, although no genes clearly involved have been so far identified. We used the Illumina RNA-Seq to study the transcriptome profiles from spinal cord of healthy and BSP *Romagnola* animals. Our RNA-Seq generated 18,949,841 short sequence reads. We used the short read aligner Bowtie to align reads on genome and TopHat pipeline to map splice junctions in RNA-Seq reads and Cufflinks to estimate transcript abundances and to test for differential expression and regulation in RNA-Seq samples. We found 965 genes significantly over/under expressed in affected animals versus healthy controls. Of these, 67 genes were more than two-fold over- in healthy animals while 564 were more than two-fold under-expressed in BSP animals. We found significant differences in gene isoform expression levels (1039 isoforms were differentially expressed), alternative use of promoters and transcription start sites between normal and BSP nervous tissue. We identified 74 alternatively spliced genes, among those the RYR1 gene, encoding for the skeletal muscle ryanodine receptor, which serves as a calcium release channel of the sarcoplasmic reticulum, showed a novel spliced isoform. This spliced variant of RYR1, which lacks intron 70, was found differentially expressed in healthy and affected animals. Also the CACNA2 and the TRDN gene expression seems to be altered in BSP animals. Together with the RYR1 gene, the dihydropyridine-sensitive calcium channels (CACNA2) and the TRIADIN (TRDN) play key roles in the generation of calcium transients during excitation/contraction coupling in skeletal muscle. The GO term enrichment analysis revealed an overrepresentation of genes associated with biological processes as striated muscle tissue development and contraction; with cellular components as sarcoplasmic reticulum, contractile fiber and sarcomere; with molecular functions as calcium ion binding. This study provides, for the first time, a transcriptomic analysis of BSP using next generation sequencing technology.

P-008

Fine mapping of calving ease in Piedmontese cattle

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Calving in cattle is affected by calf morphology and by dam characteristics. It is described by two different traits: maternal calving ease, which is the ability to generate dams with good physiological predisposition to calving, and direct calving ease, which is the ability to generate calves that are easily born. The aim of this study was to identify regions of cattle genome harboring genes possibly affecting direct calving ease in the Piedmontese cattle breed. A population of 323 bulls scored for direct calving ease (EBV) was analyzed by a medium-density SNP marker panel (54001 SNPs) to perform a genome-wide scan. The strongest signal was detected on chromosome 6 between 37.8 and 38.7 Mb where 13 SNPs associated to direct calving ease were found. Three genes are located in this region: LAP3, encoding for a leucine aminopeptidase involved in the oxytocin hydrolysis; NCAPG, encoding for a non-SMC condensin I complex, which has been associated in cattle with fetal growth and carcass size; and LCORL, which has been associated to height in humans and cattle. To further confirm the results of the genome-wide scan we genotyped additional SNPs within these genes and analyzed their association with direct calving ease. The results of this additional analysis fully confirmed the findings of the GWAS and particularly indicated LAP3 as the most probable gene involved. Linkage Disequilibrium (LD) analysis showed high correlation between SNPs located within LAP3 and LCORL indicating a possible selection signature due either to increased fitness or breeders' selection for the trait. Currently, we are genotyping the Piedmontese breed with the 800K SNP chips (BovineHD) for genetic association studies to discover genes and haplotypes that can predict breed-specific traits.

P-010

Effects of milk protein polymorphisms on milk traits: an across-breed study

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The relationships between the genetic polymorphisms of the 6 main milk proteins have been extensively investigated in the bovine species, although some conflicting results have been reported. The aim of this study was to compare the milk protein structure and their effect on milk traits in three breeds, Italian Friesian (IF), Italian Brown (IB), and Italian Red Pied (IRP). More than 450 proven sires were selected within each breed. The four milk caseins (αS1-CN, β-CN, αS2-CN, and κ-CN) and the two whey proteins, α-lactalbumin (α-LA) and β-lactoglobulin (β-LG), were genotyped at the DNA level within the corresponded genes (CSN1S1, CSN2, CSN1S2, CSN3, LGB, LALBA) by SNP genotyping. A total of 13 SNP were considered allowing the identification of the main genetic protein variants or groups of variants as well as some polymorphisms within the non-coding sequences. Associations between each SNP and the estimated breeding values (EBV) for milk, protein and fat yield and protein percent were evaluated within each breed. Favourable effect of the κ-CN B variant were observed in all breeds, mainly related to protein yield (IB, IRP) and protein percent (IF, IRP). Two casein variants were specifically typed in the IF; β-CN I was significantly associated with protein yield (+22.57 kg) and protein percent (-0.04%), whereas κ-CN E negatively affected milk yield (-257.8 kg) and fat yield (-13.2 kg). The αS1-CN C variant was favourably associated to protein percent (+0.04%) in the IB, where a higher effect was found (+0.07%) for a SNP within the CSN1S1 promoter. Significant effects were found in all breeds for LGB, both for the SNP associated to the main β-LG protein variants (A and B), and for a SNP within the LGB promoter. The trends of the effects were different in the 3 breeds for the yield traits, whereas LGB A variant was positively associated with protein percent (+0.02%) in IB and IF. Association with the traits were also calculated after reconstruction of the haplotypes considering all the 13 SNP. Results demonstrate the importance of considering milk protein polymorphisms and the classic protein variants in breeding programmes.

P-011

Identification of genomic regions influencing blood and milk traits in dairy cows

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The development of high throughput SNP platforms allows to study the genomic structure for several livestock species, and to perform association studies to identify putative genomic regions implicated in phenotypic variation. In order to explain the genetic determinism of multifactorial traits, Multivariate Factor Analysis (MFA) could be proposed.

In this work, 44 Holstein and Bianca Val Padana dairy cows were genotyped with the 50k SNP panel and 55 milk quality, 23 hematological and 25 blood plasma metabolic parameters, were determined. MFA was used to extract latent variables from covariance matrix of marker genotypes separately for each chromosome (F_gen) and for each phenotype groups (F_phen). Subsequently, genomic regions linked to phenotypic traits were evaluated computing the Pearson correlation among F_gen and F_phen scores (threshold $r>|0.55|$). MFA was able to extract 50 new synthetic and uncorrelated variables for each chromosome from 37,378 SNPs retained after editing, explaining between 98 and 99% of the total variability. At the same time, 13, 9, and 8 factors extracted from milk quality, hematological and blood plasma metabolic parameters respectively, were able to explain about 88% of the phenotypic variation. For milk quality 4 of 13 F_phen exceeded the threshold with 13 F_gen extracted from 10 chromosomes (max $r=0.67$: F_phen 12 *vs* F_gen 24 chromosome 8). For haematological parameters 3 of 9 F_phen exceeded the threshold with 5 F_gen extracted from 5 chromosomes (max $r=-0.71$: F_phen 5 *vs* F_gen 19 of chromosome 24). For blood plasma metabolic parameters 4 of 8 F_phen exceeded the threshold with 19 F_gen extracted from 17 chromosomes (max $r=0.65$: F_phen 3 *vs* F_gen 1 chromosome 18). This preliminary analysis showed that it is possible to identify genomic regions involved in some phenotypic traits by exploiting SNP variability.

P-012

Productive and reproductive performances of Alpagota sheep breed

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In the contest of recognition, characterization and management of animal genetic resources (AnGR), a research about the Alpagota sheep breed has been started. This breed, classified as a multipurpose (meat, wool and milk), is characterized by a great adaptation to the environment in which it is raised and it is suitable to exploit marginal areas. The population size is estimated to be approximately 2400 heads in about fifty flocks (APA, Belluno, 2010) and is of strategic importance to the economy and the protection of the mountain ecosystem. Moreover, this breed might be of interest in terms of genetic diversity and may constitute a pool of rare gene variants. For two consecutive years, for 567 ewes of three representative flocks phenotypic, productivity and reproductive data were recorded. In addition, milk daily yield and quality (fat, protein and lactose) were collected weekly for twenty ewes, and a wool sample was collected for forty subjects. Prolificity was slightly greater than one (1.06), the fertility and fecundity were of 0.73 and 0.77, respectively. Weight of lambs were 3.5 kg and 23.0 kg at birth and slaughter respectively, whit an average daily gain of 165 g/d. Milk production was about 21 kg in 127 days, with a good quality (fat=6%; protein=5.7%; lactose=4.7%). The wool had a fiber diameter of over 30 microns, a tenderness of 49.80 and a number of fibers of 4540. Therefore, it may be included among the medium quality wool, adequately soft and compact and suitable for particular yarns. Results obtained from this preliminary study confirm that the ancestral features of this breed have been maintained and that productive and reproductive performances are good but can be improved. Further studies are needed to define management guidelines of this AnGR with the main goal to safeguard genetic variability and at the same time improve economic performance.

P-013

Evaluation of the trend of the inbreeding from 1970 to 2011 in the Bracco Italiano dog breed

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The Bracco Italiano is one of the oldest pointing dog breed, used for hunting ever since the Renaissance time. In this study we analysed the pedigree information to evaluate the trend of the inbreeding of the breed in the course of time since 1970 to 2011. Pedigree records of 24,613 animals were considered. 16,832 dogs were inbreds. The average inbreeding coefficient in the reference population (23,997 animals) resulted 4.1%, while the average inbreeding of the inbreds was 6%. The inbreeding coefficient was <0.05 in 9849 dogs (58.51%) whereas it was >0.20 in 623 dogs (2.53%). The percentage of inbreds per year increased from 2.44% in 1976-1980 to 100% in 2003. 16 traced generations were highlighted. The evolution of inbreeding indicates a steady increase over time, from a mean value of 0.5% for dogs born in 1979, to an average value of 7.6% in dogs born in 2011. Nevertheless a regular monitoring of genetic variability of the population 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.

P-014

Genomic analysis for the valorization of *Nero Siciliano* swine breed

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Local breeds are a resource of great interest both from the genetic point of view, since they can be a repository of allelic combinations rare or absent in the selected breeds, and for the maintenance of marginal areas, since they can easily be associated to typical products helping farmers manage and protect the environment. *Nero Siciliano*, an autochthonous black pig reared in the natural park of the Nebrodi Mountains, is an example of such possibility. Used to produce high quality meat, including salami and cured ham, it has known an important increase in the number of farms and in sow reared in the last ten years thanks also to the creation of a Protected Designation of Origin label for *Nero Siciliano* meat and other related products. Genetic characterization is a fundamental prerequisite for managing genetic resources and can be exploited for setting up molecular authentication protocols. To characterize the breed a representative sample of 93 *Nero Siciliano* pigs was selected from 22 farms well distributed in the Nebrodi area and analyzed using the PorcineSNP60 Genotyping BeadChip v2 (Illumina, San Diego, CA, USA), containing 61,565 SNP. Descriptive parameters were calculated (heterozygosity, LD, etc.) and a comparison with data obtained from the Italian Large White, Landrace and Duroc breeds using the same genotyping platform was performed. Different SNP sets (200, 150, and 100 randomly selected SNP, a subset of 100 SNP monomorphic in the *Nero Siciliano* or in the other three breeds) were tested with Structure and GeneClass2 software in order to distinguish the *Nero Siciliano* from the cosmopolitan breeds. The results suggest that a 100 random SNP dataset is sufficient to discriminate among the four breeds. Structure software was also used on 80 samples for which microsatellite data were also available to evidence potential population structures among the *Nero Siciliano* breed. Similar results were obtained with both microsatellite and SNP markers.

P-015

The Rough Collie dog breed: a depth of the pedigree of eighty traced generations

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The Rough Collie is a dog breed of ancient origins. In Italy, about 1000 dogs per year were recorded to ENCI in the period 1980/1995 while nowadays about 400 puppies/year are registered. The aim of the present study was to determine the depth of the pedigree and the genetic structure of the breed starting from a single dog born in 2012 and belonging to an Italian breeder. The complete genealogy included 2713 dogs (1012 males and 1701 females) distributed among 80 traced generations. 1582 dogs were inbreds (636 males and 946 females) and the inbreeding coefficient was <0.05 in 374 dogs whereas it was >0.25 in 276 dogs. Four stallions had more than 30 descendants. The average inbreeding coefficient over all animals excluded the base of the population (496 animals) resulted 8.4% (47.7% as maximum value). Starting from the animals with 64 traced generations all animals were inbreds. The evolution of inbreeding indicates a steady increase over time, from a mean value of 0.24% for dogs belonging to the 2nd traced generations, to an average value of 28.84% in dogs belonging to the 73rd generations. Despite the attention of the breeders in the accuracy of genealogical records, the levels of inbreeding that can be deducted from this dog are very high, thus a regular monitoring of genetic variability of the population must be adopted, in order to safeguard the genetics health of the breed.

P-016

Polymorphism analysis of AMY1, AMY2, SLC5A1, SLC2A2 and SLC2A5 genes in Italian horse breeds

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The dietary demand of modern competition horse relies on high-cereal feeding daily interspersed by limited forage and it is frequently characterized by marked changes in carbohydrate fractions, above all starch, compared to natural grazing conditions. These dietary variations influence digestion, metabolism, and increase the risk for colic and laminitis, both main causes of equine mortality. Aim of this work has been to analyze the polymorphism of genes related to digestion and absorption of complex carbohydrates, particularly starch, to make a comparison between different cosmopolite and Italian native horse breeds, characterized by marked differences in feeding and management conditions. The analyzed genes were: salivary and pancreatic alpha-amylases (AMY1 and AMY2, respectively), solute carrier family 2, facilitated glucose transporter member 2 (SLC2A2) and member 5 (SLC2A5) and sodium/glucose cotransporter 1 (SLC5A1), code for GLUT2, GLUT5 and SGLT1 enzymes, respectively, main routes for the transport of dietary sugars from the lumen of the intestine into enterocytes. Horses belonging to three cosmopolite breeds, Throughbred, Purebred Arabian and Friesian, and three southern Italian native breeds, Sanfratellano, Indigeno Siciliano and Murgese, 2 DNA samples per breed, were pooled. Single-nucleotide polymorphism (SNP) detection was performed by sequencing one DNA pool per breed. A total of 13 novel SNPs were found. Six SNPs are located in AMY1 gene and 3 are in exons, of which 1 is non-synonymous. A non-synonymous SNP was also identified in exon 1 of AMY2 gene. No SNPs were found in the SLC2A2 gene region analyzed, while 4 SNPs were characterized in 3'UTR of SLC2A5 gene. Finally, 4 SNPs were found in SLC5A1 gene, all located in intron 3. Three out of the SNPs found in AMY1 and AMY2 genes were genotyped in 121 horses belonging to the analyzed breeds, with the addition of Haflinger and Sella Italiano breeds samples. The results of the SNP genotyping are presented.

P-017

Separation of donkey's caseins by cation-exchange chromatography

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Protein composition of donkey's milk (DM) has recently gained interest in research studies. In the last years, clinical trials showed promising results for the use of DM as a whole in the dietotherapy of patients affected by IgE-mediated and non-IgE-mediated cow's milk protein allergy. The mechanism of the observed tolerability is not yet clarified, but it is reasonably related to the structural features of DM's proteins as well as to their high similarity to human's milk counterpart with respect to bovine's milk. Within the context of protein characterization, the aim of this work was to separate donkey's caseins with the final goal of obtaining protein standards for use in quantification analysis. The whole casein fraction was obtained, by isoelectric precipitation, from a single donkey's milk sample. The separation was carried out by cation exchange chromatography (CEX) implemented in an ÄKTA system. A first analytical analysis was aimed to find the correct run conditions to differentially elute the caseins from the acid mixture. Subsequently, the method was adapted to a preparative column in order to collect the different protein peaks. Chromatography collections were then submitted to mass spectrometry analysis to identify the proteins and measure their purity. Three different fractions were obtained by eluting the whole casein from the cation exchanger column: in addition to the pure β-casein (F1) which clearly eluted at the beginning of the gradient, two additional protein groups were obtained, one showing κ-casein and αs-caseins (F2) and the other containing αs-caseins (F3). In view of setting up a preparative method aimed at obtaining protein standards, only F1 and F3 collections represent useful results thanks to their high amount while F2, though analytically interesting, represents a poor result on the quantitative side of the research.

P-018

Sequence characterization of the agouti signalling protein and melanocortin receptor genes in donkeys with different coat colours

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Coat colour in farm animals is one of the most important traits that distinguish breeds and populations. This phenotype is mainly determined by the distribution of eumelanins (black pigments) and pheomelanins (red/yellow pigments). Their synthesis is regulated by the melanocortin 1 receptor (MC1R/extension *locus*) that binds the α-melanocyte-stimulating hormone (alpha-MSH) and the agouti signalling protein (ASIP, coded by the agouti *locus*). Loss-of-function mutations in MC1R lead to red/yellow pigmentation, while gain-of-function mutations cause black/dark colours. Mutations at the agouti *locus* exert, in general, epistatic actions over the extension *locus*. These two key genes have been already characterized in most farm animal species, including the horse in which a few mutations are associated with different coat colours. Only partial sequence information is already available in donkey (*Equus asinus*) and no mutations affecting coat colour have been reported in this species so far. In this study, we used primer pairs designed on the horse ASIP and MC1R gene sequences to amplify genomic DNA from 10 donkeys (from Ragusano breed of bay coat colour and other not classified animals) with different coat colours and patterns. ASIP and MC1R gene sequences were obtained in Equus asinus, completing information available in GenBank database. Two polymorphic sites were identified in the MC1R gene whereas no variability was observed in the ASIP gene. Other studies are currently underway to evaluate if the identified mutations affect coat colour in donkeys and to identify other mutations associated with coat colour phenotypes in *Equus asinus*.

P-019

Analysis of PRKAG3 gene polymorphisms in Italian local pig breeds

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The PRKAG3 gene encodes for the regulatory gamma 3 subunit of adenosine monophosfate-activated protein kinase (AMPK) protein that plays a relevant role as regulator of carbohydrate and fat metabolism in mammalian skeletal muscle cells. Several single nucleotide polymorphisms (SNPs) in the porcine PRKAG3 gene have been previously identified. Two missense mutations (c.595A>G and c.599G>A, giving p.I199V and p.R200Q aminoacid substitutions, respectively) were the most studied in different pig breeds and populations and have been associated with meat and carcass traits. Pigs that carry the dominant c.599A allele (p.200Q or allele RN-) have excessive skeletal muscle glycogen content and give the so-called acid meat (also referred to as Hampshire type meat). So far, the p.200Q allele has been identified in Hampshire and Hampshire derived lines. The c.595A>G SNP has been associated with variation of numerous traits including water holding capacity, pH, colour, carcass composition, and several quality traits of dry-cured ham. In this study, we investigated the distribution of the c.595A>G and c.599G>A PRKAG3 SNPs in five Italian local pig breeds: *Apulo-Calabrese* (64 pigs), *Casertana* (28), *Cinta Senese* (77), *Mora Romagnola* (22) and *Nero Siciliano* (109). Sampled pigs were from different herds and registered to the Anagraphic Book of the National Pig Breeders Association (ANAS). Genomic DNA was extracted from hair roots or blood and SNP genotyping was performed by PCR-RFLP. None of the animals of the Apulo-Calabrese, *Casertana*, *Cinta Senese* and *Mora Romagnola* carried the c.599A (p.200Q) allele. This allele was carried by only 3 *Nero Siciliano* pigs, suggesting that Hampshire or Hampshire-derived lines could have contributed to shaping *Nero Siciliano* genetic pool, as in part supported by other studies on coat colour. All breeds were polymorphic at the c.595A>G site. In agreement with studies performed in other breeds, the c.595G (p.Ile199) allele was the less frequent in all analysed local breeds with minor allele frequency >0.10. Based on identified allele frequencies, the c.595A>G SNP can be useful in association studies with meat, carcass and ham quality traits in the Italian local pig breeds.

P-020

Advanced research in genomics and processing technologies for the Italian heavy pig production chain: AGER-HEPIGET, a project for meat quality

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In Italy more than 70% of the pig production is dedicated to the processing of high quality PDO dry cured hams and other cured products. In AGER project, research activities have been planned to introduce innovations, practical applications and know-how in several parts of pig production chain to improve efficiency, quality of meat and dry cured hams, processing technologies, product safety. In order to elucidate the molecular basis of fat deposition and fatty acid composition in pigs the collection of biological samples (blood, muscle, backfat) and the phenotyping for the most important fat traits and meat quality parameters has been realised. This research will be carried out by integrating genome-wide SNP genotyping (SNP60K Chip), transcriptomic analysis of adipose tissue (RNA-seq to obtain mRNA and miRNA profiling) and Copy Number Variation analysis of porcine genome. In order to increase the efficiency and safety of the pig production chain a biorepository and a database of new phenotypes related to immune response with particular reference to PRRS has been developed and markers and/or genes associated with the immune traits have been identified. A genomic selection plan in pig breeding schemes aimed to control immune-capacity traits will be simulated. An innovative carcass sanitization system for pig abattoirs started to be tested in the project substituting the standard procedure with an alkaline hydrolysis. The effects of the sanitizing/dehairing treatment on skin, on fresh meat and on salami have been analysed. Two aspects of the project are related to dry-cured and cooked ham technologies, to improve nutritional properties by sodium reduction and to investigate about a spoilage defect of dry cured ham of unknown origin and prevention. Salt reduction is the aim to achieve in dry-cured hams by a technological treatment aimed to the control of proteolysis and production of bioactive peptides while in cooked hams formulation, tumbling and cooking conditions will be modified to this aim. To investigate the so-called vein defect, spoiled dry-cured hams is analysed and compared with defect-free hams: the isolated bacteria will be characterized to identify their role in the origin of vein defect.

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P-021

Use of the canonical discriminant analysis to select a reduced pool of single nucleotide polymorphism markers for sheep breed assignment and traceability purposes

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Several market research studies have shown that consumers are concerned with the source of the food they eat. Then, the development of methodologies able to trace animal derived foodstuffs is become of primary interest. DNA-based technologies seem to be the most useful tools to completely prevent consumers from frauds. Moreover, to satisfy the increasing number of consumers demanding for edible products of certified origin, the development of a breed DNA traceability and authentication methods might represent an important marketing strategy. In this study, a new statistical method to discriminate among 21 different Italian sheep breeds and assign new individuals to groups was applied. For each breed, 22 animals were genotyped using the Illumina OvineSNP50 BeadChip: 20 animals were designed as training population (TP), 2 as validation population (VP). For each breed, monomorphic markers and SNP with MAF<0.15 were discharged and, at the end of the data editing, the retained markers were 26,450. The canonical discriminant analysis (CDA) was used to discriminate among breeds. CDA was first applied at chromosome level and markers whose canonical coefficients were higher than 0.27 were retained. A genome-wide CDA was then developed with only the selected markers to test the separation among breeds. Finally, the discriminant analysis (DA) technique was used to assign the VP to the proper breed. The by-chromosome CDA selected a total of 265 top discriminant SNPs and the genome-wide CDA gave a significant separation among all groups. The DA was able to assign 42 over 46 independent individuals to the proper breed. Only for four breeds, the assignment percentage was 50%. These results suggested that an assay with the selected 265 SNP could be used to routinely track monobreed products.

P-022

Identification of polymorphisms in the rabbit melanocortin 4 receptor gene and association with finishing weight in a commercial rabbit population

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Melanocortin 4 receptor (MC4R) is a member of the G protein-coupled receptor (GPCR) superfamily consisting of seven transmembrane domains with an extracellular NH2 tail and an intracellular COOH terminus. MC4R is mainly expressed in the hypothalamus in which plays a key role in controlling energy homeostasis and food intake with effects on body weight and fat deposition. Polymorphisms in the melanocortin 4 receptor (MC4R) gene have been already associated with growth performance in different species, like pigs, cattle, sheep and chicken and in humans several mutations cause severe obesity. For these reasons MC4R can be considered a candidate gene to identify markers associated with production traits in rabbits. In this study we resequenced 1729 bp of the MC4R gene in 31 rabbits from different breeds and lines and identified ten polymorphisms: one was a missense mutation (p.G34D), located in a conserved position of the extracellular tail of the MC4R protein. The missense mutation was genotyped in 516 performance tested rabbits of a commercial paternal line under selection for growth efficiency. Association analysis indicated that rabbits with the less frequent genotype in this population (DD) had a lighter weight at 70 postnatal days than animals with genotype GD (P<0.10) and animals with genotype GG (P<0.05). This result may confirm the important roles of variability in the MC4R gene in affecting growth and related traits across species.

P-023

A comparative serum metabonomic analysis between Italian Duroc and Italian Large White pigs

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Extensive metabolic profiles of body fluids, obtained by nuclear magnetic resonance (NMR) spectroscopic or mass spectroscopic data, can provide information to deduce the effects of many different factors on biological processes. In particular, genetic factors can determine differences in physiological processes that are captured by metabolomics or metabonomics analyses. In this study we compared metabonomic profiles obtained from serum of two pig breeds (Italian Large White and Italian Duroc) with the aim to disentangle breed specific biological aspects. Twelve castrated males (6 Italian Large White and 6 Italian Duroc) were raised at the test station of the National Pig Breeders Association (ANAS) in the same conditions till they reached a live weight of about 160 kg. After a period of starvation, early in the morning pigs were transported all together at a commercial slaughterhouse, electrically stunned and jugulated within 10 minutes. Collected blood sample were processed after about 2 hours and serum was stored at -80°C till use. High Resolution Magic Angle Spinning-NMR serum spectra were recorded with a Bruker AVANCE spectrometer. Each sample was analysed in triplicate. Preliminary Principal Component Analysis of the obtained NMR spectra indicated that metabonomic analysis could be able to distinguish breed specific metabolic profiles. These results might represent useful information to disclose genetically determined regulatory pathways contributing to understand breed differences in biological processes indirectly linked with production traits.

P-024

Association between twenty four candidate gene markers and milk production and composition traits in *Reggiana* dairy cattle

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The identification of quantitative trait *loci* (QTL) and the subsequent characterization of quantitative trait genes (QTG) have been the objective of several studies in dairy cattle usually using cosmopolitan and widely spread breeds. The effects of these QTG have been only in few cases investigated in local breeds. *Reggiana* is a local dairy cattle breed that account less than 2000 cows reared in the North of Italy, mainly in the province of Reggio Emilia. Its milk is used for the the production of mono breed *Parmigiano Reggiano* cheese that is sold at a higher price than undifferentiated cheese. In this study, we analysed 24 candidate gene markers that other studies in other breeds have shown to be associated with milk production and composition traits. A total of 128 *Reggiana* sires (born from 1984 to 2001) were genotyped. Estimated breeding values were provided by the National Association of *Reggiana* Breeders (ANABORARE) for the following production traits: milk yield, protein yield and percentage, fat yield and percentage and cheese production index. The effects of the 23 markers on the 6 traits were estimated with a model that accounted for the year of birth and weighted for the reliability level. Two association study methods were used: i) GLM of the software package SAS, without including any pedigree information; ii) ASREML, including pedigree information, and including in the model the random animal effect. Significant associations (P<0.001) were obtained between the DGAT1 p.K232A mutation and fat yield, protein percentage and cheese production index and between a GH1 polymorphism and fat percentage and protein percentage. These results will be useful to evaluate marker assisted selection strategies in Reggiana cattle as genomic selection programmes seem not reliable in numerically small breeds.

P-025

Evolution of alphaS1-casein polymorphism in Italian Alpine and Saanen breeds

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AlphaS1-casein, coded by CSN1S1 gene, is one of the most important goat milk protein, although, in this species, it represents on average only the 5.6% of the total casein content. On the basis of the milk content of alphaS1-casein, the CSN1S1 variants can be grouped into 4 classes: strong alleles (A, B1, B2, B3, B4, C, H, L, and M), producing almost 3.5 g/L of alphaS1-casein each; intermediate alleles (E and I; 1.1 g/L); weak alleles (F and G; 0.45 g/L); and null alleles (01, 02, and N) apparently producing no alphaS1-casein. Previous studies have reported that the CSN1S1 can affects casein, protein and fat levels, total solids, milk rheology, as well as cheese yield and quality. The aim of the study was to evaluate how the frequencies of the major CSN1S1 allelic variant have changed during the last 10 years in the two main dairy goat breeds raised in the North of Italy: Italian Alpine and Saanen. Two hundred and twenty three Alpine and 129 Saanen were sampled from 8 farms spread all over Lombardy. AS-PCR and RFLP-PCR were performed to genotype the major allelic variants at the alphaS1-casein *locus*. Only the Saanen breed proved to be in Hardy-Weinberg equilibrium at this *locus*. The allele frequencies observed in the present work showed statistically significant differences (χ^2 test, P<0.005) compared to those reported in two studies published in 2004. This difference was due to an increase of the strong allele A (12% and 18%) and the intermediate allele E (15% and 8%) nearby a reduction of the weak allele F (17% and 12%) and the strong allele B (11% and 13%) respectively in Saanen and Alpine breeds. The A allele is well known for being associated to smaller micellar casein, with a consequent better cheese yield. Recent indications of selection concerning Alpine and Saanen bucks favours both strong and intermediate alleles, consistent with the described strong frequency increase of CSN1S1^E. For the top farms analysed, with over 85% genotypes with strong alleles, these directives could lead to an increase of the allele E and consequently to a reduction of protein content and cheese yield.

P-026

NOD2 - a candidate gene for susceptibility to paratuberculosis in sheep

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This study was aimed at exploring correlations between genetic polymorphisms and susceptibility to paratuberculosis in *Sarda* sheep breed by amplifying the full sequence of the ovine NOD2 gene, currently available only for cattle. Blood and faeces of 95 individuals were collected in a flock positive to *Paratuberculosis*. We first performed an Elisa test on blood, identifying 51 positives and 44 negatives. To verify the results, faeces samples were then analyzed by PCR. Genomic DNA was extracted from blood of the same individuals using NucleoSpin Tissue kit (Macherey& Nagel) and quantified by a fluorimeter DTX Multimode Detector 880 (Beckman Coulter). In a previous study we identified 3 SNPs in the NOD2 gene, candidate for susceptibility to paratubercolosis in ruminants and to Chron disease in humans. None of the SNPs resulted significantly associated with the disease (P≤0.05) but two borderline values (P≤0.056 and P≤0.083) were obtained for two of the SNPs. This could suggest that other SNPs within the same gene could be associated with paratuberculosis in sheep. We therefore designed primers to amplify the full sequence of the NOD2 ovine gene. Seventeen primers pairs were used to amplify and sequence a panel of 10 affected and 10 control samples. The sequences were BLASTed to verify the correspondence with bovine sequence. About 12,000 bp on a total of 31,000 bp according to the bovine NOD2 sequence were successfully sequenced and submitted to Genbank. We found 36 polymorphic sites that will be tested for association with the disease.

P-027

Phylogenetics inferred from mitogenome and mtDNA regions of *Ovis aries*

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Recently, technological advances in molecular biology have given the possibility of obtaining mitochondrial genome sequences. Several studies demonstrated that the use of mitogenome, instead of mtDNA single regions ad D-loop, provide a better resolution in reconstructing species phylogeny and phylogeography. The aim of our research was to sequence the complete mitogenome of *Ovis aries* and examine the utility of different mtDNA components in phylogenetic analysis. We sequenced mitochondrial D-loop from about 940 samples belonging to 66 sheep breeds from Mediterranean and Eastern Europe and to European mouflon (*Ovis musimon*), identifying a total of 146 polymorphic sites and 383 haplotypes. From total samples, 48 more representative of species diversity were selected for complete mtDNA. Alignment of individual sequences of different breeds to GenBank reference sequence (NC_0019041.1) showed several polymorphisms in several regions of mitogenome. Phylogenetic tree analysis of the mitogenome from 48 domestic sheep showed all five haplogroups described in literature HA, HB, HC, HD and HE with a 100% bootstrap support. In addition we divided the complete mitogenome in different regions: 13 protein-coding genes, 2 srRNAs, D-loop and a concatemer of transfer RNAs. The helpfulness of different components of the complete mtDNA sequence for making phylogenetic inference was examined using median-joining network trees. We observed that a number of haplogroup combinations were indistinguishable in trees based on RNAs transfer, 12sRNA and 16sRNA sequences. As expected, the control region is the more variable and contributes the highest information to breed diversity. The analysis of the complete mitochondrial sequence may enrich data on European sheep breed diversity and establish a rationale method for assessing species genetic variability.

P-028

System dynamic modeling of dairy cattle breeding scheme

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Simulations of breeding scheme help to predict the behavioral trends of breeding systems and to support the decision making processes. They are also useful to study the relationship among technical, biological and economical variables of a certain breeding population. Recently, simulations have extensively been used to predict the effect of changes in the current breeding schemes due to the introduction of genomic information in routinely genetic evaluation of dairy cattle. Several approaches have been proposed following deterministic or stochastic models either or not including economics of breeding scheme. Within the boundary of a breeding scheme, the dynamics of animal breeding values (BV) and its accuracy are affected by several endogenous variables strongly interconnected in many feedback loops. System Dynamics approach allows to study the loop dominance and the structural effects on variable patterns. It was used in this study to focus the breeding scheme designing and to describe, in a stock and flow diagram, the main pattern and interaction of the variables involved in the breeding scheme. A conceptual model of a generic breeding scheme was firstly developed including: animals in each selection path, the phenotypic value, the true genetic value, the estimated breeding value, the trait genetic variance, the selection intensities and the accuracy of the estimates of the breeding values. The conceptual model has to be expanded in different sub models and translated in a stock and flow diagram of Vensim®. At this phase the animal sub-model was developed using published information on the Italian Holstein breeding scheme. It included the 4 classical path of selection in dairy cattle: Sire of Sires (SS), Sire of Dams (SD), Dams of Sire (DS) and Dams of Dams (DD). Firstly a progeny test scheme was designed. The initial values were set up at 1 million cows, of which 150,000 available for progeny testing and 850,000 available for proven sires. Sixty proven sires were replaced by 20 sires/year selected among the best 300 tested young sires. The developed sub-model included 41 single variables and was able to find the steady state of the size of each simulated selection line and their progeny.

P-029

Effect of L-carnitine on buffalo *in vitro* embryo development

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The *in vitro* embryo production (IVEP) technology is the best tool to increase the maternal contribution to genetic improvement in buffalo. The major current limitation is the poor cryotolerance of buffalo embryos, due to their high lipid content. It was demonstrated that supplementing bovine culture media with L-carnitine, a cofactor of β-oxidation, improves *in vitro* embryo development (Sutton-McDowall ML *et al*. 2012 Theriogenology 77:1632-1641). The aim of this work was to evaluate whether L-carnitine supplementation during IVC improves blastocyst development and cryotolerance of *in vitro* produced (IVP) buffalo embryos. Abattoir-derived cumulus-oocytes complexes (n=410, over 5 replicates) were matured and fertilized *in vitro* according to standard procedures (Gasparrini B *et al*. 2006 Theriogenology 65: 275-287). On day 1 (Day 0=IVF), zygotes were cultured in SOF supplemented with 8 mg/mL BSA, in the absence (control, n=165) or presence of L-carnitine (n=170) at a concentration (0.25 Mm) selected after a preliminary dose response trial. In vitro culture was carried out at 38.5°C under 5% CO_2, 7% O_2, and 88% N_2. Cleavage and blastocyst rates (in relation to the cleaved embryos) were evaluated on Day 5 and 7, respectively. The blastocysts were vitrified by cryotop in 16.5% ethylene glycol, 16.5% DMSO and 0.5M sucrose and the survival rate, based on morphological criteria, was assessed after 24 h culture. Data were analyzed by Chi square test. Cleavage (81.3% *vs* 82.1%, in the control and carnitine groups) and blastocyst production (40.0% *vs* 47.6%, in the control and carnitine groups) were not affected by the treatment. The percentages of fast developing embryos (expanded and hatched blastocysts), *i.e.* those of better quality, were 17.0 and 23.5%, respectively. Interestingly, the embryos cultured with L-carnitine showed higher survival rates after 24 h culture (78.7% and 96.4%, in the control and carnitine groups, respectively; $P<0.01$). These results demonstrated that L-carnitine supplementation of culture medium improves the resistance to cryopreservation of IVP buffalo embryos, without affecting blastocyst production. We speculate that the increased cryotolerance in the presence of L-carnitine may be attributed to a better utilization of the endogenous lipid stores, leading to improved embryo quality.

P-030

Flow cytometric evaluation of functional parameters in bovine semen

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Semen quality evaluation and determination of its fertilizing ability are essential aspects in the practice of assisted reproduction in domestic species. Fertilization is a multifunctional process, thus several tests related to sperm functional competence and multiparametric data analysis are required for a proper evaluation. Despite many studies are available in the literature and much progress has been made, at present a set of tests suitable for the prediction of fertilizing ability is still not available. Flow cytometry (FCM), allowing the analysis of different parameters on several sperm cells in short time, can provide a fair assessment of semen potential fertility. In this study different quality parameters of bull frozen-thawed semen were evaluated by FCM and by computer assisted sperm analysis (CASA). Frozen semen from 18 Holstein-Friesian bulls, 10 batches each, was assessed. The following parameters were tested by FCM: sperm viability (SYBR-14/propidium iodide (PI) staining), acrosomal status (FITC-PNA/PI staining), membrane lipid disorder (Merocyanine 540 hydrophobic dye), lipid peroxidation (BODIPY (581/591) C11 lipophilic probe), mitochondrial membrane potential (JC-1 potentiometric probe). Sperm kinetic was assessed by CASA. The mean values of measured parameters, standard deviations and minimum and maximum values were: proportion of viable sperm: 41.73±10.57% (16.26 to 66.44); live acrosome-intact sperm: 34.34±10.52% (8.52 to 56.55); live low lipid disorder sperm: 22.54±7.62% (6.97 to 49.84); live peroxidized sperm: 0.55±0.92% (0.00 to 8.49); live peroxidized sperm after promoter ($FeSO_4$) treatment: 4.28±2.93% (0.76 to 16.41); high mitopotential sperm: 43.89±10.74% (9.78 to 67.09); motile sperm: 63.43±14.32% (14.50 to 88.50). Values were in general fairly consistent with levels found in the literature. Sperm viability was significantly correlated ($P<0.0001$) with low membrane lipid disorder ($r=0.68$) and acrosome-intactness ($r=0.62$), both in live sperm, and with motility ($r=0.59$). Moreover high mitopotential showed moderate but highly significant ($P<0.0001$) correlations with low lipid disorder in live sperm ($r=0.51$) and with motility ($r=0.40$). FCM can be considered a helpful tool for quick multiparametric evaluation of frozen-thawed bull semen. At present the estimation of the correlations between *in vivo* fertility and FCM semen quality parameters is in progress.

P-031

Genetic progress in harmony and movements traits in Spanish Arabian horses

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Successful selection in a domestic population is conducted by gradual improvement of the average breeding values. Genetic progress is usually measured with genetic trends, thus trying to measure if any changes have been undergoing over an established period of time. In the present study, we examined the genetic and environmental trends of conformation (harmony) and mobility (walk, trot and canter) traits in the Spanish Arab horse breed. These have been observed taking into account the breeding values averages in respect to the year of birth of each individual, having animals born between 1960 and 2011. We found that the genetic trends of the harmony (+0.0007), walk (+0.0003) and trot (+0.0007) were slightly positive, finding only a negative trend for the canter (-0.00001). Noteworthy is the fact that all the characters show a positive trend from 2007, year when the modern breeding program of the breed start (based on Blup Animal Model methodology) in spite of the previous empirical selection developed by farmers based on mass selection. All these results show that since the implementation of the modern breeding program, there is a positive and more effective genetic response to selection. However, In the near future the increasing of the number of animals in the program and the number of observation will result in an improvement of the trend and accuracy of the results.

P-032

Regions in the bovine genome harboring evidence of selection signatures revealed by dense single nucleotide polymorphism panels

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Identifying positive genomic markers of selection in domestic animals can provide powerful insights into the mechanisms involved in evolution, help target *loci* for selection and may highlight the genetic basis of phenotypic diversity for complex traits. Test based on Extended Haplotype Homozygosity (EHH) are proven as the most powerful statistics for detection of signatures of selection in genome-wide SNP data, in intra-breed analysis. In particular, integrated Haplotype Score (iHS) test statistic is designed to detect regions with high level of haplotype homozigosity over a long distance (relative to neutral expectation) within population. Here we computed integrated iHS for detection of signatures of selection in Holstein (HOL) cattle breed with High Density SNP panels. SNP data from 916 HOL bulls genotyped on Illumina BovineHD Genotyping BeadChip assay where furnished under the INNOVAGEN project, after a quality control provided by ANAFI. We estimated haplotypes and missing genotype using BEAGLE.3.3.2 software. Preliminary analysis where done on chromosome BTA2, searching markers of selection in a dataset of 40,056 SNPs for 916 individuals. We calculated iHS along the whole BTA2 chromosome using rehh R package: iHS where obtained integrating EHH of ancestral and derived alleles with a minimum allele frequency (MAF> 0.05). On the basis of iHS data, we defined our markers of selection as all SNPs with a corresponding P-value<0.01 and with at list one contiguous SNP with high iHS. A total of 214 chromosomal regions were thus obtained. We searched for corresponding genes and transctipts through EnsamblDB, finding a total amount of 33 genes and 43 transcripts. A subsequent analysis was done, selecting only SNPs with a P-value<0.005 and chromosomal regions with a minimum range of 8.5Kb (over the 3rd quartile), and we obtained an amount of 27 chromosomal regions, 6 genes and 7 transcripts. Finally, we provide functional annotation for both gene lists, using DAVID 6.7, and we obtained from 2 to 8 candidate genes as cause of positive selection. EHH-based statistic test on HD SNP panels provide more accurate analysis in obtaining markers of recent positive selection, potentially reducing the number of false positive markers signal.

P-033

He-Ne laser irradiation effects on cryopreserved ram semen

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The development of new procedures aimed at improving the quality of ram cryopreserved semen is a suitable goal to be pursued. In previous research was showed that the He-Ne laser irradiation can biostimulates sperm motility in different animal species (Ocaña-Quero *et al*. 1997; Ruiz-Pesini *et al*. 2000; Corral-Baquès *et al*., 2008; Iaffaldano *et al*., 2005; 2010). However, the effect of He-Ne laser was never investigated, to the best of our knowledge, in severely stressed semen, as in the freezing/thawing procedure and, in particular, in ram semen. Therefore, the aim of this study was to investigate whether and how two energy doses of laser irradiation (3.96 and 6.12 J/cm^2) can improve the qualitative characteristics and energetic profiles of ram spermatozoa after freezing-thawing process. Cryopreserved ram sperm from *Merinizzata Italiana* breed was kindly purchased by APA association. Samples were thawed in a water bath at 37°C for 30 seconds. Each pool was divided into three aliquots: one was kept as a control and the others two were irradiated with He-Ne laser (wavelenght 632.8 nm; 6mW at two different energy dose (3.96 and 6.12 J/cm^2). The total mass and forward progressive motility (subjectively assessed by light microscopy), viability (SyBr-PI staining), DNA intactness (Acridine orange staining), osmotic-resistance (HOS-test) and acrosome integrity (FITC-PSA) were evaluated after irradiation. In parallel, the effect of irradiation on biochemical parameters of samples was evaluated by measuring the activity of cytochrome oxidase (COX) and the energetic charge (HPLC). In all cases we found that the lower dose of laser energy resulted in a decrease in both mass and progressive motility, but not in viability, acrosome and DNA integrity; in parallel, this energy dose resulted in a stimulation of cytochrome oxidase (COX). On the contrary, the higher energy dose slightly increased progressive motility, viability, osmotic resistance and acrosome integrity without affecting mass motility and DNA integrity; energy charge and COX activity were found not to differ with respect to the control. Further studies are needed to confirm these results with a larger number of samples and to evaluate the effect of laser irradiation utilizing higher energy doses.

P-034

Genotyping by sequencing protocol in water buffalo species

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Advances in high-throughput short-read sequencing technology have facilitated rapid and cost-efficient generation of gigabases of data by individual laboratories. This has led to new approaches for simultaneous discovery and genotyping of dense genetic markers on a scale that represents a step change from the state of the art prior to this technology. One such approach is GBS which is a genome complexity reduction technique that sequences at depth flanking regions of restriction enzyme (RE) cleavage sites followed by multiplex sequencing to produce high quality polymorphism data at relatively low cost per sample. Therefore we propose testing the approach to genotype water buffalo, a species important for both specialized markets and developing countries and for which we now have a draft genome assembly (International Buffalo Genome Consortium). Here, we report a procedure for constructing GBS libraries based on reducing genome complexity with PstI enzyme, which is partially methylation-sensitive, and a 48-multiplex sequencing in a single lane of Illumina HiSeq1000 producing 100bp single reads. The 48 buffalo samples used to set up and validate the GBS protocol were already genotyped with the recently developed Axiom Buffalo Genotyping 90K array (Affymetrix). The 48 adapters terminates with a 4 to 9 bp barcode on the 3' end of its top strand and a 4 bp overhang on the 5' end of its bottom strand that is complementary to the sticky end generated by PstI. After sequencing, a custom de-multiplexing was performed to sort the reads into separate files according to their barcode, with the barcode sequence removed and the remainder of the sequence trimmed that includes the initial recognition site. On average 2.12M reads were produced per DNA sample. The mean estimated coverage across all the samples is 0.14 but the coverage of different sites throughout the genome, as captured through the PstI-based protocol, is highly variable with high- and low-coverage fragments. The SNP calling pipeline is underway using BWA as alignment tool and GATK for the SNP calling. The importance of reducing genome complexity with REs coupled with multiplex NGS for high-density SNP discovery and genotyping has been already demonstrated in several species with large genomes and may be an added value in breeding, conservation, global species and population surveys.

P-035

Analysis of CSN1S1 and CSN3 genes in Murciano-Granadina goat breed

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In goats, milk caseins are encoded by four genes located on chromosome 6. Although many studies on casein genes polymorphism have been carried out also comparing the genotypes with the amount of the proteins in milk or with several technical features as the clotting ability of proteins to produce cheese, few studies are available taking in count the variation of all the four casein genes (using haplotype of SNPs). This work is part of a study aimed to analyse casein genes(CSN1S1, CSN2, CSN1S2, and CSN3) in Murciano-Granadina goat breed. Murciano-Grenadine breed is distributed in autonomous communities of Andalusia, Murcia, Valencia, Castilla-La Mancha, Balearic Islands, Catalonia and Extremadura and represent the most important breed in Spain for census and production. Here we present the preliminary results of CSN3 and CSN1S1 gene polymorphisms. We used the SnapShot multiplex (ABI) technique in order to analyze polymorphisms of CSN1S1 (located within exon 9 and exon 19) and CNS3 (within exon 4) genes on samples from 200 bucks chosen in the top rank of the breed and with pedigree and phenotypic information (record of lactation of the daughters). The results show a predominance of AB genotype in CNS3 casein (frequency 0. 45) and of the BE genotype for CNS1 casein (frequency 0.3).

P-036

Genetic diversity between three populations of Nguni (Zulu) sheep using microsatellite analysis

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Zulu sheep are found mainly in the rural KwaZulu-Natal Province (South Africa). Research flocks were established in Makhathini Research Station (MS) and University of Zululand (UZ). Zulu sheep numbers are declining hence there is a need for genetic characterization as first phase for planning conservation strategies. A panel of 29 microsatellite *loci* (FAO suggested) was used in this study to evaluate the genetic variability and population structure in three Zulu sheep populations. These three populations were sampled from MS (n=33), UZ (n=21) and from a community at KwaMthethwa (KM) (n=32). Two of the European breeds Appenninica (AP) and Spanish Merinos (SM) were used as out group. Results accurately differentiated the Zulu sheep from the two European breeds. Zulu sheep populations exhibited the lowest genetic variability (0.57- 0.60), whereas the two European breeds displayed the highest values of observed heterozygosity (0.70 and 0.72). Among the Zulu sheep, the mean number of alleles per *locus* was the lowest (3.74) in UZ and the highest (5.57) was realized in MS. On the neighbor-joining tree, UZ and MS populations formed a cluster and the MK population formed its own node. The results demonstrated by the STRUCTURE analysis showed that MK population had some genetic material for some breeds not considered in the study. Owners of MK populations confirmed to may have brought in sheep to their flocks that were crossbred with other breeds since 2008. It was concluded that actions to conserve Zulu sheep should be taken as soon as possible.

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P-037

Variability in candidate genes for meat production traits in *Romagnola* beef cattle breed

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In *Romagnola* breed cattle, a selection focused in obtaining an improvement of daily gain, fleshiness, light skeletons and correct morphology was planned by ANABIC. All these objectives are taken in consideration during the performance test. In the last years molecular markers are becoming more and more important in animal breeding. In particular the SNPs markers can be used in association with traditional selection to make more effective the animals evaluation. The candidate genes method has been tested in different species and breeds. The aim of this study was to investigate the variability of 17 SNPs situated in 12 different candidate genes involved in meat production traits, namely biometric measures and the selection indexes; a 238 *Romagnola* young bulls population in performance test was studied. Genomic DNA was extracted from 238 blood samples and the genotyping determination of all investigated SNPs were performed by KBioscience (UK) using KASPar technology. To asses genotyping accuracy 10% of the samples were genotyped in duplicates. For each SNP the genotypic and genic frequencies were estimated and Hardy Weinberg (HW) equilibrium deviation verified by a specific software. Four SNPs situated in the following genes were monomorphic: GDF8, GH and NPY; moreover in the GDF8 (promoter, -371) only two genotypes were observed (TA and TT) such as in the GHRL (exon 3, 446 - AA and AG). All the gene frequencies were in HW equilibrium, GDF8 (promoter, -371), Ob-R (exon 20, 115) and NPY (exon 2, 666) except. Growth index, loin length at the start of performance test, head length and thickness of the skin at the last rib were influenced by four SNPs. GHR (promoter, 149) was significantly ($P \leq 0.05$) associated with 18 traits, LEPT (promoter, 1759) and MYF (intron II, 1948) with 9 traits, GHR (exon 4, 300), NPY (exon 2, 666), UCP (intron 3, 1099) with 8 traits. This field must be more explored, but the obtained results allow to suppose the use of some SNPs in a marker assisted selection programme for *Romagnola* cattle.

Acknowledgments
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P-038

Molecular markers as a tool to contain inbreeding in *Lupo Italiano* dog

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The *Lupo Italiano* is a dog breed of about 400 individuals, originated in 1966 from a mating between a German Shepherd male and a wild wolf female, followed by close inbreeding, which led to the current uncommonly high F value (mean 0.34). The high degree of relatedness between individuals and the low population size make it difficult to select mating pairs in order to limit the loss of genetic variability. So, the aim of this study was to verify if molecular data, in addition to the genealogic ones, can be used as a tool for a better genetic discrimination between individuals, even in a population with such a high level of inbreeding. Twenty-four *Lupo Italiano* dogs, born between 1999 and 2011, were genotyped using 24 microsatellite *loci*, recommended by the ISAG. The sample included 12 dogs belonging to three litters and 12 dogs as little related as possible. A neighbor-joining tree was drawn to graphically express the molecular distances between all the analysed individuals; for the three groups of full-sibs, the F value, derived from the genealogical data recorded in the Anagraphic Register, was plotted against the percentage of homozygous *loci* (PHL), derived from microsatellite data. The NJ tree allowed a good differentation not only between the less related individuals, but also between the full-sibs, indicating the possibility to identify the most genetically different subjects within a litter. Moreover, quite different PHL values were found within the three analysed litters: for example, six full sibs (F=31.5) showed PHL values ranging from 0.46 to 0.67. In conclusion, microsatellite markers proved to be an effective tool in the *Lupo Italiano* dog to select mating subjects with the highest within and between individuals diversity, hence to provide additional information to implement more adequate mating programmes.

P-039

A Collaborative European Network on Rabbit Genome Biology – RGB-Net

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The European rabbit (*Oryctolagus cuniculus*) is a key species in biology. Basic discoveries have been made investigating this mammal whose genome has been recently sequenced. The rabbit is a livestock, an animal model, a wild resource, a pest and a fancy animal and comprises a large number of breeding stocks/lines. COST Action TD1101 (2011-2014) A Collaborative European Network on Rabbit Genome Biology – RGB-Net is an action granted by COST in the domain of Biomedicine and Molecular Biosciences focused on the rabbit in all genome biology related fields. Chaired by the University of Bologna and INRA, it gathers more than 100 experts from 21 European countries and USA, China, Japan, Taiwan and South Africa, working in many research fields (breeding, genetics, bioinformatics, physiology, evolution, embryology, immunology, wild life, clinical medicine, etc.). RGB-Net aims at building an open international network of research organizations, associations and companies in order to facilitate the transfer of rabbit genomic information from experimental data into usable benefits and applications. Four Working Groups are focused on i) the refinement of the European rabbit genome resources and the development of genome-based platforms, ii) genetic aspects in meat, fur and pet rabbits and biodiversity resources, iii) the rabbit as a model in basic biology and human diseases and as a tool for biotechnology applications, and iv) genetic and comparative genomic aspects for the study, exploitation and management of wild lagomorphs. The outcome is a coordination of rabbit research activities and a transfer of knowledge, that will produce a strong European added value across a broad spectrum of biology research fields. More information on RGB-Net can be obtained at http://www.biocomp.unibo.it/rabbit/.

P-040

Use of different statistical approaches to study selection signatures in sheep breeds farmed in Italy

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Natural and artificial selection affect genome structure causing genetic variation between breeds. Dense marker maps of thousand SNP disseminated across the whole genome allow for the investigation of chromosomal regions that differ between breeds. Several statistical approaches have been proposed to study selection signatures in livestock species. In this work, four approaches were used to study selection signatures in a sample of 496 sheep belonging to 20 Italian breeds, different for geographical origin and production aptitude. The four approaches were: I) Fst Outlier Detection (FOD), implemented in the LOSITAN software. II) comparison of Breed LS means of the sum of differences in SNP allele frequencies along sliding windows (SNP_DIFF). III) Correspondence analysis (CA). VI) Canonical Discriminant Analysis (CDA). Animal were genotyped with the Illumina OvineSNP50 BeadChip. The first six chromosomes were considered. After data editing, a total of 20,194 SNP were retained for the analysis. The different approaches were able to identify the same regions expressing variation between breeds. On OAR6, for example, all methods highlighted a region located between 35 and 41 Mb, where BMPR1b and ABCG2 *loci* map. Moreover, SNP able to differentiate between breeds were also detected at 76, 96 and 107 Mb, near to KIT, IL8 and SCD5 *loci*, respectively. All methods were able to discriminate breeds and, in general, a geographical pattern of variation has been detected. However each approach may supply different kind of information. FOD detected a relatively low number of markers in divergent selection but it was able to identify *loci* under balanced selection. CA and CDA decomposed the total variability of SNP markers among breeds in different and uncorrelated variables that could be useful for the identification of genes influencing complex traits.

P-041

Genomic analysis of five cattle breeds farmed in Italy using runs of homozygosity

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High throughput genotyping represents a powerful tool for studying structural variability in the genome of livestock species. Runs of homozygosity (ROHs), defined as DNA segments that harbour uninterrupted sequences of homozygous genotypes, have been proposed as measures of the inbreeding level of a breed. In this work, distribution of ROHs in five different cattle breeds farmed in Italy was investigated. Bulls of Italian Holstein (IH) (2,093), Italian Brown (IB) (749), Italian Simmental (IS) (479), Piedmontese (P) (364) and *Marchigiana* (M) (410) were genotyped with the 50K Illumina bead-chip. After edits, 44,325 SNP were retained for the analysis. ROHs were calculated with the following settings: maximum distance between adjacent SNP=1Mb; minimum number of SNP included in the run=15; only ROH longer than 1Mbp were considered. ROH lengths, calculated for each animal and chromosome were analysed with a linear mixed model that included the fixed effects of the breed and of the chromosome in which the ROH was located, their interaction and the random effect of the animal. All the factors included in the model affected significantly ROH length. On average, IB showed the longest average ROH length (about 3.5 Mb) whereas the shortest was observed in the Piedmontese (1.7 Mb). Longest ROHs were observed on BTA10 (3.7 Mb), shortest on BTA3. Within breeds, the longest ROHs on BTA10 were confirmed in all the breeds with the exception of IB, that had the highest values on BTA6. The two dairy breeds had the highest frequencies (3% and 2.6% for IB and IH, respectively) of longer ROHs (>16 Mb) compared to the dual purpose IS (0.6%) and beef (1.6% and 0.5% for M and P, respectively) breeds. The results of the present study agree with previous reports on cattle and suggesting that ROHs provide interesting information on the genomic structure, selection history, demography and inbreeding of livestock breeds.

P-042

Equine autochthonous genetic types: molecular characterization by microsatellite markers

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The ministerial decree no.24347 of year 2003, states the separate registration of *Napoletano*, *Persano* and *Salernitano* horses populations into the Book of equine population at limited diffusion. Subsequently, in the year 2009, the decree no.552 included Salenitano and *Persano* into same population. This study aimed at verifying the distinction between these three populations, by genotyping 18 microsatellite in 53 *Persano*; 56 *Salernitano* and 15 *Napoletano* horses. The following parameters were estimated: observed number of alleles (Na), effective number of alleles (Ne), Polymorphic Informative Content (PIC) for each *locus*, mean observed (Ho) and expected (He) heterozygosity, number of *loci* in significant disequilibrium from Hardy-Weinberg equilibrium (HWE), molecular coancestry (fij), inbreeding (fi), selfcoancestry (si), Kinship distance (Dk); moreover, the analysis of factorial correspondence (AFC) and the population assignment test (STRUCTURE) choosing the K value by Evanno's method were performed. The results evidenced: in *Salernitano* horse: Na=112; Ne=63; Ho=0.660±1.38, c.v.=20.96%; He=0.686±0.123, c.v.=17.90%; *locus* COR058 was the most informative; 3 *loci* [ASB23 (P=0.0001), HMS07 (P=0.0228), HMS01 (P=0.0053)] were in significant disequilibrium from HWE; fij=0.32; fi=0.34; si=0.67; Dk=0.26; in *Persano*: Na=112; Ne=63; Ho=0.621±0.160, c.v.=25.74%; He=0.658±0.171, c.v.=26.05%; *locus* ASB02 was the most informative; 5 *loci* [HTG10 (P=0.0019), ASB23 (P=0.0001), HTG04 (P=0.0008), SGV28 (P=0.0265)] were in significant disequilibrium from HWE; *locus* HMS45 was in equilibrium; fij=0.35; fi=0.38; si=0.69; Dk=0.34; in *Napoletano*: Na=69; Ne=47; Ho=0.659±0.168, c.v.=25.47%; He=0.589±0.120, c.v.=20.32%; *locus* ASB02 was the most informative; 4 *loci* [ASB23 (P=0.0013), VHL209 (P=0.0220); HTG04 (P=0.0597), HMS02 (P=0.0572)] were in significant disequilibrium from HWE, 4 *loci* (AHT04, HTG04, HMS01, COR058) were in equilibrium; fij=0.41; fi=0.34; si=0.67; Dk=0.26; AFC distributed the most of subjects in three different groups according to declared genetic type; the population assignment test discriminated almost all subjects according to belonging cluster. All results allow suggesting the separation of *Salernitano* horse from *Persano* according to ministerial decree no.24347 of year 2003.

P-043

Comparison between *Casertana* pig and *Casertana* x Italian Duroc (DuCa) pig cross

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The comparison involved 16 subjects of *Casertana* pig autochthonous genetic type (6 raised in confined system and 10 in half-wild system) and 23 DuCa (12 raised in confined system and 11 in half-wild system). The *in vivo* assessment interested: Weight Increase (WI) and Feed Conversion Ratio (FCR); the *post mortem* determinations concern: lean and adipose cuts as well as fat thickness. The results, valid in the limit of the observation field, underlined that: (i) the Genetic Type (GT) influences the FCR ($P<0.001$), the weight of the ham ($P<0.001$), the weight of *fiocco* (*culatello*) ($P<0.01$), the weight of fat (seasoned) ($P<0.003$) and the fat thickness ($P<0.001$); (ii) the rearing system influences the FCR ($P<0.05$) and the weight of the ham ($P<0.05$); (iii) the sex (whole female and castrated male) influences FCR ($P<0.002$) and fat thickness ($P<0.05$). The rearing system influences ($P<0.001$) the weight loss at ripening of some products, both for *Casertana* GT and for DuCa. These preliminary results could suggest a different productive use of the two GTs.

P-044

Insulin-Induced Gene 2 characterization and its relationship with milk traits in *Sarda* sheep breed

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The insulin-induced gene 2 (INSIG-2) is a controller of lipid metabolism and it plays a central role in the sterol-mediated regulation of SREBP, SCAP and HMG-CoA reductase. Human polymorphisms in the INSIG-2 gene sequence are found to be associated with weight gain and obesity, whereas in livestock there are no studies about the influence of this gene on productive traits. The aim of the present research was to characterize INSIG-2 gene nucleotide sequence and to detect polymorphisms and their possible relationship with milk traits. The study has been conducted on 200 *Sarda* ewes, lactating and in their third to fifth lactation. Every month, from 30th to 150th day of lactation, the milk yield of each animal was recorded and a milk sample from each ewe for fat, proteins and lactose concentration analysis was taken. A blood sample was collected from each ewe for DNA extraction. 100 ng of genomic DNA were used for the amplification of the exons 2, 3, 4, 5 and 6. For each exon 10 amplicons, randomly selected, were sequenced and compared with bovine sequences in GenBank. Then all the PCR products were denatured and analyzed using Single Strand Conformation Polymorphism (SSCP) method. The different SSCP patterns were sequenced. No mutations were found in the coding sequence of the gene. Five SNPs were found in intronic regions, namely: Intron 1 G88A, Intron 2 436TCAGdel, Intron 2 G471A, Intron 3 C90T, Intron 5 T238G. No relationship between INSIG-2 polymorphism and milk production traits was found. The *Sarda* ewes produce milk high fat level in and this certainly is the result of a genetic selection made since almost a century by farmers. So this could have led to the elimination of the genetic variants that produced low-fat milk. The results is the little variability of these analysed traits of the INSIG-2 gene. However, it would be necessary to extend the study to other gene fragments (such as introns, promoter) in order to expand the knowledge about this gene in *Sarda* sheep breed.

P-045

The viability of fresh ram semen samples for DNA extraction

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In DNA extraction, one of the several samples used is semen that can be useful as both fresh and frozen. In fresh semen, the problem is the viability of the sample after long periods. The objective of this study was to evaluate the viability of ram semen for DNA extraction at different time. The DNA extractions were done using the sperm of two rams at zero time (fresh), six, twelve, twenty-four and forty-eight hours, one week, one month and two months after the collection. The quick alkaline protocol for DNA extraction was used. During the experiment, the samples were stored at 4°C. After the extractions, the concentration and quality (purity) of genomic DNA extracted was determined by optical density in a spectrophotometer at NanoDrop 2000c device. The concentration of protein contamination in the DNA was supplied by the rate of A260/A280nm, after reading made by the spectrophotometer. The rate A260/A230nm was related to the contaminants of reagents used and the 1.8 value was considered a DNA free of contamination. Analysis of data was performed by the GLM procedure of SAS, however, due to the high coefficient of variation, the data were log-transformed. There were not significant differences in concentrations of nucleic acids, according to the storage time of the sample, with an average of 4818.21±4962.62 ng/uL. For the ratio of A260/A230nm the average was 0.61±0.23 and did not show differences in storage time of the sample, probably due to the use of only one protocol. For the ratio of A260/A280nm the average was 2.06±0.56, and the fresh semen showed higher value, 2.54±1.0. The semen stored one week and the stored in one month were the closest to the value of 1.80. With the obtained results it can be concluded that with pure semen storage at 4°C for two months there is no change in concentration of extracted DNA or alterated by contamination with reagents. In fresh semen it may be necessary to perform a purification step, because this one showed the highest protein contamination.

P-046

Genetic characterization of sheep breeds of Egypt and Italy

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A high number of breeds will be lost in the near future, before their characteristics can be studied and their potential evaluated. Therefore it is strategically important to preserve farm animal diversity for future generations. We used mtDNA to analyze genetic biodiversity between Italian and Egyptian breeds, gain information on breeds origin in both countries and to improve genetic management for maximizing biodiversity conservation of Italian and Egyptian sheep. A 721 bp fragment of the mtDNA control region (15,540 - 16,261 bp, NC_0019041.1) was amplified and sequenced in a total of 137 unrelated individuals from five sheep breeds (Egyptian Barki, Ossimi and Rahmani and Italian *Sarda* and *Laticauda*) and in Italian muflon (Ovis orientalis musimon). A 423 bp fragment excluding a zone rich of tandem repetitions (15,641 - 15,969 bp) was selected. Reference sequences for defining haplogroups were: DQ852286, DQ852287 for A, DQ852285 DQ852282, AF039579 for B, DQ097460, DQ097462, DQ852283 for C, DQ852288, DQ852289 for D, DQ852280, DQ852281 for E haplogroups; for wild sheep: O. vignei Arkal (AY091489.1), O. bochariensis vignei (AY091490.1, AY091491.1, AF039580.1), O. ammon collium (AY091492.1), O. ammon nigrimontana (AY091493.1, AY091494.1), O.orientalis (EU308498, AF039579). We retrieved 78 haplotypes and 23 polymorphic sites using DnaSP 5.00; Haplotype diversity (Hd) was 0.982. The most common of the 78 haplotypes occurs in 10 samples; others in a number of samples from 9 to. 2. The other haplotypes occurred just once. Private haplotypes were found in ITMUS, EGOSS, EGBAR, EGRAH samples. Average evolutionary divergence over sequence pairs within groups were conducted in MEGA5 on 137 nucleotide sequences using the Tamura-Nei model. The evolutionary distances were inferred using the Tamura-Nei method using the Neighbor-Joining method using MEGA5. The optimal tree showed the sum of branch length=0.217 (1000 bootstrap replicates). The breeds were grouped into three of the five maternal haplogroups. Phylogenetic analysis resulted in 1.46%y of the samples belonging to A, 2.19% to C and 96.35% to B mt DNA haplogroups. The results of this study contribute to the knowledge of Italian and Egyptian sheep breeds and will be useful to the understanding of population genetics and breeds evolution.

P-047

Reproductive performance and piglets growing rate of *Suino della Marca*

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The Genetic Type *Suino della Marca* was registered by ANAS in Swine Hybrids Bulletin Board Registers on 11/10/2010 and officially recorded with MIPAAF M.D. 28825/2010. According to local breeders requests to get pigs suitable for outdoor rearing, it resulted after crossbreeds among *Cinta Senese*, Large White and Duroc followed by composite breeding. After 4 years mating program a group of *Suino della Marca* pigs was bred indoor (377) and a group outdoor (46); during a three-year period 423 deliveries were analysed recording total born, live born and dead piglets. On 438 piglets the growing performance was observed from birth to weaning based on the following parameters using descriptive statistics: birth and weaning weight, weaning age, total weight gain (TWG) and average daily gain (ADG) in indoor (288) and outdoor (150) husbandry. The average litter performance is listed in the table below.

Table 1. Litter performance of *Suino della Marca* (mean±std. dev.).

	Total (438)	Indoor (288)	Outdoor (150)
Litter	9.39±1.93	9.56±1.69	7.91±2.90
Live born	8.81±2.12	8.98±1.95	7.43±2.84
Dead piglets	0.57±1.19	0.59±1.20	0.48±1.07

On 438 records the average birth and weaning weight were 1.49±0.29 and 10.22±4.50 kg respectively and obtained in 39.37±12.85 d. During the lactation period the TWG was 9.34±5.52 kg with an ADG 0.227±0.06 kg. Between the two different systems interesting differences were observed: 1.64±0.16 *vs* 1.21±0.27 kg for birth size; 8.70±1.62 *vs* 16.27±6.74 kg for weaning weight reached at 32.34±4.04 and 57.94±9.07 d respectively. The different length of lactation was due to the rearing system adopted, therefore the indoor TWG was lower (6.55±1.18 kg) than calculated for outdoor piglets (15.11±6.43 kg); indoor ADG was 0.213±0.04 *vs* 0.254±0.08 kg outdoor ADG. The present investigation showed *Suino della Marca* sows having satisfying litter performance even though the number of total born should be improved to increase the Genetic Type consistency. The higher outdoor ADG may be ascribed to a higher daily milk production of sows reared in outdoor system reflecting their good maternal aptitude.

Acknowledgments

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P-048

Some of the main results of Quantomics EU project: CNV detection and GWA analysis in the Italian Brown Swiss Dairy cattle

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Amongst Quantomics results here we present identified QTL regions for mastitis resistance and a medium resolution map of CNVRs obtained in the Italian Brown Swiss. A total number of 1489 bulls were genotyped on Illumina's BovineSNP50 BeadChip on UMD3.1 autosomes and a subset of 1342 bulls was used for CNV detection. Among these, 192 bulls were genotyped on Illumina's HD chip (777k) and utilized for GWA analysis jointly with the remaining bulls which genotypes were imputed to Illumina's HD chip interrogating 735,238 *loci*. PennCNV and SVS7 software were used for CNVs detection for a total of 46,728 *loci*. We corrected for GC score and wave factor and employed PCA for SVS7 to correct for technical background noise to reduce false positive calls. PennCNV and SVS7 CNVs results were summarized into 1101 (220 losses, 774 gains, 107 complex), and 277 (185 losses, 56 gains and 36 complex) CNVRs on 651 bulls, respectively. The consensus between the CNV scans was obtained using the Redon *et al*. (2006), and Wain *et al*. (2009) approaches, covering 146 Mb (5.88%) and 17.1 Mb (0.68%), respectively. CNVRs were annotated with the bovine Ensembl gene set v69 and tested for enrichment of GO terms using DAVID database. Consensus CNVRs were enriched for protein-coding genes. GO analysis identified genes in the CNVRs related to cytoplasm, intercellular part, cellular processes, cytoplasmic part, and intracellular organelles. For the GWA analysis, after data filtering, a total of 588,308 SNPs were retained for with MAF >0.02, call rate >0.90 at SNP and bull level. Stratification in the population was corrected for PCA. Success of correction for stratification was empirically assessed based on Q-Q plots of expected versus observed P-values. We employed single SNP regression and multiple SNP regression in sliding windows of three to five SNPs. Significance was declared employing a false discovery rate approach. Several QTL regions were found across the genome. The most interesting regions were located on BTA1, BTA4, BTA7, BTA13, BTA16, BTA20, BTA21 and BTA27.

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P-049

Use of epididymal semen cryopreservation in farm animal gene banking: field applications in *Brianzola* sheep and *Nero Siciliano* pig breeds

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In local breeds, farmed in marginal area, few males are available for semen collection on farm. Semen collection training would be more complicated considering that the animals are not accustomed to being handled by humans. Moreover cryopreservation procedures are complex for the lack of facilities and expertise near the farming area. In this context epididymal sperm extraction from testicles of slaughtered or castrated animals is the only option to collect gametes. The aim of this work was to implement epididymal extraction technique, associated to the possibility of managing rather long periods between animal death, sperm recovery and freezing, for the setting up of two Italian local breeds' genetic reserve: the *Brianzola* sheep (BS) and the *Nero Siciliano* pig (NSP) breeds. In case of BS, testicles from 9 rams were collected at local slaughterhouse and semen samples extracted and frozen in our laboratories. For NSP, testicles from 10 boars were collected on farm in Sicily, after surgical castration. For 5 boars epididymal sperm recovery and freezing were done on-site; for the remaining boars testicles were transported by air at 5°C and processed within 24 hours in our laboratories in Lodi. Epididymal sperm was collected by using the retrograde flushing technique. After dilution samples were equilibrated at 5°C for 2 h, subsequently loaded into 0.5 mL straws and frozen on nitrogen vapors. Semen volume, total number of sperm and sperm motility were assessed. A total of 207 and 115 semen doses were stored for BS and NSP breed, respectively. Overall the quality of the sperm recovered was acceptable, considering semen volume (BS =.45± 0.4 ml; NSP=9.69±5.8 mL), total number of sperm collected (BS=4.7±4.05×10^9 cells/mL; NSP= 26.0±32.1×10^9 cells/mL) and total motility (BS=30.9±8.0%; NSP=63.0±7.5%). The high variability among samples detected depends mainly on donor age. Access to new technologies is important for gene banks to develop their collections. These data suggest the possibility to extract viable epididymal sperm from testicles transported and processed within 24 hours.

P-050

Genetic diversity of sheep Santa Ines of the North of Espirito Santo State, Brazil

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A knowledge of the genetic diversity of animal populations is important to guide breeders in order to prevent the occurrence of undesirable genetic effects caused by inbreeding depression. DNA analysis is an important tool for identifying inbred individuals and also for studies of phylogenetic relationships, being a complementary methodology for selection and animal breeding. The objective of this study was to evaluate the genetic diversity of Santa Ines sheep breed. Were collected 80 hair sheep of males and females from four herds in the north of the Espírito Santo state, Brazil. Were used the microsatellite molecular markers OarFCB020, OarFCB049, SRCRSP05, MAF065, OarFCB129, OarFCB011, SRCRSP08, Inra63, OarFCB304 and MAF214 in order to perform a preliminary characterization of genetic variability of the four sheep herds. A firts analysis was made with polyacrylamide gels and the final analysis was made with capillary electrophoresis. The effective number of alleles per *locus* ranged from 2 to 9. All markers were polymorphic. A low level of heterozygous alleles (15%), was found, the F average was 0.45% within herds, alerting that the breeders of this region are making matings between related individuals.

P-051

Inbreeding and genetic diversity of sheep Santa Ines of Rio de Janero state, Brazil

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To start a conservation and improvement program of the sheep breeds in Brazil, is necessary a minimum knowledge of the patterns of genetic diversity existing within and between the breeds. The goal of this work was to evaluate the genetic variability of the Santa Ines herds in the Rio de Janeiro state with microsatellites markers. We analyzed 120 animals of 6 different farms, 10 microsatellites *loci* were studied per each animal totaling 1200 samples. The microsatellite DNA markers are recommended by the Food and Agriculture Organization of the United Nations (FAO) and the International Society of Animal Genetics (ISAG). The effective number of alleles per *locus* ranged 3 to 9. Nine microsatellite *loci* analyzed in this study showed polymorphism, with the exception of *locus* SRCRSP08 which showed no amplification in PCR and no allele were observed. The average observed heterosygosity was low (average of .20), but expected heterosygosity ranged from .44 to .85, with average of .59. The PIC varied from .40 to .84 with average of .55 and the average inbreeding coefficient was .50 with the maximum of .88. It is concluded that the genetic diversity of this animal population is low and the future mating's should be made between the animals with low or no parentage in order to prevent the occurrence of undesirable genetic effects caused by inbreeding depression.

P-052

Genetic marker test for carcass trait of Santa Ines lambs of Brazil

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Some studies have been performed to identify QTLs that may be associated with traits of economic importance. Microsatellite markers are co-dominant, multi-allelic and highly reproducible. Some studies show the *locus* OarHH55 as a possible microsatellite marker associated with QTLs related to carcass weight and final body weight in sheep. The aim of this study was to associate the polymorphism in the *locus* OarHH55 with carcass weight and body weight in Santa Ines lambs breeding at the IFES institute of Alegre, Espírito Santo state, Brazil. Thirty-six animals at eight months of age were weighted before slaughter and the carcasses were weighted after slaughter. The DNA of each animal was extracted from hair bulb samples with proteinase K protocol. The amplified product was separated by polyacrylamide electrophoresis and viewed after staining with silver nitrate. Analysis of the phenotypic data showed average means of 36.1 kg for body and 16.5 kg for carcass weight. When analysed the effect of the marker on the body and carcass weight, data were not different for carcass weight (P>.05) but showed difference in the body weight (P<.05). All animals showed only one allele with approximately 145 bases pairs. The absence of variability at this *locus* in this flock may explain the lack of variation in carcass weight and non-genetic factors may be the reason of different slaughter body weights. In Brazil, still there are not studies that relate the QTL with body and carcass in sheep. Therefore, further studies with larger numbers of animals must be performed to confirm this result. This is a possible target *locus* to animal breeding.

P-053

Genetic structure and composite genotype distribution at Oxytocin - Oxytocin Receptor *loci* in Italian Mediterranean river buffalo

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In the last years, several association studies between milk production traits and markers located in candidate genes have been carried out in buffalo species. In particular, an association between a G→T transversion at the 170th nucleotide of the second exon, responsible for the Arg97→Leu amino acid substitution, at the Oxytocin (OXT) *locus* and milk yield in buffalo has been already found. In this work we carried out a polymorphism detection at Oxytocin Receptor (OXTR) *locus* and an analysis of the genetic structure and composite genotype distribution at OXT and OXTR *loci* in Mediterranean river buffalo. The OXTR sequence analysis evidenced a C→T transition at 129th nucleotide of the third exon responsible for the Arg353→Cys amino acid substitution. Genotyping for above mentioned two SNPs was performed in the KBiosciences laboratory (http://www.kbioscience.co.uk). The investigation at the OXT *locus* was carried out on 562 buffaloes reared in different farms located in Campania (Southern Italy). The genotype distribution was 294 G/G, 214 G/T and 54 T/T, with a frequency of 0.713 for the G allele. This result is in agreement with that one already reported for 170 buffaloes belonging to an experimental herd located in the same region. The genotyping of the same subjects at the OXTR *locus* showed a distribution of 332 C/C, 194 C/T and 36 T/T, with a frequency of 0.763 for the C allele. The investigated population is in HW equilibrium. The observed OXT-OXTR composite genotype frequencies were: 0.3060:CC-GG, 0.2278:CT-GG, 0.1815:CC-TG, 0.1335:CT-TG, 0.0569:TT-GG, 0.0356:CC-TT, 0.0302:TT-TG, 0.0196:CT-TT and 0.089:TT-TT. The results obtained from this work represent the first example of genetic polymorphism detected at OXTR *locus* in ruminants, thus opening the way to further interesting studies. It is possible, for instance, to hypothesize that in buffalo the Oxytocin Receptor different amino acid composition by such alleles could be responsible for a different Oxytocin-Oxytocin Receptor system function and regulation. So, the detected polymorphism at OXTR *locus* such as the OXT-OXTR composite genotypes could represent useful genetic markers to estimate their effects on milk production traits in buffalo, but further association studies with milk recording data are needed to evaluate their potential use.

P-054

Sequence analysis of the tyrosinase gene (*Albino locus*) in wild and domesticated rabbits (*Oryctolagus cuniculus*) and in other wild Lagomorph species

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Tyrosinase (TYR) is the key enzyme involved in melanin production. It is expressed in melanocytes, located mainly in the skin and in the pigment cell layers of the eyes. Mutations in the TYR gene are associated with several pigmentation phenotypes in mammals. In particular, disrupting mutations cause different forms of albinism in mice, humans, cattle and several other species. Classical genetic studies have already reported a few alleles at the rabbit Albino *locus* that have been preliminarily characterized by sequencing the TYR coding region in a few rabbit breeds. In this work we further investigated the TYR gene in 11 rabbit breeds with different coat colours (Belgian Hare, Burgundy Fawn, Californian, Champagne d'Argent, Giant Chinchilla, Giant Grey, Havana, Leprino di Viterbo, New Zealand White, Silver and White Vienna), in wild rabbits from Sardinia and in other wild Lagomorphs (Lepus timidus, L. europaeus and Sylvilagus sp.). Four out of five exons composing the TYR coding region were amplified using primers designed on intronic, coding or non-coding regions of the gene sequence assembled in the rabbit oryCun2.0 genome. Sequencing was carried out using a combination of Sanger sequencing (Big Dye v3.1 cycle sequencing kit and sequences loaded on an ABI3100 Avant sequencer; Applied Biosystems) and Ion Torrent PGM sequencing. Three new polymorphisms (two synonymous and one missense mutation) were identified in exon 1 of the rabbit gene. A quite high number of nucleotide differences were identified comparing Oryctolagus cuniculus sequences with those obtained in Lepus and Sylvilagus sp. These data will be useful to evaluate if selection sweeps shaped variability at the Albino *locus* in Lagomorphs.

P-055

Immunodetection of new peptides involved in steroidogenesis in the testis of *Marchigiana* cattle

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Recently, some peptides notoriously involved in food intake, were also detected in mammalian reproductive system. Among them, *in vitro*, Orexin A (OXA) was able to increase testosterone production and regulate stem cell cycle throughout the interaction with its receptor 1 (OX1R), while NUBC2/ Nesfatin-1, increases testosterone secretion only after hCG stimulation. In this study we investigated both the presence and expression of orexinergic system (OXA, OXB, OX1R and prepro-orexin) and Nesfatin-1 in the testis of *Marchigiana* cattle using immunohistochemistry and western blotting analysis. By immunohistochemical analysis we have identified: OXA- and OXB-immunoreactive Sertoli, Leydig and peritubular cells; OX1R-immunopositive Leydig cells and spermatocytes; Nesfatin-1- immunoreactive Leydig and peritubular cells. In *Marchigiana* cattle testis, the presence of orexinergic system and Nesfatin-1 were found by western blotting analysis. Our previous study reported the production of OXA in the urethra-prostatic complex of bovine. Here, for the first time, we described the presence and localization of orexin system and Nesfatin-1 in bovine male gonads. Our results are in agreement with literature data concerning both laboratory and farm animals. Until now, the presence of Nesfatin-1 has been described only in rat testis. Considering that bovine reproductive activity was influenced by photoperiod, we are evaluating testosterone, OXA and Nesfatin-1 blood levels in *Marchigiana* cattle adult breedings during the four seasons, throughout the collection of blood samples from four male breedings, bimonthly, by ELISA assay. In this way, we would evaluate the linkage among testosterone, OXA and Nesfatin-1 blood levels, during the four seasons in *Marchigiana* cattle male breedings.

P-056

Morphological evolution of Martina Franca donkey

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Aim of the research was to describe some morphological traits of Martina Franca donkey breed and their variations over time. The research was carried out on two databases collected in different ways. The first included 41 Martina Franca donkeys (28 females, 13 males), born between 1998 and 2012 in three farms; the animals were measured (wither height: WH, chest circumference: CC, cannon bone circumference: CBC), and the anamorphosis index (AI: CC^2/WH) and the dactylo-thoracic index (DTI: CBC*100/CC) were calculated. The second database included the body measures from 343 adults registered in the herd book (275 females and 68 males), born between 1979 and 2008. The two databases were then combined (384 animals) and two subgroups were isolated: youngs (age <1 year; n=10, 2 females and 8 males) and adults (age >2 years; n=374, 301 females and 73 males). Animals aged between 1 and 2 years were not included. Measures and indexes were submitted to analysis of variance (youngs: fixed effect of sex, random effect of sire and, as a covariate, the age at measurement; adults: fixed effects of sex, year of birth [five classes: <1991, 1991-1995, 1996-2000, 2001-2005, >2005], two-way interaction, random effect of sire and the age at measurement as an intraclass covariate). The results obtained in youngs must be considered as preliminary, due to the reduced number of cases. In young donkeys, sex was never significant ($P>0.05$), while in adults it was highly significant ($P<0.001$); this indicates that sexual dimorphism could be evident from the second year of age. In relation to the effect of the year of birth, the results indicate that morphology changed over the years (WH +7.32%; CC +1.19%; CBC +14.90% higher in animals born after 2005 than those born before 1991). Consequently, AI was reduced by 4.63% between donkeys born before 1991 and those born after 2005, confirming an increase in dolicomorphism in the recent population. It can be concluded that the morphology of Martina Franca donkey evolved over the years; the variations can be explained, in part, by the improvement of breeding and feeding, and in part by the change in the genetic evaluation which is no more oriented to produce heavy animals.

P-057

MUC1 in nelore cattle (*Bos indicus*): analysis of sequence and polymorphism

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MUC1 is a high-molecular-weight glycoprotein located at the apical pole of epithelial cells. The DNA sequence analysis revealed the presence of a repetitive region constituted of variable number of 60 bp units that code for motifs of 20aa including important glycosylation sites. The length polymorphism analysis revealed the existence of five and six different alleles, in cattle and goat respectively. Association studies reported a significant relationship between the VNTR polymorphism and productive and reproductive traits. In this work we carried out the sequencing of MUC1 repetitive region of five alleles observed in Nelore cattle. The sequence analysis of ten PCR fragments (two individuals for each allele) was performed on ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, CA). Using the Tandem Repeats Finder Server (Benson 1999) and the ExPASy Server (Benson 1999; Gasteiger *et al*. 2003) we analysed the repetitive region and forecasted the translation. All five alleles are confirmed to contain the VNTR structure, typical feature of MUC1. Therefore the units do not share 100% sequence homology. The alignment of 170 repetitive units revealed 18 variants, encoding for 9 different amino-acid units; four units are the most frequents. The first 10 aa in each unit are highly conserved, in particular 4 proline residues that play a key role for protein backbone structure; whereas number and position of glycosylation sites (serine and threonine) are not conserved among the last 10 aa. The comparison of the 5 alleles shows differences not only in length, due to the variable number of units, but also in composition and succession of the units. As consequence the structure and glycosylation of the corresponding protein may results modified. Furthermore, comparing the allele 1 sequences obtained from two individuals we observed different arrays of units; this provides evidence of allele variants not identifiable by length analysis.

P-058

Analysis of copy number variations in Italian sheep breeds using the Illumina OvineSNP50 BeadChip array

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Copy number variations (CNVs) are defined as intraspecific gains or losses of ≥1 kb of genomic DNA. These polymorphisms represent the most important source of variability in mammalian genomes in terms of interested nucleotides. Many CNVs represent neutral polymorphic variants, whereas several others cause or are associated with both Mendelian and complex traits. We already investigated CNVs in the ovine genome by cross-species array comparative genome hybridization and produced a first sheep CNV map based on the cattle genome. In this study we used the Illumina OvineSNP50 BeadChip data generated in Italian sheep breeds to identify CNVs in the *Ovis aries* genome. Genotyping data were obtained for 24 animals for each of 15 different breeds (Altamurana, Appenninica, *Bagnolese*, Bergamasca, Biellese, Delle Langhe, Fabrianese, Gentile di Puglia, Istrian Pramenka, *Laticauda*, Massese, Pinzirita, Sambucana, Sopravissana and Valle del Belice). Single nucleotide polymorphisms (SNPs) included in the Illumina OvineSNP50 BeadChip were first mapped on the Oar_v3.1 build of the sheep genome. CNVs were called after filtering genotyping data using the PennCNV software with stringent parameters and correction for waviness effects to reduce false positives. In total, we identified 656 CNV events, covering on the whole 2.62 Mb, and corresponding to 24 CNV regions (CNVRs): 21 were characterized by a loss and 3 by a gain of copy number. Some of these CNVRs harbour coding genes involved in important metabolic pathways and signalling processes. Additional studies are needed to evaluate the effects of these CNVs on phenotypic and production traits in sheep and their usefulness in defining relationships among breeds.

P-059

Genetic diversity in different Italian pig breeds evaluated using Illumina PorcineSNP60 BeadChip data

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High throughput genotyping tools make it possible to extract interesting genetic information from farm animal populations that could be applied to evaluate genetic relationships and the degree of inbreeding, to analyse selective sweeps, and to identify useful markers for breed allocation and authentication of monobreed products. Pork products derived from local pig breeds are usually considered of high value and sold at a higher price, compared to undifferentiated products, creating the needs for combating possible frauds derived by the financial incentives to substitute them with low value products. In this work, we tested the possibility to use the Illumina PorcineSNP60 BeadChip array to identify single nucleotide polymorphisms (SNPs) that could be useful to authenticate pork products derived from pig breeds. On the whole, 500 pigs from 3 Italian breeds were genotyped (360 Italian Large White, 92 *Apulo-Calabrese* and 48 *Nero Siciliano*). Then, 61,177 SNPs were filtered for subsequent analyses. Among these SNPs a subset of 96 polymorphisms was subsequently selected considering their informativity in the pair comparisons, that means SNPs with the larger allele frequency differences between the pairs of populations were chosen. Principal Component Analysis using this subset of SNPs showed a sharp separation between Italian Large White pigs and those of the two local pig populations that were not completely separated to each other. The same SNPs were also analysed using STRUCTURE software with 10,000 Burnin length period, 1000 repetitions and K=3, obtaining an Fst value of 0.37 indicating that intra-population variation is slightly higher than inter-population variation. This works provided useful information on the use of Illumina PorcineSNP60 BeadChip data to identify markers able to distinguish pork products from local and cosmopolitan pig breeds.

P-060

Identification of polymorphisms in the porcine GPR120 (FFAR4) gene and association with production traits in Italian Large White pigs

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The G protein-coupled receptor 120 (GPR120) gene, also known as free fatty acid receptor 4 (FFAR4) or O3FAR1, encodes for a receptor for long-chain free fatty acids that regulates various physiological processes, including adipogenesis, appetite control, food preference, gut hormone secretion, osteoclastogenesis and anti-inflammation. In humans, a missense mutation that inhibits GPR120 signalling activity is associated with obesity and insulin resistance. In this study, we selected the GPR120 gene as candidate for meat production, carcass and growth efficiency traits in pigs. Identification of polymorphisms in the porcine GPR120 gene was carried out using the Ion Torrent PGM sequencer. DNA pools constructed using equimolar DNA concentration from 8-50 pigs of different breeds/populations (Italian Large White, Italian Landrace, Italian Duroc, *Casertana*, Pietrain, Meishan and Wild Boar) were used to amplify the three coding exons, intronic regions and 5'- and 3'- flanking regions. PCR products were sequenced on a 316 Ion Torrent chip and ionograms were automatically called and aligned with TMAP aligner, included in the Ion Torrent suite v3.2, to the GPR120 gene sequence available from the Sscrofa10.2 genome version. Three single nucleotide polymorphisms (SNPs) were identified across breeds/populations. A SNP in the putative 3'- flanking region was genotyped on 560 Italian Large White pigs with extreme and divergent estimated breeding values (EBVs) for back fat thickness (BFT) selected within a performance tested population of about 12,000 animals, 280 with the most negative and 280 with the most positive BFT EBVs. Allele frequencies differed between the two extreme tails ($P<0.10$). Association analysis between GPR120 genotypes and EBVs for other traits carried out using the GLM procedure of SAS showed that the investigated SNP was associated with average daily gain, feed-gain ratio and ham weight EBVs ($P<0.01$).

P-061

Analysis of variability in the goat RXFP2 gene, a candidate for horn size in ruminants

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Phenotypic differences are usually used to distinguish different breeds within the same livestock species. For examples, breeds are recognized because particular coat colours or patterns, size or height of the animals, presence or absence of horns, their shape, etc. Revealing genes contributing to trait variation might provide, on one hand, new insight into the biological mechanisms affecting these traits and, on the other hand, could provide useful markers for forensic applications, including authentication of mono-breed products. Recently a genome wide association study carried out in a wild sheep population suggested that the relaxin/insulin-like family peptide receptor 2 (RXFP2) gene is an important candidate to explain variability in the horn size and shape in *Ovis aries*. This gene is involved in determining primary sex characters in mice and humans. In this study we investigated the goat RXFP2 gene to identify polymorphisms useful to evaluate its role in determining horn features in this small ruminant species and, eventually, useful for authentication of mono-breed products. Five primer pairs were designed on the bovine RXFP2 gene sequence to encompass 5 out of 18 coding exons of this gene. Goat genomic DNA from 30 animals of different breeds with different horn shape and size (Girgentana, Saanen, Camosciata delle Alpi, Derivata di Siria, Maltese and Murciano-Granadina) was amplified and obtained products were sequenced using Sanger sequencing and a capillary sequencer. Two SNPs were identified in intronic regions and one missense mutation was identified in exon 4. Minor allele frequency ranged from 0.05 to 0.12 across breeds. This low level of polymorphic sites might indicate a quite high level of conservation across breeds. As these polymorphisms were not very informative, additional regions will be sequenced to identify other SNPs in the goat RXFP2 gene.

P-062

Exploring genomic differences between *Nera di Arbus* and *Sarda* dairy sheep breeds

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The aim of this work was to assess the genetic diversity between the two autochthonous sheep breeds of Sardinia by using the Ovine50K Beadchip (Illumina). Genotypes of 44 300 SNP were obtained for 94 *Sarda* rams from the artificial insemination center (SW) and 116 *Nera di Arbus* (PN) sheep sampled from 32 private flocks. Genomic relationships (GF) between individuals were estimated following Hayes *et al*. (2009). The degree of LD was calculated as r^2. The extent of haplotypes sharing between breeds was taken as the correlation of r statistic for SNP pairs at different distances. Reynolds genetic distance and Fst were estimated. Furthermore, average difference in allele frequencies (VAD) was calculated in overlapping sliding windows of 11 SNP (Hayes *et al*., 2009) to identify divergently selected regions. LD decay with SNP distances was sharper for SW than for PN. The genomic relationship matrix revealed a slightly higher average GF within SW (0.104) than PN (0.0952) and an average GF between them equal to 0.058. The Reynolds genetic distance was 0.0163 and the average correlation between r ranged from 0.7 to 0.3, indicating a high level of haplotypes sharing between the two breeds. The highest values of VAD were estimated on OAR 19. Despite the improved *Sarda* and the *Nera di Arbus* breeds were considered to be the same breed in the past, this study confirms that selection for milk yield led to some genetic differences between them.

P-063

Salernitano horse breeding program: first report

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The *Salernitano* is a warmblood type of horse native of Southern Italy in Campania Region, a very ancient breed currently considered as endangered. At present only about 200 heads are registered in the Official Record Book with some small groups of mares living in the Campania Region, still not registered. In 2010 the Administrative Committee of Campania Region sponsored a breeding plane to increase the *Salernitano* horse population, involving 4 stallions of the Regional Center of Equine Improvement in S. Maria Capua Vetere (CE). The artificial insemination (AI) program started from April to July on *Salernitano* mares of private owners of the Region. The aim of the project was to increase the number of the *Salernitano* horse registered in the RA by optimizing the employment of stallions during the breeding season and monitoring their fertility. Nineteen farms, located in the different provinces of the Region, signed on to the plane with 39 mares in total. Forty-five ejaculates were collected from 4 *Salernitano* stallions and gel-free semen was evaluated for gross appearance, volume, concentration, visual motility and viability. The followed up mares for more than one cycle, requiring on average 1.25 inseminations for cycle and 1.7 inseminations for pregnancy. The mean fertility rate for oestrus cycle was 44.6%, with no differences among stallions and months; the overall pregnancy rate was 64.7%. Twenty-one mares foaled (18 fillies and 3 colts), 2 mares aborted and two fillies dead. The projects ended with an active of 13 mares identified as belonging to *Salernitano* breed and 16 new fillies that could be enrolled in the main section of Official Record Book, while the 3 colts could be inserted in the secondary part of the same Book with a potential increase of the actual official data on *Salernitano* horse breed of about 20%.

P-064

Use of Locally Weighted Scatterplot Smoothing regression to study selection signatures in Piedmontese and *Marchigiana* cattle breeds

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Selection is the main *locus*-specific force that affects genetic variation in species. A consistent change in the pattern of allelic frequencies is the result of the action of this force. The fixation index (Fst) is an estimate of population differentiation based on genetic polymorphism data. Currently, several statistical methods have been developed to analyse genomic data for the detection of chromosomal regions under selection. In the present work, the Locally Weighted Scatterplot Smoothing regression (LOWESS) analysis combined with the Control Chart for the statistical significance, were used to investigate selection signatures in two cattle breeds with beef production aptitude. Fst was calculated for 43,116 SNP marker *loci* distributed across the genome in 364 Piedmontese and 410 *Marchigiana* bulls. Animals were genotyped with the Illumina Bovine bead-chip containing 54,001 SNP. The LOWESS and Control Chart approach was able to highlight selection signatures in chromosomes known to harbour genes affecting muscling trait. Examples are peaks detected for BTA2 in the regions where the myostatin and lactase genes are located.

P-065

Quantitative trait *loci* mapping for milk fatty acids in italian Brown Swiss dairy cattle breed

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The detection of genomic regions affecting complex traits has leaded the interest in using large panels of single nucleotide polymorphisms (SNPs) to identify quantitative trait *loci* (QTL). Selective DNA pooling strategy is a method to reduce costs in genomic studies by genotyping pooled DNA samples from selected individuals at each of the two phenotypic extremes. The identification of genomic regions responsible for genetic variation in milk fat composition may help to understand the biological pathways involved in fatty acid synthesis. In this study, a selective DNA pooling approach in Italian Brown Swiss cattle was applied to identify QTLs for Δ9-Desaturase (D9D), conjugated linoleic and vaccenic (CLA and VA) acids. A total of 120 daughters for each of the five selected families (60 animals with higher residual values and 60 animal with lower residual values) were pooled and genotyped using Illumina Bovine SNP50 BeadChip. In this study the generation of B-allele frequency was performed automatically using the self-normalization algorithm of Illumina BeadStudio software. Statistical analysis was performed with respect to SNPs for which the sires were heterozygous. Using the R software a procedure has been implemented in order to perform a single marker sire test. A multiple testing correction was applied using the proportion of false positives (PFP) among all positive test results. Association tests were carried out in order to identify genes with an important role in pathways for milk fat and fatty acids metabolism. Several chromosome regions were significantly associated with the traits studied, being some of these regions harboring genes known to be involved in fat synthesis as reported in literature.

P-066

Morphological characterisation of the Amiata sheep

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This study aims the morphological and biometric characterization of the Amiata sheep. This ovine population has the common Tuscan or *Nostrale* or *Vissana* sheep ancestral, that lived in central and southern Tuscany during the XIX century. Merino derived sheep where after introduced in the original population, forming a small sized and merino type sheep, that lived in the Amiata Mount area, and in particular in the Labbro Mount. This genotype had meat, milk and wool aptitudes. The modern Amiata sheep, living in the homonym area, has shown Frontal index (FI= head width/length x 100) of 47.7 cm in males and of 44.0 in females, and all individuals have shown ears in horizontal position. The 62% of rams was horny, and the 95.6% of ewes didn't have horns. The 95.6% of sheep was white, with a medium quality merino fleece type. The medium-low develop breast was symmetric and well attached. The height at withers was 73.8 and 70.6 cm in males and females respectively, and the chest circumference was 100.4 cm in rams and 96.9 cm in ewes. The croup width was 22.4 cm, and did not show variations between males and females. The breed standard of *Sopravvissana* and *Gentile di Puglia*, two Italian Merino derived sheeps, have shown that these breeds are slightly smaller than Amiata sheep (ASSONAPA, 2013). The Dactyl-Thoracic Index (DTI=front shank circumference/chest circumference x 100) has shown a average value of 9.4 and did not show differences between rams and ewes.

P-067

Effects of varying dietary protein and lipid levels on the growth performance of the European grayling (*Thymallus thymallus*)

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Despite a growing interest for the European grayling (*Thymallus thymallus*) as a new candidate for freshwater aquaculture, little information is available on growing records and feeding behaviour of this fish species in captivity as well as on its nutrient requirements or suitable nutrient levels to be adopted in formulating complete dry feeds. As a first approach in this direction, a feeding trial was carried out to study the growth response of grayling to diets varying in macronutrient levels. Four practical-like complete feeds containing different crude protein and lipid levels (45/20; 50/16; 55/12 and 55/20% DM), were prepared. Each diet was fed twice daily to visual satiety over 20 weeks to triplicate groups of fish, each consisting of 15 specimens (age 1+, ind. wgt. 112±6.5g), according to a single factor random design. Fish groups were kept indoor in 250-L tanks supplied by well water at nearly constant water temperature (12.8±0.4°C), flow-through rate (5 L*min^{-1}/tank) and exposed to an artificial day-length set at 12h. Three specimens were sampled at random from a spare group at the beginning and 3 fish per dietary group were collected at the end of the trial for whole body nitrogen analysis and calculation of nitrogen retention (N gain*100/N intake). Irrespective of the diet, voluntary dry feed intake ranged between 4.9 and 5.5 g/kgABW/d and was found to be much lower than that of salmonids reared at the same temperature. Final body weight-FW, specific growth rate-SGR and feed conversion ratio-FCR of fish fed diets high in protein (55/12 and 55/20) were similar and significantly better than those exhibited by grayling given the diets 45/20 and 50/16 which did not differ from each other (FW, 220 *vs* 196 g; SGR, 0.44 *vs* 0.39; FCR, 1.2 *vs* 1.4, $P<0.05$). Nitrogen retention (32±1.5%) was unaffected by the dietary treatment. The results obtained so far suggest that grayling takes advantage of high protein diets also at post-juveniles stages and that increasing dietary lipid above 12% DM results in poor protein sparing effect in this fish species.
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P-068

Identification of fish species by isoelectrofocusing

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The significant increasing consumption of fish and related products in Italy, in recent years, has led to the frequent occurrence of fish from foreign sea areas further some processed products that make difficult a proper identification not only to the consumer but also to the veterinary inspector. Since many fish species are naturally toxic whereas some accumulate environment heavy metals in their tissue or are highly allergenic it is necessary to get a reliable and simple identification methods in order to protect human health and to support enforcement and compliance with labelling legislation. The inspection of the fish is made by observing morphological characteristic of the species. However, it is difficult when seafood is processed (filleted, sliced, smoked, cooked, canned, breaded). In these cases, laboratory techniques based on species-specific biomarkers are essential to provide for a proper identification. Many analytical techniques have been set for seafood species identification. Two methods guarantee reliable results as Isoelectrofocusing (IEF) and PCR. This study aims to develop and validate a reliable analytical method for fish species identification in order to control and certify products labelling through isoelectrofocusing (IEF). Therefore, 20 fishes of 11 different species, collected at the market, were analysed. First, the samples were identified and classified by the specific morphological characteristics. Then they were photographed and analyzed by IEF in order to create species-specific protein maps based on the different number of bands and range pI distribution. These latter were processed by densitometer GS 800 Biorad with software Quantity One. As a result, the study detected the protein maps of some fish species belonging to the order Pleuronectiformes as Sogliola (*Solea vulgaris*) with 7 bands (range 4.40-6.89); Platessa (*Pleuronectes platessa*) with 14 bands (range 4.66-7.31); Passera (*Plathichtys flesus*) with 10 bands (range 4.32-6.67) and Limanda (*Limanda limanda*) with 11 bands (range 4.50-7.43). IEF is an easy and economical method able to distinguish fish species and therefore useful to discover commercial and sanitary frauds. Moreover it represents a useful tool in the process of certification of providers in GDO.

P-069

An improved GC-Mass method to assess the short chain fatty acid in fish gut fecal compounds

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Butyric acid, a short chain fatty acid (SCFA), has been reported to protect fish gut structure and having an anti-inflammatory property, becoming evident when high percentage fish meal (FM) substitutions with vegetable proteins (VP) is operated. To achieve the control of microbial butyrate production in fish intestine, a reliable method to assess small concentrations of such SCFA in fecal compounds is envisaged. The volatility of butyrate allowed to quantify the molecule in fish feces by the headspace method with GC-Mass. The method consisted in acidification and heating of feces in order to evaporate the butyrate. Many parameters (initial temperature and the characteristics of the ramp) have been experienced, in order to find the best analytical conditions and to avoid errors due to contaminants. The high sensibility of the protocol allowed to detect 0.11 mM as butyrate in feces within 250 µL of injection, with 13 minutes of run. The sensibility of GC-Mass has been tuned by the calibration with butyric acid in methanol and the lowest concentration measured by machine was 0.05 mM. The composition of sample and the contaminants prevented the achievement of 0.05 mM (27.5 nmol butyrate/250 µL of injection). Further details and calibration diagram are reported on the poster.

P-070

Lipids and fatty acids composition of eggs in relation to larval quality from cultured common sole (*Solea solea*) broodstock

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Eggs quality optimization is a fundamental aspect in the standardization of the production cycle of a fish species. Lipids content and fatty acids composition of common sole (*Solea solea*) eggs were measured and correlated to eggs and larval viability parameters along an entire reproductive season. Seventeen batches of fertile eggs obtained from natural spawning of captive breeders were characterized for lipid content (L), fatty acids content (FAs), total weight (TW), spawning temperature (T), floating rate (FR), hatching rate (HR) and survival rate of larvae (SR) at 0-6 days post hatching (dph). Based on univariate regression models it was possible to formulate significant relations using L, FAs, TW and T as independent variable. T, L, and 20:5(n-3)+20:4(n-6) (EPA+ARA), were significantly ($P\leq0.05$) correlated to FR. T, 14:0, 18:0, 22:6(n-3) (DHA), $\sum$monounsaturated FAs, $\sum$polyunsaturated FAs of the (n-3) series, and EPA+ARA were significantly correlated to HR and SR. It was also found that L, 16:0, 16:1(n-7), 18:2(n-6), 20:4(n-6), saturated FAs, $\sum$polyunsaturated FAs of the (n-6) series had a significant effect to HR, while TW, 18:1(n-7), 20:5(n-3), $\sum$(n-6)/$\sum$(n-3), DHA/EPA were significantly correlated to SR. The results indicate that quantitative eggs lipids and fatty acids composition can predict eggs and larval quality of common sole. Since breeders' diet is one of the main factors affecting eggs composition, the results of this study could provide useful information for broodstock nutrition.

P-071

Space use and activity of cattle on pasture monitored with GPS/GSM collars. Preliminary results

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Animal tracking by GPS collars is becoming a widespread technique both for wildlife and for grazing livestock, in order to describe their use of space and habitat preferences. GPS collars are now frequently equipped with activity sensors, which represent a new challenge as well as a possibility to derive the behaviour of free ranging animals and their activity over long periods of time. In this work we report the preliminary results of an attempt to detect the main activities of cattle on confined pasture, by means of activity sensors located on Pro-light 4 GPS/GSM collars (Vectronic Aerospace® - Berlin). Some indications on their space use at pasture are also reported. A triaxial accelerometer continuously records activity (2.5 minute/interval) and delivers x and y values respectively for forward-backward and lateral head movements, on a scale from 0 to 255. However, since the values are not directly associable to a specific activity, the animal behaviour is not automatically detectable. In order to distinguish different behavioural categories we fitted GPS collars on two cows, a *Chianina* and a Limousin, and simultaneously we performed direct observations of their behaviour. For each animal, values for different behavioural categories were compared statistically using Welch ANOVA and Games-Howell post hoc test (SPSS 16.0). As preliminary result we found significant differences ($P<0.05$) in forward-backward movements between resting, feeding, and slow locomotion for the Limousin cow; furthermore, feeding and resting differed significantly ($P<0.001$) in the *Chianina* cow both in forward-backward and in lateral movements. In relation to the monitoring of the space use, preliminary results seem to indicate a greater mobility for the *Chianina*, particularly considering interfix distances in different time of the day.

P-072

Temporal gene expression profiling of liver from peripartal dairy cows during spring and summer

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Thermal stress during hot seasons has been shown to render dairy cattle more susceptible to metabolic disease, including liver lipidosis. We examined temporal gene expression profiles during the dry period and early lactation in liver of 12 Holstein cows that calved in the spring (SP: March-April) or summer (SU: June-July) using a whole-transcriptome bovine microarray (Agilent) and quantitative RT-PCR (qPCR). Liver biopsies were performed at -30, 3, and 35 days relative to parturition. Bioinformatics analysis of data was performed using the Dynamic Impact Approach. The 23 target genes selected for qPCR were associated with heat shock response (HSP70A1A, HSTF1), fatty acid oxidation (CPT1A, PPARA, ACOX1), hepatokines (FGF21, ANGPTL4), esterification and VLDL assembly (MTTP, APOB100, DGAT1, SREBF2), glucose metabolism (PC, PCK1, PDK4), inflammation and oxidative stress (TNF, GPX1, SOD1, SOD2, SAA3, HP, HAMP), and endoplasmic reticulum stress (EIF2AK3, XBP1). Results from qPCR revealed a postpartal increase ($P<0.05$) for the expression of HSP70A1A in SU. In contrast, HSTF1 was greater overall in SP due to upregulation after calving. Heat stress was associated with lower overall expression of CPT1A and also PPARA and ACOX1. Expression of FGF21 and ANGPTL4 increased from -30 to 3 days in SP and SU; however, cows in SU had greater overall expression after calving. Except for MTTP, expression of APOB, DGAT1, and SREBF2 was greater ($P<0.05$) overall in SP. The gluconeogenic enzymes PC and PCK1 increased from -30 and 3 days in both SP and SU but expression of PC was greater overall in SU; whereas, PCK1 was greater overall in SP. Expression of acute-phase proteins and oxidative stress indices increased between -30 and 3 days in SP and SU, but there was greater overall expression in SU. This response contrasted that of TNF, which was greater overall in SP. In contrast to nonruminants, no change was observed in endoplasmic reticulum stress-related genes. Overall, results revealed that during hot seasons the liver transcriptome in peripartal cows is markedly altered and likely contributes to the susceptibility of those cows to develop disease. The increase in oxidative stress and inflammatory mediators due to heat stress might make the transition cow more susceptible to disease.

P-073

Microbial inoculum for litter conditioning in calves rearing: preliminary results on litter quality and health

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Litter is considered a potential source of animal pathogens for livestock, affecting both animal health and behaviour, and its sanitation is one of major concerns especially in intensive rearing of young animals, more subject to gastroenteric infections. To evaluate the effect of a commercial microbial inoculum for litter conditioning, supposed to affect ammoniacal and pathogenic microflora level of litter for its content in saprophyte microorganisms specialised in degradation of organic matter, a trial was carried on for 9 weeks on two groups of Holstein male calves. Animals, with an initial average age of 2 months, were reared in 6 pens of 13 heads each on a permanent straw litter: 3 pens were assigned to control (C) and 3 to treated (T) thesis. From trial day 8th, the inoculum was distributed weekly on the litter at the recommended dose (7 g/calf/week, hydro-solution). Litter samples were taken at the beginning and every 2 weeks of the trial (5 sampling), and physical (temperature, humidity), chemical (pH, total N) and microbiological (coliforms, aerobic mesophylic and sulphite-reducing clostridia, *E. coli, Salmonella*, verocytotoxigenic *E. coli*; 9 replication for sample) analysis were performed. Data were analysed by ANOVA according to treatment and sampling. No statistical differences were recorded between groups for litter temperature (14.75 *vs* 15.62°C in C and T respectively), humidity (80.80 *vs* 82.57% in C and T respectively), pH (8.83 *vs* 8.67 in C and T respectively), and total N (1.77 *vs* 1.80% on DM in C and T respectively). Differences were instead found in coliforms (9.7 *vs* 4.4×10^5 CFU/g in C and T respectively, $P<0.01$) and aerobic mesophylic bacteria (13.4 *vs* 4.0×10^7 CFU/g in C and T respectively, $P<0.05$), but not in *E. coli* (20.4 *vs* 6.9×10^4 CFU/g in C and T respectively) and sulphite-reducing clostridia (2.7 *vs* 26.5×10^4 CFU/g in C and T respectively). No Salmonella were found, while verocytotoxigenic E. coli were found only in alternate samples (1st, 3rd, and 5th samplings). In conclusion, the tested microbial inoculum seems to reduce the level of some bacteria without affecting litter characteristics, with a useful role in containing microbial pathogens population in calves' intensive rearing systems.

P-074

Feeding preference in horses: comparison between different pasture forages grazed as micro-swards by Anglo-Arab stallions

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Full-time stabling and feeding excess of concentrates to horses can result in colic, laminitis, obesity and stereotypies. Hence part-time grazing and exercising on pastures are desirable. A short term test was conducted to evaluate horse preference for pasture forages. Treatments (T) were established by sowing forage species in boxes to create micro-swards as follows: 4 boxes of a control forage (Lolium perenne L.), LPLP, and 7 binary associations composed by 2 boxes of control and 2 of test forages offered side by side. Test forage and corresponding treatments were: Avena sativa L., LPAS; Bromus catharticus Vahl, LPBC; Dactylis glomerata L., LPDG; Festuca arundinacea Schreb, LPFA; Lolium multiflorum Lam, LPLM; Cichorium intybus L., LPCI; Trifolium repens L., LPTR. Eight stall-fed Anglo-Arab stallions (S) were exposed to the treatments for 4 minutes daily (test period) in a 8x8 Latin-Square design-trial lasting 8 days (D). Herbage intake was measured by weighing the boxes before and after the test, accounting for evapo-transpiration losses. Feeding behavior was recorded by a video-camera. Bite mass and intake rate were calculated on both fresh (FMBM, g, FMIR, g/min) and dry matter (DMBM, g and DMIR, g/min) basis. The behavioural variables were analysed by GLM with T, S and D as fixed effects to study i) total intake components considering the whole dataset ($N=64$) or ii) test forage intake components, using a subset excluding LPLP ($N=56$). T means were separated by Tukey test ($P<0.05$). On the whole, LPLP showed higher total bite rate (BR, n. bites/min) than all binary associations ($P<0.001$). LPBC displayed the highest while LPCI the lowest total DMIR ($P<0.05$), being the other treatments intermediates. Among the binary associations, stallion BR was higher while eating F. arundinacea than C. intybus ($P<0.05$) but the reverse was true for DMBM , resulting in similar DMIR between these forages. The highest test forages DMIR was recorded on B. catharticus while the lowest were on F. arundinacea and C. intybus ($P<0.05$). To conclude, on the basis of total and test forage DMIR, LPBC was top while LPCI and LPFA bottom ranking for preference, the other treatments being intermediates.

P-075

Physiological measurements and related indices around calving to validate welfare assessment models at dairy herd level: an example

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The objective assessment of the animal welfare in dairy farms is a very complex and controversial task. The main problems are related to welfare definition, choose of the indicators to include in the model, aggregation of the scores of each indicator in a weighted overall welfare score. Then, the model has to be validated though a gold standard that does not exist. The aim was to compare the results obtained with the application of our model of welfare assessment (IDSW: Integrated Diagnostic System Welfare) with more objective welfare assessment based on physiological blood indices. The IDSW considers indirect and direct indicators and calculates a standardized (0-100 scale) overall welfare score (OW). The welfare in a dairy herd (850 dairy cows) was monitored by using IDSW for a period of 30 months. During the trial 101 blood samples from early lactating cows (from 5 to 30 DIM) were also collected, and analyzed to assess energy, protein, and mineral metabolism, as well as markers of inflammatory conditions. The Liver Functionality Index (LFI) and the Liver Activity Index (LAI), which includes some negative acute phase proteins or related parameters (albumin, cholesterol, bilirubin and vitamin A), were used to evaluate the severity of inflammatory conditions occurred around calving. The data of the selected blood indices used for LAI were transformed to z distributions and the final value of each cow was calculated from the average of the z scores. The results obtained with IDSW show an increase of welfare during the trial, from a low value (67.9%) observed at first check, to an acceptable value (76.5%) observed at the last check. The welfare improvement was related to the improvement of management, with enhancement of direct welfare indices (mainly health, reproductive and behavioural type). At first check poor LFI and LAI values were observed (-3.29 and -0.26, respectively). During the trial these values increased, and reached values considered acceptable at the last check (-0.13 and 0.54, respectively for LFI and LAI). These results seem promising in order to develop methods, based on physiological indicators, to validate the applied models of welfare assessment in dairy herds.

P-076

Plant extracts in rabbit diet: effects on welfare and performance

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In order to investigate the effects of some dietary phytoderivates (*Origanum vulgare* L. and *Rosmarinus officinalis* L.) with antimicrobial and/or antioxidant properties on rabbit welfare and productive performances a total of 100 New Zealand mixed-sex rabbits were weaned at 30 days of age and immediately split into homogeneous groups submitted to the following dietary treatments: 1) Standard diet (C); 2) Standard diet +150 ppm Vit E (E); 3) Standard diet +0.2% oregano (O); 4) Standard diet +0.2% rosemary (R) and 5) Standard diet +0.1% oregano + 0.1% rosemary (OR). The plant derived ingredients were obtained with an enzyme aided extraction of leaves using water as solvent (Phenbiox®). All rabbits were housed individually in flat-deck cages measuring 600×250×330 mm. Rabbit welfare was evaluated analyzing the natural immune responses by the lysozyme concentration (Lys), serum bactericidial activity (SBA) and hemolitic complement activity (CH) in blood serum; the productive performances were evaluated by measuring individual live weight and feed intake weekly. Blood samples were collected from 10 rabbits/group at 30 days (Time 0) and 80 days of age (Time 1). At 80 days, 8 rabbits/group were slaughtered and the carcass weight were rated. Starting from similar weights at the beginning of the trial, the highest final live weight (2517g OR *vs* 2408g C, $P<0.386$) and the best feed conversion ratio (3.52 OR *vs* 3.62 C, $P<0.43$) were registered in OR group. The natural immune responses were influenced more by an age-related effect rather than by the diet. Indeed, SBA drastically felt from 30 to 80 days, whereas Lys and CH increased (for all parameters: $P<0.05$). A diet effect was registered in OR group for the CH which significantly increased at Time 1 *vs* Time 0 ($P<0.05$). The CH assay is very useful for assessing the onset risk of infectious disease or the gravity of diseases already in place. The OR diet seems to be able to improve this immune activity.

P-077

Orange pulp silage and dehydrated citrus pulp on parasitological parameters of sheep naturally infected with gastrointestinal nematodes

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In sheep production, gastrointestinal nematodes are responsible for considerable economic losses. Farmers and costumers are interested in health, welfare and sustainability. Brazil produces 90% of oranges from South America. After juice extraction (82% humidity) is often discharged and requires proper destination. This project goal was to evaluate the supplementation of silage made with orange pulp and dehydrated citrus pulp, in parasitological parameters from sheep and to raise alternatives for health maintenance, and the sustainability. The orange pulp is rich in essentials oils, mostly d-limoneno, that can cause toxic interference in biochemical and physiological functions of the parasites, being alternatives for maintenance, effectiveness of the anti parasitic and the sustainability of the agriculture production. Accomplished in Nova Odessa Institute, São Paulo (22º46'39" South, 47º17'45" West), Southeastern Brazil. Forty-five lambs Santa Inês breed, naturally infected with gastrointestinal nematodes, with 23.9 Kg average weight, kept in pastures of Aruana grass (*Panicum maximum*). Lambs were separated in three different pastures to compose 3 groups: Control: corn silage *ad libitum* and 1% from its body weight of a meal with 18% crude protein. Group 2 and 3 – corn silage *ad libitum* and 1% body weight of 18% CP meal with dehydrated citrus pulp and corn silage *ad libitum*, 4.5% of orange pulp silage and 0.3% body weight of meal with 45% CP, respectively. The amount of food was adjusted according to animal weight. Fecal egg count (FEC), coprocultures and egg hatch test (EHT) were performed every 14 days, totaling seven collections. ProcGLM (SAS), with repeated measures at time and Tukey test were performed. No difference were found for FEC (P>0.05) from groups 1, 2 and 3 with respective averages of 1687.88±3223.23, 1299.02±1925.97, and 1688.94±3873.18. Coprocultures showed predominance of the genera Haemonchus, followed by the Trichostrongylus e Strongyloides (65.7%, 33.3% e 1%, respectively). Viability of eggs were evaluated by EHT and also do not presented differences (P>0.05) between groups 1, 2 and 3 with average hatchability of 90.2%, 89.5% and 85.2% respectively. Orange pulp silage or dehydrated orange pulp did not help to control gastrointestinal nematodes.

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P-078

Parasitological parameters of lambs artificially infected with *Haemonchus contortus* fed orange pulp silage or dehydrated citrus pulp

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Haemonchus contortus is the most prevalent and pathogenic parasite found in tropics, and its control is based on conventional anthelmintics. Alternatives for anthelmintics should be found, because multiresistant strains of this pathogen is widely spread. Natural compounds have been studied for its anthelmintic activity, reducing costs and residues on meat products. Orange pulp is a residue from the orange juice industry and is often discharged requiring proper destination. The use of byproducts from agriculture can be an interesting way to contribute to low residues in nature. The orange pulp has essential oils units composition that consists in a mixture ofterpenes, such as citronellal, citral and d-limonene. Those compounds are secondary metabolites that can cause toxic interference in biochemical and physiological parasites functions, and presents the advantage of being less toxic to mammals. The project goal was to evaluate the supplementation of orange pulp silage and dehydrated citrus pulp parasitological patterns of Santa Inês lambs artificially infected with *H. contortus.* Accomplished at Instituto de Zootecnia, Nova Odessa-São Paulo, southeastern of Brazil, twenty four lambs were dewormed with Neguvon® and infected with 3500 *H. contortus* larvae. After 28 days lambs were divided in three treatments and alocated in individual stalls. Treatment 1 (T1) received corn silage and soy meal Treatment 2 (T2) and 3 (T3) received corn silage and dehydrated citrus pulp mixed with soy meal and orange pulp silage plus soy meal, respectively. Total diet protein from all treatments were 11%. Parasitological parameters as FEC and egg hatch test (EHT) were done every 14 days. ProcGLM (SAS), with repeated measures at time and Tukey test were performed. Orange pulp silage was analyzed by gas chromatographyand indicated that 65% of the oil extracted consisted in limonene. No statistical difference was found for FEC and averages were: 3.682±3.706, 4.579±5.254 and 4.625±5.004, respectively for T1, T2 and T3. Also in EHT, no statistical difference was found between treatments (91.34±3.14%, 69.0±33.27% and 89.48±4.72%) however T2 showed a decreasing curve in the course of collections, with average of 15.06% of hatchability in the last collection, which can represent a minor pasture contamination by *H. contortus.*

P-079

Jenny behaviour during machine milking

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This study was aimed to investigate the effect of machine milking on behaviour, milk production and milk quality of jennies. Four Martina Franca and four Ragusana jennies were used. Five jennies had no experience of machine milking (group NE), whereas other three had (group E). Milking was performed by a portable milking unit once a week from 20 to 140 days in milk and residual milk was dripped by hand. Before milking foals were separated from the mothers at different time intervals. The behaviours, recorded from the attachment to the removal of milking cluster, were step (foot lifted less than 15 cm off of the ground) and kick (raised more than 15 cm off of the ground, even if a clear kick was not visible). In addition, defecation, urination and vocalisation were recorded as a single behavioural activity, but this activity was observed so rarely that was not included in the statistical analysis. Duration of milking, milk yield and quantity of dripped milk were also registered. Data were analysed by using the mixed procedure of SAS with day of lactation as repeated factor, experience as non repeated factor and time of separation of foal as covariate. The jenny was considered random. In addition, Pearson correlation coefficients (r) were used to determine the correlation between variables. The number of steps (1.29±0.56 *vs* 1.61±0.43 n/min; for groups E and NE, respectively) and kicks (0.34±0.26 *vs* 0.56±0.20 n/min; for groups E and NE, respectively) expressed by jennies during machine milking was very low. These variables along with duration of milking were not affected by experience (2.87±0.29 *vs* 2.94±0.22 min; for groups E and NE, respectively). Conversely, time of separation from foals had a significant influence on duration of milking and number of kicks (P<0.01). Duration of milking increased with the time of separation (r=0.294; P<0.01), whereas, even if not significantly, the number of kicks decreased as the time of separation from foals increased (r=-0.147). It can be concluded that jennies rapidly habituate to machine milking, although their behavioural response may be affected by the time of separation from foals.

P-080

Directive 74/1999/EC on the protection of laying hens: analysis of implementation issues by means of focus group and qualitative interview methods

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The aim of the present preliminary research was to understand the reasons which determined, in the Italian context, the delayed implementation of the 74/1999/CE Directive, and the issues that poultry operators have faced and are currently dealing with in order to comply with the law. A further aim of the research was to assess the possible consequences of the correct implementation of the Directive on the whole poultry industry on a national and European level, both in terms of farm management and in the market. The research was carried out through the application of two qualitative techniques: 1) a self-contained focus group, which involved experts from the University, from the Public Veterinary Services (AUSL) and from the business world, 2) a semistructured qualitative interview, which involved two veterinarians from the AUSL, two veterinary professionals (which are currently employed by two of the most important poultry groups in Italy and in Europe), two experts on the welfare of laying hens, a representative of the Italian Association of Poultry Producers, and two poultry farmers. From the information gathered it was possible to understand which factors determined the delay in the law implementation: 1) from an economic standpoint, the adoption of modified cages requires an investment of 10-15€ per hen, and a reduction of hen number up to 30%; 2) the interpretation of the directive is not straightforward as concerns construction of the cages, 3) the reduction in the number of hens implies, also in barn systems, a decrease of production (-6-8%), 4) farm management is more difficult and expensive, 5) farmers that have chosen alternative systems have to face more sanitary problems (*i.e.*: *Salmonella*).

P-081

What foods are identified as animal friendly by Italian consumers?

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In the EU, and in particular in the Italian market, consumers have difficulties in recognizing "animal friendly" products, *i.e.* those products obtained from animals reared under increased level of animal welfare in comparison to the minimum requirements set by the European legislation on the protection of farm animals. Europe is currently working to create an animal welfare label to be used on food, with the aim to make it easier for consumers to identify and choose animal friendly products. Our research is based on the collection of 335 consumer interviews (conducted near and within supermarkets and hypermarkets in Bologna and in its province) and 57 interviews with veterinary experts, in order to analyze consumers attitude towards animal welfare and their knowledge of farming conditions to understand how this perception can influence the consumption behavior of animal friendly products. The results of the research lead us to conclude that there are different degrees of interest and knowledge of this subject and that consumers which are most interested in animal welfare would be willing to pay up to a 20% premium price for animal friendly products. Increased knowledge and information in relation to animal welfare have a positive effect on purchasing behavior. Consumers that are more informed judge animal friendly products as more ethical and greener and not healthier and more tasty. However, the survey confirms a significant difficulty in the identification of animal friendly products by all interviewees, including experts. Therefore, a European label and an increase of information for consumers would be desirable.

P-082

A novel index to quickly assess the severity and the consequences of the inflammatory status in the periparturient dairy cow

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Inflammation is a common occurrence in periparturient dairy cows but, if too severe, it could be source of further health problems and reduction of performance. The level of inflammation widely varies among cows, but just some of them show clinical symptoms. Several plasma indexes have been used to describe the inflammatory phenomenon, like the acute phase proteins

(APP), whose plasma concentration increases (positive APP) or decreases (negative APP) with the enhancement of inflammation. Our first target was to set up an index composed by some plasma inflammatory parameters able to give a more exhaustive information on the inflammatory phenomenon than the examination of individual parameters. In addition, we aimed to provide as soon as possible after calving an evaluation of the consequences of inflammation on the following lactation. The experiment involved 56 multiparous dairy cows, frequently checked through all their transition period for the health status, milk yield, BCS, many plasma parameters. A Principal Component Analysis was carried out to choose the parameters to be included and the best time of sampling. According to this analysis, haptoglobin, reactive oxygen metabolites, cholesterol and paraoxonase sampled 7 days after calving was the best combination. A range of variation for each parameter was defined basing on literature data. The extreme values were assigned scores 10 (best) and 0 (worst), and the linear correlation calculated accordingly can give a score for every concentration level of the parameter. The Post-calving Inflammatory Response Index (PIRI) is the summation of all the four scores. The calculation of the index for the investigated population showed a good agreement with the profile of other plasma parameters related to inflammation (*e.g.* ceruloplasmin, bilirubin, retinol) and to energy metabolism in the early lactation. In addition, the cows with a higher PIRI underwent also fewer post-calving health troubles, showed a higher milk yield and a lower lipomobilization. According to these results, the PIRI index is able to promptly identify the cows with inflammatory problems immediately after calving. However, the index has to be further validated on a wider population and possibly improved by anticipating the identification of the cows at risk.

P-083

Do antiseptic interfere with pain due to surgical castration in piglets?

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In 2 previous phases of this study about pain in piglets subjected to surgical castration the latency to move, defined as the time required by the piglets to make the first step when placed back within the farrowing crate after castration or handling, was investigated. In the first study the variable resulted affected by treatment but not in the second. This relevant difference in the behaviour encouraged the investigation of other possible factors interfering with castration's pain. One hypothesis was related to the use of different type of antiseptics in the post-castration wounds that may cause additional pain. To verify the hypothesis, 92 piglets were castrated using 4 different antiseptics in the post castration wounds. In particular, the active substances of the antiseptics were: 1) Clorexidin digluconate, 2) Rifaximine, 3) Chlortetracycline hydrochloride, and 4) Micronized silver carbonate plus calcium carbonate. Antiseptic number 2 contained ethyl alcohol. Variables latency to move and presence of pain-related behaviors (such as vocalization during antiseptic's administration and scratching the back on walls or floor when placed in the farrowing crate) were recorded at the moment of castration (T0) and 24 hours later (T24). Data regarding presence of pain-related activity was processed adopting the Marascuilo procedure to simultaneously test the differences of all pairs of proportions. For statistically significant differences ($P<0.05$), odds ratio were calculated. Data about latency to move were analyzed using a mixed model of SAS considering as fixed effects antiseptic, time, presence of pain-related behavior, interaction antiseptic*time, and interaction time*pain-related behavior, while animal within antiseptic was the random effect. The results regarding the variable pain-related behavior demonstrated that at T0 piglets treated with antiseptics 2 and 3 showed more painful behaviors. Variable latency to move showed to be significantly affected by the interaction time*pain-related behavior ($P<0.05$), indeed piglets with no pain-related behaviors used 1.2 seconds to move at T0 versus 0.6 seconds at T24. No antiseptic effect was evidenced for this variable, so the above formulated hypothesis did not explain the difference found in the first phases of the study in the latency to move.

P-084

Social interaction between male and goats synchronized estrus

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This study investigated the social and sexual interaction with goats synchronized estrus compared to male. We used 35 Saanen goats, which were divided into five groups to verify the hierarchical positions. We observed the following agonistic social behaviors: chasing, threatening, pushing, give halter, biting and retreating. The goats were classified according to the hierarchical positions in high, medium and low, as the matrix sociometric. The female of high rank from each group were separated into new pen observations of the restoration of social and sexual interactions with the male during estrus, which was synchronized with hormone use. The main sexual behavioral patterns exhibited by females were approaching, sniffing, licking, Flehmen, rubbing, circular motion, wagging his tail, homosexual behavior, vocalize, urinate and mounts. SNK test was performed using the software R. The results for social agonistic behavior of the goats showed that females of high rank were more threatened than the male. However, the other agonistic behaviors did not differ between positions. It is observed that the goats with high rank held more frequently the sexual behavior of rubbing, vocalizing and ride on the male. Other behaviors did not differ with respect to hierarchical position of the goats. It is concluded that goats high rank exhibit strong interactions with the male sex, which allows them a greater mating success in relation to goats medium and lower position.

P-085

Investigation on some wood materials and their use as environmental enrichment in post-weaning and fattening pigs

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The objective of this research is to identify a system of environmental enrichment to accomplish the Directive 2008/120/EC which requires the pigs must have permanent access to a sufficient quantity of material to enable proper investigation and manipulation activities. The study has taken into account both the improvement of animal welfare and the economic impact for farmers. The features of the manipulable usable materials and their possible applications in farming have been examined in details. Wood has been considered the best material to develop systems of environmental enrichment in relation to the most common type of flooring of the fattening farms in northern Italy (slatted flooring) with sustainable costs. The wood essences and kind of saw timber have been selected among the available resources in the area involved in the research. They are cost-effective and can be inserted into the farm without compromising the health of the animals, neither by contact nor by ingestion. The selection process has led to focus on core of veener poplar logs and wood sawdust pressed briquettes. The next step has been to design systems consisting of a fixed structural component to be installed inside the pen for the pigs to which identified manipulable materials can be added and replaced quite effortlessly. Field trials carried out involving 72 post-weaning piglets in 3 pens and 349 growing pigs in 4 pens showed that: a) wood sawdust pressed briquettes are not suitable in the post-weaning phase both for environmental reasons (excessive moisture in the pen) and for an overconsumption of the material (1 briquette crumbled in less than 2 days). b) The system with log placed horizontally (in such a way that the animal can rotate it) and the system with fixed vertical log result the most attractive among the several devices tested in post-weaning phase. c) The wall grid containing logs of poplar timber to raise with the snout, to spin and to bite seems to be the most desirable device during the growing-fattening phase. d) In general, a decreasing interest has been evidenced over time for all systems, from a maximum involvement of the animals recorded during the first days (for some devices up to 15 heads at the same time), to a minimum after a few weeks, in some cases no interaction during all day.

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P-086

Management and stereotypies of thoroughbreds in Central- Northern Italy

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The study deals with horse management and incidence of behavioural anomalies, considering 504 thoroughbreds for flat and national hunter races, housed in 30 barns located in 3 racecourse and training facilities: Merano, Pisa, Livorno. The horses were examined by one of the authors, veterinary surgeon employed in the racecourses. Two kinds of questionnaire were used to collect further information asking owners and personnel. Form 1 considered housing (type of box, bedding); feeding (number of meals, concentrates and hay); training (sessions frequency and intensity, use of horse-walker, paddock). Form 2 provided clinical and management information about each horse evidencing behavioural problems. Twenty-eight horses (5.5%) showed stereotypic behaviours: 17 crib biting (3.4%), 8 weaving (1.6%), 3 box circling (0.6%). These percentages are lower than findings in other studies. In 8 cases the stereotypy appeared during a period of inactivity after an incident. The results showed trends in accordance with other studies: higher incidence of behavioural anomalies was evidenced when horses were individually housed in box with internal window compared to horses in box with external window; feeding 3 meals/day caused more anticipation anxiety, expressed especially by weaving, than 2 meals/day. All stereotypic horses were fed 6-8 kg of concentrate, with a low hay/concentrate rate (1:1-1.5:1): this was confirmed a risk factor especially for crib biting. The use of horse-walker and individual paddocks of small dimensions seems to cause more frustration, expressed equally by crib biting or weaving, than distraction. Training sessions on short distances at fast canter cause an increase, even if not significant, of the incidence of behavioural anomalies than sessions on long distances at slow canter, performed in national hunter racing (4.1% *vs* 3.1%, $P=0.345$). Nevertheless in two cases weaving appears after daily heavy work on short distances. In one case box circling disappeared after changing training from short to long distances.
These management aspects are considered of concern for poor welfare by scientists, but often these notions aren't applied into practice in the farms. Moreover, the complexity and interconnection of factors affecting horse welfare suggest that evaluation is

necessary for each horse: in two cases after management changes the stereotypy disappeared.

P-087

Carrageenan skin test to assess inflammatory response in periparturient dairy cow: preliminary results

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Carrageenan is a high-molecular-weight sulphated polysaccharide that induces a local inflammatory response in mammals after intradermic or subcutaneous injections. Thus, in laboratory animals the carrageenan challenge is used to test the efficacy of non-steroidal anti-inflammatory drugs. The aim of this experiment has been the study of the carrageenan skin test (CST) as a new tool for the evaluation the susceptibility to inflammation in periparturient dairy cows. Local inflammation was induced by subcutaneous injection of sterile carrageenan (λ-Carrageenan, Wako Pure Chemical Industries, Japan), dissolved in saline to form a 0.7% solution. The injection of 4.2 mg of carrageenan was done on the shoulder region of 7 dairy cows at dry off and at -45, -20, -3, 0, 7, 28 days from parturition. The injection area was marked by a pen and the site of treatment was different for each injection. The response to CST was measured as skin thickness, using a plicometer just before the challenge (T0), then 2 (T2) and 9 (T9) days later. The overall response of each challenge was calculated as the area under the curve of the increasing thickness between T2 and T9 in respect to T0. Blood samples were collected at T0 and used to determine a wide metabolic-inflammatory profile. Health status and milk production were also recorded during the experimental period. The results showed a wide variation of the responses to CST throughout the peripartum. At dry off the response was quite low, then showed the maximum 20 days before calving, whereas the minimum level was observed around calving (-3, 7 days from parturition). The response to CST rose again at 28 days of lactation. Several plasma parameters were negatively (*e.g.* albumin, bilirubin, zinc) or positively (*e.g.* reactive oxygen metabolites) correlated with the response to the challenge after calving. These results suggest that dairy cows became very susceptible to inflammation 2-3 weeks before calving, when most of the plasma inflammatory indices are steady and when immune competence is reported to start worsen. The high susceptibility to inflammation seems quickly lost around calving when, oddly, the positive acute phase proteins showed the typical marked raise.

P-088

The influence of environmental temperature on physiological parameters of Santa Ines sheep breed in the northern of Rio de Janeiro, Brazil

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The objective of this study was to evaluate the effect of the time of day and the pelage color on the physiological parameters and body surface temperature of Santa Inês sheep bred under extensive conditions in the northern of Rio de Janeiro, Brazil. The experiment was conducted with fifty-four animals in September 2012 (spring) at 8 a.m and at 3 p.m; this month is characterized by low rainfall. The climatic parameters evaluated were environment temperature (T), black globe temperature (BGT) and relative humidity (RH). The physiological parameters analyzed were respiratory rate (RR), heart rate (HR) e rectal temperature (RT). An infrared thermometer was used to measure the surface temperature of withers (WT), rump (RT), knee (KT) and eyes (ET). The BGT was higher in the morning (39.5±1.2°C), and no difference were found in T (26.5±0.9°C) and RH (62.6±2.5%) due to the time of observation. The lowest BGT (35.0±0.4°C) in the afternoon is probably due to the fact that there was high prevalence of winds, probably lowering the thermal sensation. The analysis revealed significant effect of the time on HR, RR ($P<0.05$), RT ($P<0.001$) and KT ($P<0.05$). Animals with black hair had higher RH (31.3±3.3 bpm) and RT (39.4±0.69°C) than the animals with brown coat color ($P<0.05$). The data obtained show that in the afternoon the animals experienced more thermal discomfort than in the morning. This fact was confirmed by the increasing of HR, RR and RT and black animals may be more difficult to dissipate heat, so that they increase RT and RR, especially for black animals. Despite the influence of time of day and pelage color, the characteristics were within the physiological values for the species, demonstrating adaptation to the region's hot climate.

P-089

Hematological parameters of Saanen goats in different stages of pregnant, bred in the state of Espírito Santo, Brazil

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The hematology is important to establish diagnosis, prognosis and in monitoring the treatment of many diseases, thus it is crucial to know the hematology profile in different phases of the animal's life, as in pregnancy and early lactation. In these phases the ruminants have profound metabolic and endocrine changes. The aim of this study was to evaluate the hematological parameters of Saanen goats bred in southern Espírito Santo, Brazil. The blood samples were collected via jugular venipuncture from twenty-two goats, using evacuated tubes containing EDTA. Blood sampling was performed every fifteen days; a total of eight samples per animal were collected. The goats were pregnant from the first to the sixth blood sampling and they were lactating in the last two. All samples were processed by using an automated analyzer. The global leukometry (GL) and platelets did not differ between pregnancy ($12.08\pm3.94\times10^3$/mm^3) and lactation ($403.86\pm120.78\times10^3$/mm^3). In addition, the erythrocyte counts (H), packed cell volume (CV) and hemoglobin concentration (Hgb) decreased during pregnancy. Between the two blood sampling made during lactation, there was significant difference only for GL, which was higher in the last sampling. Comparing pregnancy and milking pregnant and lactating females, there was no significant difference only in platelet values. The CV, H, Hgb were higher in pregnant females, while GL was higher in lactating goats. On the basis of these findings, it may be generalized that erythrocytes decrease while leukocytes increase during the pregnancy to lactation transition in goat. However, it is in the lactation stage that goats require more attention.

P-090

The influence of environmental temperature on physiological parameters of mares in the north of Rio de Janeiro State, Brazil

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The aim of this study was to evaluate the influence of the environment on physiological parameters. This study used fifteen mares bred in northern Rio de Janeiro, Brazil. The data were collected at 8:00 a.m., three times a week from October 2012 to February 2013 (summer). An infrared thermometer (ICEL® model TD-965) was used to measure the surface temperature of wither (WT), rump (RT), knee (KT) and eyes (ET). The physiological parameters analyzed were rectal temperature (RT), heart rate (HR) and respiratory rate (RR). The climatic parameters were environmental temperature (T), black globe temperature (BGT) and relative humidity (H), which were measured with a Heat Stress Meter® model HT30. Blood samples were collected and the hematological parameters analyzed (packed cell volume, hemoglobin concentration, erythrocyte counts, white blood cell count and platelet). Analyses of data were performed by the GLM procedure (SAS). The highest averages temperatures were observed in the first collection on WT (35.51±0.65°C), RT (35.76±0.57°C), KT (34.82±0.39°C) and ET (35.51±0.73°) ($P<0.001$), and also on HR (58.86±14.16 bpm) and RR (36.57±15.44 breaths per minute)($P<0.05$). These could be explained by the high temperatures in October presented by the highest average (47.75±3.76°C) of BGT which showed a highly significant difference ($P<0,001$) compared to the other months (30.34±7.53°C; 28.86±0.85°C; 28.37±1.42°C). The averages of T were 29.47±2.01°C; 28.47±0.68°C; 28.10±0.78°C; 26.48±1.85°C. No difference was found between the months ($P>0.05$). The increase of HR and RR of the animals can be explained because these parameters are the first line of physiological defense of the animals when subjected to thermal stress. The RT was stable despite the high temperature of the BGT. The hematological parameters had no differences ($P>0.05$) between the months and averages was inside the normal limits for the species. The increases in RR and HR in the hottest period were effective to maintain unchanged the internal body temperature which is measured by the rectal temperature, showing the adaptability of these mares in this region.

P-091

Effect of lactational status on plasma indices of redox homeostasis in bovine and buffalo cows

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Lactation is associated with an increased rate of metabolic processes, that may result in increased generation of reactive oxygen species (ROS), leading to oxidative stress, that may impair health, fertility, and zootechnical performance of dairy cows. Here we characterized blood redox status of early and mid lactating cows, in order to evaluate the effect of lactation on redox homeostasis, and to define potential targets of oxidative damage in blood. The investigation was carried out on lactating bovine and buffalo cows, reared in a dairy farm localized in Campania, southern Italy. Cows from 20 up to 80 days in milk (DIM) were grouped as early lactating cows, while those from 85 up 160 DIM were grouped as mid lactating, thus ensuring that several cows, in each category, were analysed twice during the sampling period. We evaluated seven samples from early lactating bovine, twelve samples from early lactating buffalo cows, eighteen samples from mid lactating bovine, and twenty-six samples from mid lactating buffalo cows. Plasma concentration of antioxidants was measured by HPLC. Hydroperoxides (LPO) concentration, and the activities of both glutathione peroxidase (GPX) and superoxide dismutase (SOD) were assessed by commercial kits. Significance of statistical differences was evaluated by t test using the GraphPad Prism 5.01 program. Plasma concentrations of alpha-Tocopherol and Ascorbate, the total antioxidant capacity, GPx and SOD activities were significantly higher ($P\leq 0.01$) in mid lactating cows, both in bovine and buffalo. These results suggest that early lactation is associated with a higher consumption of antioxidants, likely due to the increased metabolic activity needed for milk production. The concentration of protein-bound carbonyls and nitro-tyrosine, as measured by ELISA, and the level of LPO were significantly higher ($P\leq 0.02$) in plasma of early lactating cows, thus demonstrating that the lower antioxidant capacity, observed in early lactating cows, was associated with a higher extent of oxidative modifications. We suggest that alpha-Tocopherol and Ascorbate play a key role in the protection from oxidative damage during lactation, and that lipid peroxidation and peroxynitrite production are crucial in determining oxidative modifications in plasma. So, the characterization of blood redox status might represent an useful tool for evaluating animal wellbeing during lactation.

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P-092

Study on innovative and manipulable wood materials able to improve the welfare level of piglets

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The aim of the study was to evaluate the effects of two environmental enrichment tools on productive parameters and welfare level of post-weaned piglets (7-30 kg LW, corresponding to 48 days of trial). 60 castrated males piglets were allotted to two experimental groups (6 replications per treatment): in one group, environmental enrichment was provided by hanging chains (control group, CTR), whereas in the treatment group, animals were allowed to manipulate wood logs placed into specifically designed containers (WL). The type of wood (poplar) and the containers were previously tested in field. The following data were collected at regular intervals: growth parameters (ADG and FCR), skin lesions, tail and ear biting, cleanliness score, health status (according to the Welfare Quality protocol) and blood parameters (a wide inflammometabolic profile) on 15 pigs per group. In order to get an early information about possible body damage (tail biting), the body surface of 20 animals (10 without any sign of lesion and 10 with initial lesions) was examined by means of a thermocamera. Environmental enrichment didn't affect ($P>0.05$) growth performance, although CTR group showed a higher ADG (506 g/d *vs* 485 g/d). No relationship was observed between body surface temperature and lesions onset. Depending on the type of environmental enrichment, some transient changes in the plasma were observed: 21 days after the addition of the enrichment tools, the levels of glucose, fructosamine, albumin, GOT, GGT, paraoxonase were lower in WL group (*vs* CTR), while the levels of cholesterol and globulin were higher. Despite some of these difference seem beneficial, they disappeared at the end of the trial. Because all piglets showed a satisfactory welfare level and good production outcomes, our preliminary results seem to indicate that no significant permanent differences exist between the two types of environmental enrichment.

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P-093

Partial carbon footprint of dairy sheep farms: simulated results from four different scenarios

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The ecological footprint approach aims to stimulate human-food production and reduce its environmental impact. Very few studied have focused on carbon footprint of dairy sheep farms. This work studied the differences in primary and secondary CO_2 equivalent (CO_2-eq) emissions among 4 simulated scenarios of dairy sheep production in Sardinia. Using a TIER 3 approach of the International Panel of Climate Change (IPCC, 2006), a spreadsheet was built on Excel® to simulate: animal categories, land use, soil management, biomass available (pasture or hay), and purchased feeds. A specific sub-model was built to estimate farm CO_2-eq emissions, including methane from enteric fermentation, methane and nitrous oxide from manure management, CO_2-eq from fertilizers and fuel and from purchased feeds. Animal requirements, dry matter intake and nitrogen excretion were estimated based on locally developed equations; enteric emissions were based on IPCC sheep coefficients, whereas emissions from manure management were based on dairy cattle IPCC tables. Simulated scenarios considered 4 farms: A) high production level, no pasture, 100% feed produced on-farm; B) high production level, no pasture, 100% forage produced on-farm, and purchased concentrates; C) medium production level, pasture, 100% of forage produced on-farm, and purchased concentrates; D) low production level, pasture, 100% of forages produced on-farm, and no concentrate use. It was found that to produce 65,000 L/y of sheep milk (6.5% fat; corresponding to 90,000 L of cow milk 4% fat), the farms A, B, C and D had, respectively, 340, 340, 421 and 547 heads, 44, 13.5, 37 and 57 ha, 49, 100, 78 and 100% of feed produced on-farm, and 235, 235, 188, 144 L/y of milk per present ewe. Enteric and manure management emissions in farms A, B, C and D were, respectively, 117.3, 119.4, 126.1 and 157.6 tons of CO_2-eq/y or 1.78, 1.81, 1.92 and 2.40 kg of CO_2-eq/L of milk. When CO_2-eq from produced and purchased feed were included, total emissions of farms A, B, C and D were, respectively, 161.2, 201.7, 201.4, and 207.4 tons of CO-$_2$eq/year or 2,45 3.05, 3.05 and 3.16 kg of CO-$_2$eq/L of milk. Concluding, reduction of emissions were observed with high production level and on-farm feed production.

P-094

How different farming systems can affect nutraceutical and traceable components of cow milk?

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The aim was to study the effect of farming and feeding systems on nutraceutical composition and traceable components of cow milk. A group of 29 farms located in Northern Italy and members of a cheese factory producing Grana Padano P.D.O were visited to collect information regarding herd composition, milk production, feeding system and land utilization. In each farm samples of bulk milk were collected for chemical and microbiological analyses. Fat and Protein Corrected Milk (FPCM) production was, on average, 27.0±4.21 kg/d per cow and fat and protein contents were 3.94±0.15% and 3.43±0.15% respectively. All cows were Italian Holstein. A cluster analysis was performed on farm characteristics (ha of land, no. cows, milk production , dairy efficiency, feed self-sufficiency) and three different groups of farms were identified. Cluster 2 included 11 farms characterized by low intensity: few lactating cows (32.4), low stocking density (2.97 LU/ha), high grass hay intake (27.5%) and low milk production (24.7 kg FPCM/cow). The ten farms in cluster 1 were more intensive: high stocking density (4.71 LU/ha), high milk production (28.9 kg FPCM/cow), low grass hay intake (15.6%); the seven farms in cluster 3 showed intermediate characteristics. Milk microbial contamination (SPC, coliform count and clostridium spores count) was not statistically different among the clusters; only lactic bacteria were higher in milk of cluster 2 compared to the others. Nutraceutical components (CLA, MUFA, omega-3 and omega-6 fatty acids) were similar among the clusters although the inclusion of maize silage in cow diets was different (30.4%; 22.8%; 34.7% on DMI for clusters 1,2 and 3 respectively). Some non-volatile hydrocarbons in the neutral lipid fraction of milk fat (phytane, phytene, phytyl C16, phytyl C18 unsaturated e phytyl C18 saturated) were significantly higher in farms of Cluster 2 than in the others. We supposed that these molecules were linked with the high grass hay and low maize silage intake of cows in Cluster 2. The results showed that nutraceutical components of milk are not significantly affected by farming and feeding system. Some non-volatile hydrocarbons in milk could be used as markers of forage composition of cow's diet.

P-095

Milk flow curve in *Amiata* donkey breed

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Mechanized milking is of recent use in equids. Milk flow curves and milk flow rate could be used to evaluate donkey mares milk-ability. These traits are influenced by anatomical, physiological, environmental factors and udder health status. Donkeys present a low cisternal capability and, in comparison with other dairy species, they require a great number of milkings per day, with a short interval among milkings. The trial was carried out along the whole lactation (early, pre mid, mid and late lactation), on 16 Amiata donkey breed mares, raised in Pontecorvo (Frosinone, Italy), milked twice a day, using a portable milking machine set at 42 kPa of vacuum level, 60 cycles/min and pulsation ratio 60:40. The milkmeter LactoCorder® (WMB-AG-Balgach) was used and 128 milk flow curves were recorded on two milkings per day (at 11:00am and at 3:00pm) with the previous separation of the foals from the dams 4 hours before each milking session. 128 individual milk samples were analysed: fat (0.49±0.20%), protein (1.63±0.13%), lactose (6.54±1.34%), casein (0.71±0.23%), urea (33.2±7.1 mg/dL) were determined on Milkoscan FT 6000, using a specific calibration curve, while SCC (4.27±0.59 $\log_{10}$/mL) was determined by Fossomatic 5000. Milk yield was 0.57±0.02 kg per milking, while average peak flow and average milk flow were 1.14±0.07 kg/min and 0.46±0.02 kg/min respectively. The observed flow curves were characterized by short plateau phase (0.08±0.13 min), long increase phase (0.47±0.27 min) and long decrease phase (0.54±0.37 min), while total milking time lasted 1.75±0.49 min. During the first minute of milking 90,9% of total milk yield was extracted. Surprisingly, 11/128 (8.59%) of the observed milk flow curves resulted bimodal (two peak curve), with clear evidence of two subsequent phases, representing the cisternal and the alveolar fractions. Significant differences among the curves obtained by different milking sessions (11:00 am *vs* 3:00 pm) were observed for increase phase (0.52 *vs* 0.43 min; P=0.041), for peak flow (1.44 *vs* 1.12 kg/min, P=0.024) and average flow (0.41 *vs* 0.52 kg/min, P=0.013). In conclusion, in Amiata donkey breed mares, the short plateau phase and high peak flow in the observed curves indicate a good adaptability of donkey to milking routine and mechanical milking.

P-095bis

Effect of supplementary feeding on lactating fallow deer and fawns performance

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The experiment aimed at evaluating the effect of dietary concentrate supplementation on fallow deer does and fawns performance. Twenty fallow deer (Dama dama) females were assigned to two homogeneous pastures according to stocking density and number of pregnant animals. Consequently, the first group (EXP) was composed by 11 animals, 7 pregnant does and 4 yearling females, the second group (CON) was composed by 9 animals, 6 pregnant does and 3 yearling females. From first birth, EXP group received a supplement (0.6 kg/d per doe and yearling female on average), while CON group was fed only on herbage. Every 45 days animals' body condition score and body weight were assessed and milk composition analyzed. Also fawns were weighed at birth and every 45 days until the end of the experiment, when their carcasses and meat characteristics were recorded. Dietary concentrate supplementation increases decreases fat content of milk (16.3 *vs* 13.7%; P<0.01) and increases body condition score of does (2.3 *vs* 2.7; P<0.05). Regards the performance of fawns, the concentrate supplementation increases: dressing percentage (47.9 *vs* 52.0%; P<0.01), conformation and fatness of carcasses (P<0.01). In summary, supplementing with concentrate the diet of grazing lactating fallow deer several beneficial effects can be observed in does and fawns performance.

P-096

Automatic rumination time monitoring after calving to early identify cows with health disorders

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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 typical method to monitor rumination is through visual observation, and recently using indirect methods based on the analysis of vocal signs rumination time. The aim was to investigate the possibility to early detect, through the continuous control of RT during the puerperium, the animals affected by clinical mastitis in early lactation. The trial was carried out in an experimental barn and involved 32 Italian Friesian cows (9 primiparous: PR; 23 pluriparous: PL) from 20 d before calving till to 40 days in milk (DIM). On all the cows the RT was continuously measured by using a rumination monitoring system (HR-Tag), consisting of rumination loggers, stationary readers, and software for processing the electronic records. The system summarizes the RT in 2-h intervals. On all the cows the daily milk yield and all health-related problems were recorded. The RT observed on cows affected by clinical mastitis (M) was analyzed using the data observed from 3 d before the drug treatment of clinical disease until 3 d after it. The data collected on these cows were compared with those observed on healthy cows (same days in milk and parity: H cows), using the data simultaneously collected. The RT reached the minimum value at calving, and after increased until 15 DIM. On average the RT from 15 to 40 DIM was 504 min/d in PR and 562 min/d in PL. The M cows, besides a reduction of milk yield (29.1 kg/d on the day of drug treatment), have shown a significant reduction of RT 2 d before the drug treatment (423 min/d), and a return to baseline values was observed 1 d after drug treatment (551 min/d). The H cows simultaneously controlled did not show RT changes (range 548 to 571 min/d) or milk yield changes (range 36.3 to 38.5 kg/d). These results seem to indicate the usefulness of monitoring the RT during the transition period to early detect of animals with health problems.

P-097

Natural variation of vitamin D in cow's milk

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Vitamin D is the collective name for a group of closely related lipids. Its main biologic function is to maintain serum calcium and phosphorus concentrations within the normal range by enhancing the efficiency of the small intestine to absorb these minerals from the diet. In addition, it has potent antiproliferative, pro-differentiative and immuno-modulatory functions and plays important roles in promoting oral health and preventing colon cancer. The main portion of vitamin D_3 derives from the conversion of 7-dehydrocholesterol upon exposure to ultraviolet rays. Vitamin D naturally present in the diet in larger quantities is cholecalciferol (vitamin D_3) contained in foods such as oily fish. Smaller quantities of vitamin D_3 occur in meat, eggs, milk and dairy products. In many countries milk is fortified, mainly by adding vitamin D3. Most of the milk sold at retail in the United States is fortified, while the fortification is less common in Europe. We used a commercially available ELISA method for the determination of vitamin D in milk. 55 individual samples of unfortified cow's milk from different herds were analyzed as well as 10 different types of milk for human consumption on the market. The enzyme immunoassay method used was confirmed as a useful tool to measure the vitamin D in the milk as it greatly reduces the time required to perform the conventional HPLC analysis. An interesting variation was found among individuals that may be associated with management factors and specific genetic effects. In this regard an interesting relationship was highlighted between vitamin D content and the genetic polymorphism of β-lactoglobulin, the main milk whey protein involved in the transport of small hydrophobic molecules such as retinol and vitamin D. The relatively high content of vitamin D in the samples analyzed justifies the limited use of the fortification of milk in our country and suggests an opportunity to improve the content of vitamin D naturally present in milk. This result could be obtained either by acting on the herd management or evaluating the possibility to select individuals genetically predisposed to produce milk with a higher vitamin D content.

P-098

Angiotensin-converting enzyme inhibitory activity of water-soluble extract of *Parmigiano Reggiano* and *Grana Padano* cheeses

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During cheese ripening, proteolysis induces the release of numerous peptides from caseins that differ depending on the variety of cheese and ripening times. Some of these peptides show biological action including angiotensin-converting enzyme (ACE)-inhibitory activity. The aim of the present work was to determine the ACE-inhibitory activity of one sample of *Parmigiano Reggiano* (PR) and one sample of *Grana Padano* (GP) cheeses. *Parmigiano Reggiano* and GP, 32-months old, were obtained from Italian manufacturers in their respective areas of production. The water-soluble fraction (WSF) was extracted from cheeses and the WSF<3000 Da was obtained by ultrafiltration. The WSF was also digested *in vitro* by pepsin, trypsin and α-chymotrypsin method. ACE-inhibitory activity was tested for both cheeses and was evaluated as IC_{50} (50% inhibitory concentration). The IC_{50} for each sample was estimated in triple preparing an inhibition curve using the sample concentration (x axis) and ACE-inhibitory activity (inhibition rate%; y axis). Cheeses revealed the presence of a consistent ACE-inhibitory activity. The IC50 was 8.94 μg/mL for PR and 7.68 μg/mL for GP in WSF<3000 Da, and 13.89 μg/mL and 10.05 μg/mL for PR and GP respectively in the WSF. Digested WSF showed the following IC_{50} values: PR=14.82 μg/mL; GP=21.20 μg/mL. These preliminary results suggest that all fractions of PR and GP WSF had interesting ACE-inhibitory activity. The potential of peptides present in PR and GP might serve as a basis for the development of functional foods with ACE inhibitory activity and blood pressure-lowering property.

P-099

Application of low density single nucleotide polymorphism arrays for the analysis of bovine milk fatty acid profile: from nutrition to genetic

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Milk is composed by multiple essential nutritional constituents, among which a significant amount of lipids (3%-5%) that may influence both positively and negatively on human health. Bovine milk fat is mainly composed by triglycerides (±98%), consisting in free fatty acids (FA) esterified to a glycerol backbone. The FA composition influences milk nutritional value and product properties, containing the highest percentage (60%-70%) of the assumed health negative trans FA and the highest amount of the health beneficial CLA (0.4-0.7%) and vaccenic acid (0.7-2%). Changing FA composition could therefore result in milk fat with additional value for the dairy industry. Feeding regimen may significantly affect milk fatty acid composition, by modulating both rumen and mammary lipid metabolism, but single individual reply differently to the diet showing differences in milk composition. Among the other factors acting on milk FA composition, genetics is of particular importance since discovering the genes responsible for the differences can allow the selection of individuals with favourable genotypes for milk with better FA composition. We analysed the individual milk samples of 319 Italian Friesian cows randomly collected in 24 herds at 96 single nucleotide polymorphisms (SNP) in genes expected to play a role in milk composition and fat synthesis. A total of 59 SNP were finally used to calculate the associations of the SNP with milk production traits and FA composition using the SAS software (ANOVA). Significant associations ($P<0.05$) were found for all the analysed phenotypes with a number of SNP ranging from to1. Information on the feeding regimens allow to divide the farms into 4 different groups and adding this information to the model the SNP significance generally increased together with the number of significant SNP (*i.e.* from 3 to 6 for CLA), giving a better understanding of the interaction among diet and genetics.

P-100

Effect of dry forage of grasses and legumes species on the fatty acid profile of goat milk

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The aim of this study was to evaluate the fatty acid profile and Health Promoting Index (HPI) of milk obtained from goats fed with dry forage of four grasses and three legumes. During the experimental period, 15 days, forty Derivata di Siria goats homogeneous for milk production, days in milk and BCS were allotted to seven groups and fed with: *Lolium perenne* (LP), *Hordeum vulgare* (HV), *Avena sativa* (AS), *Triticosecale* (TS), *Medicago sativa* (MS), *Vicia sativa* (VS) and *Trifolium incarnatum* (TI). Fatty acids were detected by gas chromatography as shows as g/100gFA. HPI was evaluated according to Chen *et al*., (2004). Data were analyzed by ANOVA and mean values were compared by Tukey-test. Fatty acid profile and HPI were affected by forage species. The ALA (α-linolenic acid) content of milk from legumes (TI, MS and VS) showed a significant ($P<0.05$) increase (0.73, 0.81, 0.16) compared to grasses species AS, TS, HV, and LP (0.30, 0.40, 0.64, 0.66). On the contrary, CLA (Conjugated linoleic acid) content of HV, AS and LP (0.69, 0.66 and 0.57) grasses forage was higher than legumes TI, MS and VS (0.56, 0.47 and 0.41). Milk from HV and TI groups showed the highest content of MUFA (Monounsaturated fatty acids) and LCFA (Long-chain fatty acids), and the lowest content of SFA (Saturated fatty acids). OBCFA (Odd-and branched-chain fatty acids) values significantly decreased from LP to HV group (4.64 to 3.76), within legumes species TI showed the highest content (4.33). The best HPI for grasses and legumes was observed in milk of HV (0.82) and TI (0.62) group, respectively. Dry forage seems to characterize in different way the fatty acid profile and HPI of milk. Milk of HV and TI groups were the most interesting from a nutritional point of view.

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Fatty acid profile of triglycerides in raw and digested donkey milk

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Donkey's milk (DM) is commonly known to be similar in chemical composition to human's milk (HM) and recently investigated as breast milk substitute. Notwithstanding the fat content is evidently different, being much lower in DM with values below 0.5% compared to the average of 3.5% in HM, the qualitative lipid profile of donkey's and human's milk reveals an interesting similarity, both milk showing high levels of unsaturated fatty acids. Also, DM is particularly rich in essential fatty acids which assume a crucial role in human nutrition. In this study, 10 individual milk samples of Ragusano donkey were analyzed for their triglycerides profile both in raw milk and in digested aliquots. Milk samples were submitted to a simulated digestion through one hour treatment with human gastric and duodenal juices, to find out whether changes occur in the digestion process. Human gastric and duodenal juices were collected in one batch from 6 healthy volunteers by aspiration using a triple lumen tube (Maxters catheters, Marseille, France). Analysis of fatty acid methyl esters was carried out by means of Gas Chromatograph (Agilent Technology 6890, USA) coupled with Mass Spectrometer (AutoSpec™ Ultima, Micromass Ltd, England) whereas identification was achieved using MassLynx software version 4.0 (Waters, USA). Under the action of pancreatic lipase together with the bile salts found in the duodenum, some fatty acids were hydrolyzed more than others: remarkable differences were observed for caprylic C8:0, capric C10:0 and oleic C18:1 acids which were much lower ($P<0.005$) in the digested samples, assuming a relevant degradation for these fatty acids. On the contrary, myristic acid (C14:0) was found significantly higher ($P<0.001$) in the digested samples as relative percentage of total fatty acids, thus a very low rate of hydrolysis is hypothesized.

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Magnitude of mobilization of body reserves of Alpine goats in early lactation

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This study aimed at monitoring the state variables of body energy in Alpine goats during the first 60 days of lactation, when milk production increases faster than dry matter intake. Forty-eight multiparous Oberhasli Alpine goats (initial mean BW 57.19±8.47 kg, BCS 3.0±0.51) in good health status were studied in a completely randomized design with six replications and eight treatments (weeks of lactation). Animals were slaughtered (following the procedures given by the Ethics committee of the Universidade Federal de Viçosa) sequentially to measure energy of internal fat mass and analyze chemical composition of animal body. Six randomly selected goats were slaughtered every week during lactation. The data were analyzed by using the MIXED procedure of SAS. Empty body weight (EBW) decreased over the eight weeks of lactation (from 57.19 to 46.50 kg per goat) and carcass fat also decreased (from 1.78 to 0.46 kg per goat and from 6.6 to 2.6% of EBW). Omental fat mass decreased gradually from the moment of parturition to the eighth week of lactation (from 3.77 to 1.06 kg per goat; 6.6 to 2.6% of EBW), whereas fat visceral mass was mobilized with greater intensity from the third to the eighth week of lactation (from 4.33 to 1.40 kg per goat; from 7.57 to 3.01% of EBW). Empty body energy decreased gradually from parturition until the eighth week of lactation (from 384.60 to 192.90 MJ per goat; from 8.78 to 5.95 MJ/kg of EBW). Body fat (from 15.58 to 9.95%) and protein (from 11.76 to 8.93%) also decreased, mostly because of the mobilization of noncarcass fat (from 17.17 to 6.74%) and protein (from 14.81 to 10.11%). During the first eight weeks of lactation the weight decreased in the liver (from 1.2 to 1.0 kg), heart (0.33 to 0.18 kg), kidneys (0.20 to 0.14 kg) and mammary gland (2.80 to 1.48 kg). Weight of uterus decreased markedly from the first to the fifth week of lactation (from 1.89 to 0.10 kg). In conclusion, Alpine goats in early lactation mobilized energy reserves not only from internal fat but also from organs and viscera.

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Preliminary investigation of colorimetric and sensory characteristics of some buffalo cheese of the Lazio region

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The Lazio region is one of the principal producers of buffalo milk in Italy. 99% of the milk produced is trasformed into cheese. The aim of this study was to evaluate colorimetric and sensory characteristics of some traditional products made from buffalo milk in a specific area of Lazio region where PDO *Mozzarella di bufala campana* is produced. 40 samples of 10 types of buffalo cheese, produced by 3 dairies located in the province of Latina were analyzed. These types of cheese are so classified: soft, semi-hard, hard and smoked. Samples were analyzed in a preliminary way also for colorimetric analysis (Minolta colorimeter CR-400 with an illuminant D65) and for sensory analysis. The sensory evaluation was performed by a sensory panel consisting of 20 consumers aged between 20 and 60 years. The panel was asked to assess different types of cheese using a score from 0 to 5 for each of the following attributes: odour (O), flavor (F), salty taste (S), acidity (Ac), bitterness (B), homogeneity (H), consistency (C) and adhesiveness (A). Colorimetric analysis results: The L* value (brightness) of the samples undergoes a decrease from 93.51 (Marzolina cheese) to 78.85 (Scamorza cheese). Values of index a* and b* obtained are in accordance to other studies even if made by sheep cheese. The color change is determined by the lipolytic and proteolytic processes that occur during aging (2 days Scamorza cheese *vs* 180 days Provolone cheese). Sensory profiles: although the used panel test has a scale from 0 to 5, no samples were scored higher than 4 for any parameters. The different sensory profiles depend on the type of cheese, on maturing days and smoking. The odour" reaches the highest value in Provolone cheese (180 days of maturing) and the lowest value in Muzzotta cheese (45 days of maturing). In addition, there were no flaws in the sensory characteristics of samples cheese as spicy and sour flavor. This study is a preliminary analyse of some traditional products made from buffalo milk because the market for this 'new' products in recent years are expanding nationally and internationally.

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Pecorino flavoured cheese: physicochemical, textural and colorimetrical characteristics of different varieties

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The quality of distinctive artisanal cheeses is closely associated with the territory of production and its traditions. Safeguarding these products means also safeguarding the uniqueness of their historical and cultural environment. The Mediterranean ewes' cheeses are included in this sector of the market. This cheese is produced in the Province of Rieti. *Pecorino* flavoured cheese is a semi-hard or hard ewes' cheese, made from raw milk. Therefore, the aim of the present research was to monitor, for the first time, its physicochemical state and textural and colorimetrical characteristics, to determine if it is necessary to regulate the productive process of this distinctive ewes' cheese regarding these aspects. 25 samples of ewe cheese (n° 5 pepper flavored, n° 5 chili flavored, n° 5 oregano flavored, n° 5 basil flavored and n° 5 walnuts flavored) were analyzed from September to November last year. For each sample, characteristics have been analyzed: chemical and physical parameters (fat (F), fat SS (FSS), protein (P), dry matter (FDM) total solids (TS) and moisture (M)) were determined with analyzer FoodScan (FOSS, Denmark) and pHmeter with glass electrode (Table 1); each cheese sample was determined three times. Colorimetric analysis has been carried out using a Minolta colorimeter CR-400 using an iluminant D65 (Table 2). Calibration has been done by white plate Minolta 13833057 with Y=94, x=0.3155 and y=0.3319, obtaining coordinates L*, a* and b*, where L* indicates brightness (0=black, 100=white), a* is a red index (-a*=green, a=red) and b* is a yellow index (-b*=blue, b*=yellow). Rheological parameters were performed on an INSTRON dynamometer, (Universal testing machine model 3343) (Table 3). The following parameters were measured from the force–time curves: hardness (N), cohesiveness (adimensional), adhesiveness (mJ), gumminess (N, as hardness x cohesiveness), springiness (mm) and chewiness (mJ, as gumminess x springiness). This study has defined the physicochemical, textural and colour characteristics of *Pecorino* flavoured cheeses. The results obtained may be used as a basis for defining the main characteristics of these products for definition of standard start. Physicochemical results are similar to reported by other authors. This study is a preliminary analysis to evaluate textural and colorimetric characteristics of *Pecorino* flavoured cheeses.

Table 1. Chemical (%) and physical characteristics of different *Pecorino* flavoured cheese.

	F	FSS	P	FDM	TS	M	pH
Pepper	30.6±0.45	0.47±0.02	29.6±1.80	34.9±2.71	65.5±3.16	34.5±3.16	5.06±0.21
Chili	30.8±2.27	0.48±0.01	27.3±0.10	33.2±0.84	64.0±3.11	36.0±3.11	5.07±0.08
Oregano	30.2±1.76	0.47±0.00	28.4±2.16	33.8±2.11	64.00±3.87	36.0±3.87	5.11±0.09
Basil	29.3±1.21	0.47±0.01	28.4±0.83	33.9±0.37	63.2±1.58	36.9±1.58	5.11±0.24
Walnuts	29.3±0.18	0.47±0.01	28.9±0.80	33.8±1.10	63.2±1.28	36.9±1.28	5.06±0.21

Table 2. Colorimetric analysis of different *Pecorino* flavoured cheese.

	L*	a*	b*	C*
Pepper	73.9±3.69	-4.41±0.78	19.9±2.43	20.4±2.41
Chili	77.2±5.04	-3.02±0.89	19.5±1.24	19.7±1.15
Oregano	60.7±6.52	-2.41±0.74	11.3±2.12	11.6±2.19
Basil	68.7±5.02	-4.57±0.37	15.7±3.14	16.4±3.11
Walnuts	74.4±3.93	-3.50±0.81	16.3±1.78	16.7±1.89

Table 3. Rheological parameters of different *Pecorino* flavoured cheese.

	Hardness (N)	Cohesiveness (adimensional)	Adhesiveness (mJ)	Gumminess (N)	Springiness (mm)	Chewiness (mJ)
Pepper	100±47.9	0.59±0.09	33.3±16.4	56.8±22.3	359±210	6.14±2.88
Chili	92.9±59.6	0.59±0.06	38.5±28.1	53.5±34.2	235±128	4.88±1.87
Oregano	114±55.6	0.61±0.09	52.0±20.86	70.5±39.28	344±309	4.55±2.30
Basil	79.8±20.93	0.61±0.08	36.0±20.80	49.6±16.28	261±240	5.29±4.12
Walnuts	89.0±42.1	0.61±0.08	41.2±25.5	55.2±27.93	312±205	5.86±2.26

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Evaluation of the sustainability of dairy goat production systems: success and main strength and weakness of their activity in Tuscany

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Many researches were carried out and published in important scientific reviews on characterisation of goat farming systems in EU and in Italy, but not in Tuscany. In this region the amount of goat farms is small, mainly in comparison with sheep, but their productions are very relevant from a qualitative and economic point of view. Dairy goat production systems in Tuscany have been stimulated by the use of natural resources, by the interest on typical productions of high value (as a gourmet food), by the social and environmental role on rural land protection and biodiversity promotion. But, little is known about the success and the main strength and weakness of these systems. A research had taken place in Tuscany in 21 goat farms (2 in AR, 5 in FI, 1 in GR, 1 in LI, 1 in LU, 4 in MS, 4 in PT and 3 in SI provinces), which represent about 80% of the total regional goat farms. The information was obtained through a collection of primary data from a structured questionnaire consisting in direct interviews with the shepherds. The interview questionnaire included 100 questions relative to a general description of farm characteristics and overall management practices, strength and weakness points of the farm and success of the farm business. About the production system, 48% farms resulted organic, 38% conventional and 14% biodynamic. All 21 producers of goat milk interviewed, said they want to continue their activity, and 76% of those who have children, 44% said that theirs sons want to continue the activity, 31% don't know and 25% not, more 1 without children but with a nephew who will continue the activity. 17 producers answered about the main weakness of the goat production systems: 12% have difficulties and problems in marketing the products, 29% complain excessive bureaucracy in conducting the activity, but only 6% declare insufficient income. As the main strength, the quality of the products was emphasized 10 times, followed by the characteristics of the goats themselves, emphasized 3 times, and the short food supply chain, only 2 times. When our database will be completed, with the 100% of the regional goat farms, a statistical analysis will be carried out in two steps: principal component analysis and successive cluster analysis.

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Buffalo farming in Sannio Beneventano area: productive and feeding issues

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The introduction of twenty pregnant buffalo cows in a dairy cattle farm of Benevento province was used as case study of farm diversification. For the whole lactation period, milk traits and reproductive performances were assessed monthly. Sensory properties of mozzarella cheese at 12 and 36 h of storage were assessed twice at 1 week interval. Sensory data were subjected to ANOVA with assessor (10), replication (3), batch of production (1° *vs* 2° batch), time after production (12 *vs* 36 h), and the interactions as factors. In order to verify the suitability of locally produced forages for buffalo farming, from 35th to 235th day of lactation cows were allotted into two groups fed diets based either on maize silage or hays. Productive data were analysed by a mixed model for repeated measures including the effects of diet, time and the interaction diet*time. The effect of diet on BCS and reproductive data were analysed by one-way-ANOVA. No significant differences between the two dietary groups were observed. Therefore, all parameters are expressed as overall means. Length of lactation was shorter than the standard value of 270 d: 253.5±75.9 d. Milk yield was 1546±622 kg/head/lactation, percent milk fat and protein were 8.64±0.83 and 4.43±0.32%, respectively. Milk yield was below the mean of buffalo cows raised in Campania and lower than the yield of the same cows in the previous lactation completed in the farm of origin (2.463±632 kg/head/lactation). These poor results are probably due to mismanagement practices, since the body condition was scored as satisfactory. The calving interval was 486.5±120.2 d, longer than the average value of 400 d, most likely as instrumental insemination was unsuccessfully used. Sensory properties of mozzarella were quite stable and did not change after 24 h of storage ($P>0.05$ for most of the attributes). Nevertheless, the sensory profile largely varied between the two batches of production indicating that the cheese-making technology was inadequate to obtain consistent cheese quality. Therefore, expanding buffalo farming outside the traditional areas has to be closely monitored in order to minimize detrimental effects on lactation length, increase insemination techniques efficiency and standardise cheese-making technology.

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Comparison of proteolytic profile of Podolian *Caciocavallo* and *Pecorino Laticauda* in relation to ripening time

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The ripening process of cheese is a complex phenomenon characterized by a combination of chemical-physical, biochemical and biological events closely related. The endogenous enzymes of milk, the enzymes of the starter, rennet enzymes as well as those of dairy interest and contaminating microbial flora are responsible of main biochemical reactions occurring in the ripening process. One of the most complex biochemical events is the casein hydrolysis leading to the formation of unsoluble peptides with high molecular weight that are further fragmented into small peptides and free amino acids during long ripening. The profile and the number of fragments produced can give information about the time and the way in which proteolysis occurs. The research considered the *Pecorino* of *Laticauda* hard cheese and *Caciocavallo* of Italian Autochthonous Grey Cattle (BovGrAI- ex Podolian), a semi-hard pulled curd cheese, both ripened for 3 and 6 months. The aim of this research was to investigate the biochemistry of the proteolytic processes that take place during the cheese maturation by 2D-SDS-PAGE/image analysis / MALDI-TOF-MS. For both types of cheese it was considered the formation of: (i)fragments of β-CN identified as γ1-CN [β-CN f (29-209)] derived from the primary proteolysis of the β-CN by plasmin action, A group; (ii)fragments with molecular weight of ~ 12 kDa, among which the γ2-CN [β-CN f(106-209)] and the γ3-CN [β-CN f(108-209)], derived from primary and secondary proteolysis, B group. At the same curing time, *Caciocavallo* cheese showed a significant greater relative volume of the spots; this greater volume could be an indicator of faster proteolysis to ascribe both to the acidification curd due to the action of lactic acid bacteria contributing to the secondary proteolysis and to the greater water activity due to a different size of curd pieces obtained from cutting step; the curd is cut into pea-sized pieces in *Caciocavallo* and rises grain-sized pieces in *Pecorino*.

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Water balance and nitrogen excretion in buffaloes in different physiological conditions

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The aim of this study was to evaluate water balance and nitrogen excretion in Mediterranean buffaloes. Sixteen subjects were divided into 4 groups (n=4/group), according to their days in milk (DIM): Group L50 (DIM=50±8); Group L125 (DIM=125±55); Group L225 (DIM=225±26); Group NL (non-lactating). The study comprised a 14-day adaptation to diets and 14-day data collection during which feed intake and milk production were recorded daily. Measurements for individual buffaloes were made for water intake, diet, refusals, milk, faeces and urine during the final 3 days. The analyses were performed according to AOAC. Lactating buffaloes had greater ($P<0.05$) DM and OM intake than NL buffaloes. Buffaloes in Group L50 had a lower ($P<0.05$) CP apparent digestibility than buffaloes in Groups L125 and L225, while Group NL showed a CP apparent digestibility similar to the other Groups. Group NL had a higher ($P<0.05$) real CP digestibility than Group L50, suggesting decreased N clearance by the kidneys and a greater N recycling by the gastrointestinal tract in the former buffaloes. Buffaloes in Groups L50 and NL had a negative N balance (-79±32 and -12±25 g, respectively) whilst buffaloes in Groups L125 and L225 had a positive N balance (26±27 and 16±45 g, respectively). Buffaloes in Group L50 had more ($P<0.05$) N in urine (204±28 g) than Groups L125 (157±16), L225 (171±26) and NL (87±22 g). The water balance was positive for buffaloes in all groups without differences. Drinking water intake (DWI) represented around 93% of total water intake (TWI) and this was similar across treatment groups. Buffaloes in Group L50 had a greater ($P<0.05$) DWI relative to TWI (96.3±1.2%) compared with buffaloes in Group NL (90.9±2.0%), L125 (89.1±2.2%) and L225 (92.8±0.6%).Overall, lactating buffaloes ingested a greater ($P<0.05$) amount of total water than non-lactating buffaloes and the former buffaloes excreted a greater ($P<0.05$) amount of water in both faeces and urine. There were high correlations between total water excretion and N intake ($r^2=0.722$; $P<0.01$) and total water excretion and N excretion ($r^2=0.976$; $P<0.01$).

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A survey on the rearing systems of young replacement stock in Holstein dairy farms located in the Veneto region

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Replacement calves and heifers are often raised in not appropriate facilities and in poor welfare conditions despite their rearing has relevant implications on costs and long-term effects on the future dairy cows' performance. This preliminary study aimed at inspecting the management and rearing systems used for replacement young stock in 24 Holstein dairy farms in the Veneto region. The survey was carried out by a trained assessor through a questionnaire and direct on-farm observations. Farms reared on average 90.9±79.2 dairy cows and 76.8±64.7 replacement stock divided in 4 age groups: calves below 2, calves 2-6, heifers 7-12, and heifers over 12 months of age covering respectively 7.8, 14.8, 22.9, and 50.9% of the total replacement stock reared. In 18 farms the farmer himself cared for the replacement stock. Dehorning was carried out in all farms except 1. Early weaning was practiced in 1 farm while in most farms (17) calves were weaned when over 90 days old. Calves below 2 months were kept outdoors in individual crates with straw bedding in 17 farms while they were reared in deep littered group pens of 2-7 calves in the remaining farms. Calves 2-6 and heifers 7-12 months old were mainly kept in deep littered group pens of 2-16 calves and 5-50 heifers. They had access to an outdoor paddock in only 3 farms. Older heifers were in deep littered group pens in 17 farms, tethered in 4 farms, and in loose housing with cubicles in the remaining farms. In most farms the replacement stock after weaning was fed a total mixed ration containing maize-silage plus hay and/or pellets. The mostly used litter material was straw with a variable, from daily to monthly, renewal frequency. A high variability was observed also for the space allowance that increased with age and ranged from 1.3 to 8 m^2/calf and from 3 to 13 m^2/heifer. For all age groups, manger space was sufficient while drinker availability was limited with 1 drinker/pen in most cases. It can be concluded that replacement stock are reared in variable conditions likely due to the lack of dedicated guidelines for their rearing.

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Gross composition of yak (*Bos grunniens*) milk

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In China, yaks (*Bos grunniens*) are grazed extensively on the western plateau in alpine and subalpine regions at altitudes from 2000 to 5000 m with a cold, semi-humid climate. The centre of the yak distribution is the Qinghai-Tibetan Plateau. Qinghai province covers an area of 722,000 square km, is situated in Northwestern China and borders the yak territories of Tibet, Sichuan and Gansu. The average altitude of Qinghai province is 4000 m a.s.l., but 80% of the land area lies between 3000 m and 6800 m. Qinghai plateau yak now is classified as a breed: its population is about five million. Technology in the management is not diffused, so yaks are usually manually milked and the average daily milk yield per animal ranges from 1.1 to 1.8 kg/d. The aim of this research was to characterise gross composition and nitrogen fractions of milk from Qinghai plateau yak. Four different yak farms were considered, located in different zones of Qinghai. For each farm, four samplings of herd milk, representative of the manual milking of all yaks in the farm, were carried out, in 4 different months (March, June, July and August). In total, 16 herd milk samples were collected. Average (±SD) milk contents (g/100 g) for dry matter (18.70±2.95; by stove drying at 102°C), ash (0.79±0.13 by muffle calcination at 530°C), fat (6.55±1.89; by IR), protein (4.76±0.57; by Kjeldahl) and lactose (4.72±0.40; by IR) are consistent with data present in literature for other yak breeds. Scarce information are in literature for nitrogen fractions; in this research, by Kjeldahl method, we determined average milk contents (g/100g) for true protein (4.53±0.59), casein (3.69±0.46), true whey protein (0.84±0.13), non protein N x 6.38 (0.23±0.06). Average value for casein number (77.45±1.03;%) resulted analogous to cows' value. In literature there are no data also for other physico-chemical properties; in this research we have registered a pH of 6.70±0.07 (similar to cows'), a density at 15°C of 1.0325±0.0053 (g/mL; with a Quevenne lactodensimeter; a value consistent with cows' average value), an urea content of 26.9±7.4 (mg/100 mL; by IR), a somatic cell count of 114 (±61) 1000 cells/mL.

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Effect of a diet with less crude protein integrated with rumen protected methionine on milk yield and quality in Mediterranean Italian buffalo cows

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Sixteen lactating Mediterranean Italian buffalo cows were divided in two groups: low protein group (LP: n=8) and high protein group (HP: n=8). The groups were homogenous for age, body weight, body condition score, phase and number of lactations and for milk production as recorded in the previous lactation. LP group received a total mixed ration (TMR; crude protein, CP =142.4 g/kg on dry matter basis; DM) with 0.90 MFU (milk feed units)/kg on DM basis, containing: 44% corn silage, 9.5% soybean meal, 18.5% corn meal, 26% lucerne hay plus 2% of vitamin/mineral supplement and 12 g/head/d of Mepron® (85% rumen-protected methionine; RPM). HP group received a TMR (CP=156.1 g/kg DM) with 0.90 MFU/kg on DM basis, containing: 44% corn silage, 13% soybean meal, 15% corn meal, 26% lucerne hay and 2% vitamin/mineral supplement. The trial lasted 120 days. To determine protein, casein, fat and urea content, milk samples were collected, twice a day, every 15d. Milk yield (7.66 kg/head/d) and protein content (46.31 g/kg) were similar in the two groups. No differences were found either in casein (38.55 g/kg) or in fat (93.65 g/kg) content. On the contrary, milk urea level was significantly lower (P<0.01) in LP than in HP group (35.53 mg/100 mL *vs* 41.09 mg/100 mL, respectively). These results indicate that, in buffalo, a diet with low CP supplemented with RPM doesn't negatively affect milk yield and quality and decrease milk urea level. The value of urea in milk may also represent in buffalo, as in bovine, an important tool to calculate the nitrogen losses of urinary tract, the resulting economic losses and environmental impact.

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Milk flow traits and udder health in Mediterranean Italian primiparous buffalo cows during lactation

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Milk production and milk flow profiles are important parameters to be recorded and evaluated as they give various advices on milking management. Milk ejection representation occurs through flow curves (FC) that are typical and characteristic for buffalo, cattle, sheep and goat. The milkmeter Lactocorder (WMB AG-Balgach) is the instrument normally used for real-time recording of milk-FC and associated parameters. Milk-FC have been studied in cattle, in sheep and goat and recently also in buffalo. The objective of this study was to evaluate milk production, principal milk-FC parameters and health status in primiparous Mediterranean Italian buffalo cows, during the whole lactation (Days in milk – DIM, 0-60, 61-120, 121-180, >180 days). On 29 buffalo cows, 232 FC have been recorded (116 during milking in the morning and 116 during milking in the evening), using an electronic milkmeter Lactocorder. Oxytocin administration was useful on 6.9% (16/232 curves) of the observations. Milk production linearly decreased (P<0.001) from 5.84±0.16 kg to 2.53±0.17 kg during lactation (from DIM 0-60 to DIM>180). Maximum and average milk flow decreased from 1.83±0.06 kg/min to 1.03±0.06 kg/min (P<0.001) and from 1.11±0.04 kg/min to 0.65±0.04 kg/min (P<0.001), respectively. Principal milk emission phases showed an opposite trend: Plateau Phase and Decreasing Phase linearly decreased and the Increasing Phase only decreased in the first two stages of lactation (at DIM, 0-60 and at DIM 61-120), to grow up till the end of lactation. Consequently, considering the total milking time, the length of Increasing Phase, in percentage, was higher (43.8%) than the length of Decreasing Phase (31.7%) and than the length of Plateau Phase (24.5%). This trend was similar during milking in the morning and in the evening. Milk produced in principal milking phase, when compared to the total milk production, was 85.8%. Somatic cell count (SCC log10) increased during lactation, with the higher value of 5.55±0.05 at DIM>180 and were significantly (P<0.01) correlated to Plateau Phase (r=0.35) and to Decreasing Phase (r=0.21). Buffalo total milking time, that is higher than in bovine, showed the predominance of Increasing Phase and Decreasing Phase respect to the Plateau Phase.

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Global warming potential of a fluid milk produced and consumed in central Italy: a life cycle assessment

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Production of milk causes environmental side effects which include emission of greenhouse gases (GHG) with a global warming potential (GWP). A life cycle assessment (LCA) approach was used to evaluate GHG emissions intensity (baseline scenario) related to production of one litre of drinking fat and protein corrected bovine milk (FPCM). Additionally, an anaerobic digester system of cow manure (63%) and residuals crop (32%) for biogas production was implemented, its functioning was applied to the baseline scenario and the impact on the intensity of GHG emissions was assessed. The boundaries of the system were extended from the field to the distribution of milk to retailers and included inputs and outputs related to the following unit processes: crop and milk production, anaerobic digester, transports, plastic disposal, processing and packaging of raw milk. The LCA was based on data referred to one large size dairy farm located in central Italy and consisting of 190 hectares of land use and 380 lactating cows. Data used were relative to year 2011 and to the annual production of 3,546,000 litres of high quality milk. The LCA yielded a GHG intensity of 1.22 kg of CO_2 Eq/litre FPCM (baseline scenario). When the anaerobic digester was considered, the LCA yielded a GHG intensity of 1.10 Kg of CO_2 Eq/litre FPCM. In practice, the analysis pointed out that manure management by the anaerobic digester leads to a reduction of 0.12 kg of CO_2 Eq/litre FPCM corresponding to 9.8% of GHG emissions abatement. Enteric fermentation was the greatest source of GHG and represented the 43 and 48% of the total GHG emissions for baseline and digester scenarios, respectively. The implementation of an anaerobic digester in dairy cows' farm represents an effective mitigation measure which permits a relevant reduction of the GWP associated to milk production.

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Pre-stimulation effects on teat anatomy in Mediterranean Italian buffalo

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The importance of oxytocin release for milk ejection is well documented for cow, goat and sheep but less for buffalo. As buffalo species is harder to be milked with evident difficulty to milk removal, this study aims to investigate on the effect of the subsequent three treatments on teat anatomy and milk removal: two pre-stimulation treatments of 2 and 3 minutes (2MP, 3MP) without administration of oxytocin and one exogenous oxytocin treatment (OT) without pre-stimulation. Fourteen Mediterranean Italian buffalo cow (mid lactation) raised in the central Italy, were submitted to these different treatments to study their effect on the following parameters: teat anatomy, measured by ultrasound imaging (Honda Electrics HS101V) on the right front and right rear teat before and after each treatment; milk yield, and milking time measured for each of the two analysed quarters by electronic milkmeter. Data on teat anatomy collected before treatments are presented as mean and standard deviation: teat length (55.4±10.9 mm); teat canal length (25.2±3.0 mm); teat cistern diameter (13.9±4.3 mm); teat wall thickness (9.6±1.3 mm) and teat diameter (33.1±2.7 mm). All these parameters showed significant variations after each treatment: teat canal significant ($P<0.001$) reduced its length with a decrement ranging from a minimum of 21.0% (2MP) to a maximum of 24.6% (3MP); cisternal diameter significant ($P<0.01$) increased in size comprised between a minimum of 15.11% (2MP) and a maximum of 46.76% (OT); teat wall thickness showed a significant ($P<0.001$) reduction ranging from 8.83% (2MP) to 23.57% (3MP). Results on milk yield extracted by quarters did not show statistically significant differences among treatments. They ranged from 1.35±0.72 kg per quarter (2MP) to 1.59±1.01 kg per quarter (OT). Milking time data revealed a significant difference ($P<005$) between pre-stimulation treatments and oxytocin administrations: they show that time decreased from 9.84 minutes (2MP) to 8.9 minutes to 6.40 minutes (OT). In conclusion the results show that all the treatments cause a similar reduction of teat canal length. Our study also shows that a 2MP is enough for the removal of alveolar milk fraction in Mediterranean Italian buffalo cow.

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Influence of breeding techniques on fatty acid profiles and sensory properties of *Longissimus* muscle of Podolian cattle

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The objective of this study was to evaluate the effects of fattening diet on fatty acid profiles and sensory properties of *Longissimus* muscle of Podolian calves. The trial was carried out on 9 castrated and 10 entire male calves. The animals were dam-reared to the age of natural weaning (6–9 months of age), then they moved in paddocks and received hay and straw *ad libitum*, and a concentrate mixture (15% corn, 15% barley, 15% middling, 5% faba beans and 50% dehydrated alfalfa) in variable amount according to the weight. After the fattening period, 7 calves (4 castrated and 3 whole) were maintained on a *Hedysarum coronarium* pasture, while the remaining 12 calves (7 whole and 5 castrated) received a ration constituted by hay of spontaneous essences *ad libitum* and a mixture (4 kg concentrate, 1.5 kg whole faba bean and corn, 1.5 wheat bran) in shed. The finishing period lasted 4 months. Animals were slaughtered at 22 (whole) – 29 (castrated) months of age. Slices of the *Longissimus* muscle (between the 9th and 13th ribs) were sampled from all calves for sensory properties, evaluated by a panel of 8 trained members, and for fatty acid composition of intramuscular fat. Data were analysed by the least-squares method using the GLM procedures with fattening, gender and interaction as fixed factors. The fattening system influenced significantly the n-3 fatty acids percentage. Animals fattened on pasture showed a higher percentage of n-3 fatty acids (4.76 *vs* 2.48, $P<0.05$) than subjects fattened in shed and, although not significant, higher linoleic acid level. The n-6 fatty acid proportions were tendentially higher in pasture group, therefore, the n-6/n-3 ratio was 3.25 and 5.63, respectively. Meat of castrated males had higher ($P<0.5$) MUFA (40.73 *vs* 33.65%) level and MUFA:SFA ratio (0.96 *vs* 0.75) than shed groups. Among sensory properties only tenderness and chewiness were influenced by fattening system: meat produced by animals fattened on pasture was judged more tender ($P<0.001$) and requiring less chewiness ($P<0.001$). Castrated animals, in comparison to whole, gave meat more tender ($P<0.001$), less juicy ($P<0.05$) and requiring less chewiness ($P<0.05$).

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Relationship between pork quality and animal welfare status

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The sensory quality of meat, in particular appearance and texture attributes, influences consumer behaviour. These attributes depend on the one side on animal welfare in farms, and on the other on pre-slaughter handling which is in any case a cause of stress which consequences may lead to economic damages for the whole production chain, consumer included. The objective of this research was to evaluate the influence of animal welfare status on pork quality. The study was carried out on 22 lots of 10 pigs (about 9 months old and 165 kg average alive weight) each one coming from 11 farms. The temperature range observed during transport and lairage was 10-25°C. The activity was performed over 2011 and 2012. To reach the above objective, management indicators of animal welfare were recorded in all the stages before slaughtering. In farms, the farm welfare index (FWI) was calculated. For each lot, the distance and time from the farm to the slaughterhouse were recorded as well as anomalous behavior (slipping, falling and pileups) in loading/unloading operations which was measured by direct observation. After slaughtering, physiological parameters (aldolase, creatine kinase, cortisol levels) as well as pork quality and biochemical indicators (water holding capacity, pH) were measured. Sensory quality of pork was evaluated through the quantitative descriptive analysis (QDA) which describes a complete product profile (appearance, olfactory, taste and flavour-related and textural). From the results it is possible to assume that sensory characteristics of pork, mainly those related to texture and appearance (intramuscular fat ($P<0.01$), tenderness ($P<0.05$), juiciness ($P<0.05$)) improve when the animal welfare indicators were higher. It was observed an increase of green hams haematomas ($P<0.05$) and blood level of aldolase ($P<0.05$) when the incidence of abnormal behaviour during loading/unloading operations was higher. Moreover, transport time increases the creatine kinase levels in blood ($P<0.05$). At slaughterhouse, greater lairage times produced better qualitative parameters of the pork (juiciness ($P<0.05$) and chewiness ($P<0.01$)).

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Computer vision system for image analysis of visible fat in dry sausages

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The research aimed to investigate the effect of the raw material origin (5 producers used meat bought from the market and 5 used meat from pigs bred on their farms) and of the brand (10 salami factories) on fat/lean ratio in Lucanian dry sausages. The sausage slices were photographed and either used for a consumer test or analysed with the Computer Vision System to evaluate the fat/lean area ratio using image binarization. This analysis converts an image of up to 256 grey levels to a black (lean) and white (fat) image. 349 male and 394 female consumers (n=743) were asked to look carefully at the 6 sausages slices in the photo and to identify the slices they considered unacceptable (each assessor was allowed to choose zero to six slices) and the slice they preferred (each assessor was allowed to choose only one out of six slices). Data gathered from the preference test were analyzed to determine statistical significance based on the frequency of each response (chi-squared one sample test) where the expected frequency was 16.6%. Data concerning acceptability were analyzed using chi-squared for k samples with an expected frequency of 50% (acceptable *vs* not acceptable). The visible fat/lean ratio of the sausages slices was higher ($P<0.001$) in the product from salami factories without pig farm. The fat percentage was highly variable ($P<0.001$) among the sausages of each salami factory, although this heterogeneity can be considered a typical characteristic of these products. On the whole, the products the consumer considered unacceptable had a higher fat percentage ($P<0.001$). Consumers (about 70%) preferred the two leanest products ($P<0.001$). Female consumers showed a higher preference for lean products as compared with male ($P<0.001$).

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Sensory properties and shelf life of Lucanian sausages

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Ten panellists used quantitative-descriptive analysis to assess sensory properties on 10 Soppressata sausages, 5 based on commercial pork (MO) and 5 obtained from pigs reared on farm (FO), at 3 different storage time (0, 3, 6 months). A 104-member consumer panel was asked to rate its liking for 6 out of 10 products in 3 conditions: tasting without information (blind B), information about meat origin without tasting (expected E), tasting with information (actual A). Odour was higher in FO products ($P<0.05$), whereas elasticity ($P<0.001$) and flavour ($P<0.05$) were perceived as more intense in MO. Storage time influenced fat colour, seasoned flavour and cohesiveness ($P<0.001$) that showed lower intensity with increasing time, whereas smoked flavour, chewiness, bitterness ($P<0.001$), saltiness ($P<0.05$) and rancid flavour ($P<0.01$) increased with storage time. PLS discriminant analysis, combining descriptive and consumer liking (explained variance: Factor 1=34% and 57% for X and Y, respectively; Factor 2=12% and 35% for X and Y, respectively), showed that liking was positively correlated with fat hue and fat diameter and negatively correlated with bitterness. In addition, PLS allowed to identify attributes more important in products differentiation such as meat and fat colour, brightness, fat diameter, seasoned flavour, chilli-odour, bitterness and elasticity. Consumers rated 5 of the 6 products above the central point (5=neither pleasant nor unpleasant) for perceived liking, indicating their good eating quality. Both expected liking scores (FO and MO) were higher then perceived liking expressed in blind conditions ($P<0.001$) and above the central point, due to a satisfactory consumer trust in legislation transparency in the case of FO and local processing methods in the case of MO. Expected liking scores were higher for FO than for MO ($P<0.001$). Two products were rated very pleasant with blind scores similar to expected ratings (no disconfirmation). Surprisingly, for all products MO information produced a contrast effect as a consequence of a negative disconfirmation, which means that actual liking moved in the direction opposite to the expectations. Conversely, when FO information was given, in most cases no effect was found on actual liking, therefore sensory properties prevailed in orienting consumer liking.

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Evaluation of *Cinta Senese* meat quality

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Cinta Senese (CS) is an autochthonous pig breed native of Central Italy (Tuscany), characterized by a good meat and high fat quality and its commercial value is strongly linked to high quality seasoned products. This trial was aimed to verify the effect of age of slaughter on meat quality of CS (PDO *Cinta Senese*). One hundred and thirty nine (139) meat samples of CS breed, collected by I.N.E.Q. (Istituto Nord Est Qualità) were analysed by IZSLT (Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana – Roma). Meat samples were subdivided in three groups according with slaughter age: group 1 (<480 days), group 2 (480-720 days) and group 3 (> 720 days). Chemical composition using Near Infrared Spectroscopy in Transmittance (FoodScan™ Meat Analyser; FOSS) and tenderness (Dynamometer Instron 3343 with shear Warner-Bratzler) were determined on the *Longissimus dorsi* muscle during 2010-2012. Data were analysed by ANOVA procedure (SPSS ver. 13.0); differences of means were localized by Bonferroni's t-test and considered significant if $P<0.05$, in order to classify the effect of the different age at slaughter (3 groups). Overall mean of chemical composition of meat were: collagen (1.41±0.30%); fat (7.19±3.49%); protein (21.03±1.72%); total solid (28.85±2.81%); moisture (71.15±2.81%). The average of shear force was 4.40±2.88 kgf/cm^2. Fat content decreases from group 1 to group 3 (8.01±4.02% *vs* 6.24±2.46%; $P<0.05$). Total solid decreases significantly with age of slaughter from group 1 to group 3 (29.44±3.16% *vs* 28.20±2.29%; $P<0.05$) while moisture showed an opposite trend increasing from group 1 to group 2, (70.56±3.16% *vs* 71.80±2.29%; $P<0.05$). All groups showed appreciable meat tenderness although results highlight a significant difference between group 1 and group 3 (3.79±2.73 *vs* 5.91±3.28 kgf/cm^2; $P<0.05$). Shear force was correlated significantly with fat content ($r=-0.27$, $P<0.05$) and protein content ($r=0.17$, $P<0.05$). In conclusion results highlight that all the analysed samples correspond to the standard of PDO. Although the results confirm the valuable characteristics of meat CS they show that the shear force increases with the increasing of the slaughter age.

P-120

Safety of honey bee products: trace metals occurrence in honey produced in the Viterbo Province

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Honey is a very appreciated food item for its nutritive and organoleptic characteristics. However, agro-environmental contamination may affect the safety of this bee product. Honey bee itself may be considered as a bio-indicator/bio-accumulator species. Beekeeping in the Lazio Region is normally sized in small production unities and honey is normally sold near the site of production. So, the control of honey may give a direct evaluation of contaminant intake by population living around. This is a very important issue in making reliable exposure/risk assessment at local-scale level. The present study aimed to assess the presence of trace elements (Cd, Pb and Cr) in honey samples collected in three study areas (AS01, AS02 and AS03) of the Viterbo Province as indicator of toxic metals occurrence in the agro-ecosystems explored by the bees. In total 23 samples were obtained from professional beekeepers during the 2010 and 2011 production seasons and submitted to Atomic Absorption Spectroscopy for the cadmium (Cd), lead (Pb) and chromium (Cr) quantitation. Cadmium was always found to be below the instrumental limit (0.005 mg/kg) while in four samples Pb was found at low level (from 0.02 to 0.046 mg/kg). On average, the Pb contamination of honey samples was lower than previously reported for the Lazio and Toscana regions. In total, 14 out 23 samples were found positive for the Cr presence. No differences were found comparing the year of production. Because there are not industrial activities in the areas where honeys were sampled, the occurrence of Cr may be explained by geopedological conditions even though the impact of vehicular traffic cannot be excluded. In fact, honeys becoming to the Viterbo city area (AS02) showed the higher mean value (0.282 mg Cr/kg) if compared with the AS01 and AS03 study areas (0,070 and 0,087 mg Cr/kg, respectively). Overall, as far as the trace elements investigated, the honey produced within the areas in study may be considered as safe. However, further studies are necessary to explain the occurrence of chromium in honey belonging to the investigated area.

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Effects of ageing on fatty acid composition and texture of Lucanian sausages processed with or without nitrates

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The Lucanian sausage is a mild seasoned sausage appreciated for its organoleptic characteristics. It is produced by processing a mixture of coarse-grained pork meat obtained from the shoulder, which is characterized by low fat, reduced water content and good compactness. Portions of belly fat (<30%) are added in order to provide greater softness. The mixture is flavoured with salt (NaCl, 20-30 g/kg), wild fennel seeds (*Foeniculum vulgare* Mill., 1 g/kg) and local sweet chilli (2 g/kg) and it is added with a very low amount of nitrates (<250 mg/kg). The total removal of nitrates would give greater wholesomeness to the product, providing worthy benefits to consumers. The aim of the study was to evaluate the effects of ageing on the fatty acid composition and texture of Lucanian sausages processed with or without nitrates. Sausages were tested at seasoning (which lasted 30 days) and after 3 and 6 months of vacuum storage. Fatty acid composition was determined by gas chromatography. Rheological characteristics were measured in order to define texture. Regardless of ageing, the total removal of nitrates did not change the unsaturated fatty acid composition of the sausage, as well as the P/S (polyunsaturated/saturated) ratio (0.22±0.01), but it lowered the elasticity (2.80±0.07 *vs* 3.17±0.07 mm; $P<0.01$) and the chewiness (66.66±8.30 *vs* 114.70±8.30 N x mm; $P<0.001$) of the sausage cube, as well as the cohesiveness (0.90±0.00 *vs* 0.92±0.00) of the sausage slice. Regardless of the type of processing, ageing significantly reduced ($P<0.01$) the unsaturated fatty acids C18:2 omega6, C18:3 omega3 and C20:2 (597.71±56.97 *vs* 1033.92±56.97; 36.81±4.79 *vs* 62.77±4.79; 48.84±3.00 *vs* 67.14±3.00 mg/100 g WM, respectively), probably due to lipolysis which occurs naturally during storage, and markedly ($P<0.001$) lowered the elasticity (2.67±0.09 *vs* 3.31±0.09 mm) and cohesiveness (0.83±0.01 *vs* 0.86±0.01) of the sausage cube, as well as the cohesiveness (0.89±0.00 *vs* 0.92±0.00) of the sausage slice. It may be hypothesized that changes of the fatty acid profile affect the sausage compactness due to a decrease of the extent of fatty acid unsaturation. Results showed that the sausages keep optimal characteristics for up to 3 months of storage.

P-122

Growth performance of purebred *Sarda* pigs in commercial farms: evaluations and perspectives

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The local swine *Sarda* population is mainly farmed in small size not specialized herds. Since 2006, it has been recognized as a breed in the native breed register. In order to suggest appropriate breeding strategies aimed at improving production traits, average pig performances in the current farming conditions should be assessed. In this work, a survey was carried out in five commercial farms in order to yield baseline information on the growth performance of purebred *Sarda* pigs. In four farms pigs were kept outdoor, whereas in the remaining animals were housed in indoor pens. Weight of 12 pigs, the backfat thickness and feed intake were monthly recorded, from weaning to final market weight, in each farm. The average daily gain (ADG) and the feed conversion ratio (FCR) were calculated. As expected, growth performance of pigs exhibited a relevant variability between farms. The maximum ADG values were in the interval 0.45-0.65 kg/d, and were observed for pigs whose BW ranged between 85-95 kg. In the best performing outdoor-farm, pigs reached 100 and 155 kg of BW at about 8 and 12 months, respectively. The growing rate was larger for pigs housed indoor that reached 100 and 155 kg of BW at about 6.5 and 9 months, respectively. Average backfat thickness in pigs at about 100 kg of BW was 24 mm both for indoor and outdoor farms. However, pigs weighing more than 150 kg had a larger backfat in outdoor farms. During the fattening phase (from 100 to 150 kg of BW) the FCR was about 5.2 for both indoor and outdoor pigs. In conclusion, the growing performances of pigs of *Sarda* breed are not comparable to those of the most common breeds of pigs used in Europe. However result of the present study gave insight for a further development of management strategies aimed at exploiting this breed in farming systems aimed at obtaining typical high quality products.

P-123

Expression of lipoic acid in edible parts of *Marchigiana* cattle

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Lipoic acid (LA) is a small molecule that contains two oxidized or reduced thiol groups. Its oxidized form is usually defined as α-lipoic acid or just lipoic acid, and the reduced form of LA is known as dihydrolipoic acid (DHLA). It is known as the antioxidant of antioxidants, inasmuch it has an influence on the second messenger nuclear factor KB (NF-κB) and attenuates the release of free radicals and cytotoxic cytokines. In current studies, it has been reported that dietary supplementation of LA induces a decrease in oxidative stress in many pathologies such as cardiovascular, neurodegenerative and autoimmune deseases, diabetes, cancer and AIDS. LA is synthesized by plants and animals. In this report, we analyzed the presence and expression of LA in edible parts (liver, heart and meat) of 10 *Marchigiana* cattle by immunofluorescence and western blotting techniques. The animals were born and farmed in the province of Benevento (Italy) and their meat is a PGI (Protected Geographical Indication) product and the name of the trade mark is Vitellone Bianco dell'Appennino Centrale. The results highlighted LA-immunoreactive materials in the hepatocytes, muscle cells and cardiomiocytes, as its presence by western blotting analysis. This is the first step of a research project in which we would compare the level of LA in edible parts of various cattle breeds raised in province of Benevento. The aim of our study is to know how the different animal feed influence the LA content in the edible parts of bovine. In particular, we have chosen *Marchigiana* as cattle model for its peculiar type of breeding, feed and genetics. Subsequently, we will quantify the LA/Kg levels in bovine meat and compare them with the safety dose shown in literature data.

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Effects of different diets on traditional salami of *Nero di Parma* pigs

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Nero di Parma pig is a crossed population obtained after a preservation program of a local black pig breed across Appennino Emiliano. In the last century the local breed reduced its number in favour of more productive breeds. Nowadays *Nero di Parma* is a black coated pigs suitable in outdoor system; fresh and seasoned products show high quality characteristics (dry cured ham, salami*, culatello*). In Garfagnana district the breeders often integrate rations of pigs using some local by-products obtained from cereals (spelt meal, spelt bran) and chestnut (chestnut meal), in order to reduce nutritional costs. Numerous studies show as Italian autochthonous breeds are suitable to be grown outdoors or with integration of by-products (*Nero Siciliano, Cinta Senese*). Aim of the study was to evaluate the characteristics of typical salami obtained from *Nero di Parma* pigs. Experimental study was developed with ten castred males fed with conventional (CN) and by-products (BP) diet. Average weight at slaughter was 149.8 kg CN *vs* 127.6 kg BP group, dressing was 84.8% CN diet *vs* 84.1% BP diet. At sectioning lean and fat cuts were separated; leg trimmings (75%) and belly (25%) were used for preparation of single mixture for each pig, salt and spices (3%) were added moreover. Traditional salami (TS), after a ripening period (60 days), were obtained at the following conditions: 18°C, 80% RH. On the TS samples proximate chemical composition were carried out according to AOAC methods: dry matter, crude protein, non protein nitrogen, ash and total lipids. Data were analyzed by one-way analysis of variance; t-test was used for statistical significance comparisons between means. Samples of TS were evaluated for technological parameters as reported: fresh weight 0.48 kg CN diet *vs* 0.32 kg BP diet; fresh length 20.75 cm CN diet *vs* 19.43 cm BP diet; weight loss at 60 days 35.19% CN diet *vs* 32.17% BP diet.

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Casertana × Italian Duroc pig: fatty acid profile of subcutaneous adipose tissue (lard)

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Fatty acid (FA) profile of lipids in animal origin products (AOP) plays an important role for its effects on consumer health. Numerous studies showed that FA composition is affected by epigenetic factors. The aim of this study is to estimate the effect of rearing system and sex on fatty acid composition of subcutaneous adipose tissue of *Casertana* × Italian Duroc pig. Pigs were slaughtered at the age of 15 months and at net live weight of about 175 kg. Fresh lard samples were taken, after slaughtering, in correspondence of the fourth up to the sixth lumbar vertebra from 16 pigs (8 females and 8 castrated males), 8 reared at half-wild (HW) system and 8 reared at confined (C) system. At slaughter, lard thickness was, on average, 3.3 cm and 3.2 cm for HW and C systems respectively and 3.4 cm in castrated males and 3.5 cm in females. Lipids were extracted by Folch method and FA fraction was analyzed by gas-chromatography. The acidic fraction considered consisted of: lauric, myristic, myristoleic, palmitic, palmitoleic, margaric, stearic, elaidic, oleic, C18:1 cis12, linoleic, arachidic, γ-linolenic, α-linolenic, eicosenoic, dihomo-gamma-linolenic acid, eicosedienoic, erucic, eicosatrienoic n-3. The results showed that: (i) sex does not affect FA composition of lard; (ii) rearing system influences the content of the margaric ($P<0.05$), arachidic ($P<0.05$) and linoleic acids ($P<0.05$); in particular, margaric acid content is 0.28% and 0.23% in C and HW rearing systems respectively; arachidic acid content is higher in C system (0.17%) in comparison with HW system (0.15%), while, linoleic acid is, on average, higher in pigs reared at HW system (18.39%) than those reared at C system (16.71%); (iii) the interaction between the sex and rearing system does not exert a statistically significant effect. Overall, the results show that the rearing system would affect only the margaric, arachidic and linoleic acids and not all fatty acid profile of subcutaneous adipose tissue.

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Effects of Curcuma longa ethanol extract on physical properties and oxidative stability of rabbit patties

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Curcuma longa (CL) is a perennial plant that belongs to the Zingiberaceae family; the main components of this spice are curcuminoids, considered the major responsible for the biological activities of the spice. These compounds show a wide range of important properties as a strong antioxidant capacity. Curcumin is the most abundant, the most active and the most studied curcuminoid. Lipid oxidation is one of the main factor limiting the quality and the shelf-life of meat and meat products. Nowadays the acceptance of the synthetic preservatives is decreasing and the consumer demand is increasingly turned to natural products. The aim of this study was to assess the effect of the employment of CL ethanol extract in rabbit raw minced meat samples, during storage at 4°C over a period of 13 days. At 2, 6, 8 and 13 days of storage physical properties (color and pH) and Thiobarbituric Acid Reactive Substances (TBARS) production, in order to evaluate lipid oxidation entity, were evaluated. Meat samples treated with CL extract were compared with control samples and positive control samples (treated with ascorbic acid). CL extract reduced the formation of TBARS in comparison to control samples at all experimental times considered , demonstrating the capacity of this spice to limit lipid oxidation process in meat products and therefore to improve its shelf-life. CL extract is able to affect color properties; lightness (L*) and redness (a*) indexes significantly decreased, while yellowness (b*) increased, compared to control meat sample. pH value increased in meat treated with CL extract after 13 days of storage. CL extract reduced the formation of TBARS in comparison to control samples at all experimental times considered, demonstrating the capacity of this spice to limit lipid oxidation process in meat products and therefore to improve its shelf-life.

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Effect of thyme and orange essential oils on chicken meat quality traits

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Poultry meat is particularly prone to oxidative deterioration due to its high concentration of polyunsaturated fatty acids. Plant essential oils are natural compounds studied for their potential antioxidant properties. Two separate experiments were conducted to evaluate the effects of a mixture of thyme and orange oils (TO) on the broiler breast and wing meat quality and lipids oxidation. For each experiment 24 fresh breast fillets and 24 wings were procured from a local grocery store. TO were added to marinade solution (water 91%, sodium chloride 6% and polyphosphate 3%) to achieve a final concentration of 0.5% v/v of TO blend. Breasts and wings were split in two experimental groups with homogenous pH and lightness and subjected, through vacuum tumbling, to two treatments, a 0.5% TO and a control (no TO). Each group was tested for pH, color (L*, a*, b*) and moisture content after marinating and after cooking, purge loss, cook yield and shear force in order to evaluate the meat quality traits. Lipid oxidation was determined on fresh and frozen meat by the TBARS analysis. In the first experiment, the TO-breasts had higher pH after marinating, higher shear force and lower purge loss and redness than the control. The TO-wings presented higher pH after marinating, higher redness and yellowness before and after marinating and lower purge loss and shear force than the control. In the second experiment, the TO-breasts showed higher pH after cooking than the control. The TO-wings presented higher lightness and redness after marinating and lower purge loss than the control. From the TBARS analysis, in both experiments, when compared to the control group, both fresh and frozen TO-breasts were less susceptible to lipid oxidation. Fresh TO-wings showed less sensitivity to lipid oxidation, but frozen TO-wings showed less sensitivity only in the second experiment and in the first only if temperature abused. In conclusion, the TO had a positive effect on the lipid oxidation without negatively affecting meat quality traits.

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Modification in tocopherol and fatty acid content in *Marchigiana* cattle meat after frying in extra virgin olive oil

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With this report, we would evaluate the modification in tocopherol and fatty acid content in *Marchigiana* cattle meat, collected from different time of aging. Actually, literature data described the fatty acid content in meat preparations (meatballs and cutlet) during frying with different cooking fats (peanut butter, olive oil, butter and etc…). However, it has never been studied the interaction between meat, at different times of aging, and cooking fats. During aging, meat undergoes changes in nutritional profile. The examined meat was collected from *Marchigiana* cattle at 7, 14 and 21 days post mortem and storage at 2°C. Tocopherol and fatty acid content was detected in extra virgin oil and cutlet ingredients (meat, eggs and breadcrumbs) after and before frying, using HPLC and HRGC method for tocopherol and fatty acid, respectively. The results showed that after frying tocopherol and MonoUnsatured Fatty Acid (MUFA) content in meat increase, while Saturated Fatty Acid (SFA) content decrease. Also, at 14 days post mortem fatty acid content showed better nutritional profile because of a major presence of oleic acid. It is know that the oleic acid is cardioprotective because it reduce the level of cholesterol. Considering fatty acid content, the atherogenic and thrombogenic indexes in the different times of collecting were calculated. We found out that the best values are referred to 14 days aging. With these results we could say that to ameliorate cutlet fatty acid and antioxidant content after frying, we recommend both the use of extra virgin oil and the meat at 14 days of aging.

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Effect of age on fat thickness, adipocyte morphology and fatty acid content of subcutaneous fat in *Nero di Parma* swine

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During the last 4 months of fattening, subcutaneous fat characteristics of 8 *Nero di Parma* pigs (4 females, 4 castrated males) were evaluated. The animals were reared in the same environmental conditions and under the same feeding programmes. At 570 and 641 days of age, by biopsy, and at slaughter (mean age: 683 d), a withdrawal of subcutaneous fat at point P2 was made. At the same moments, thoracic circumference, BW and backfat thickness (P2) were estimated. The fatty acid content (GC HP 6890; Agilent Technologies Italia, Milano) was analysed and also adipocyte morphometric analyses, by digital camera (Zeiss Axiocam MRc5), were assessed on fat samples. The images were processed with Axio Vision software (rel. 4.5). Data were submitted to analysis of variance, using a mixed model including fixed effects of sampling (3 levels), sex (2 levels), interaction and the random effect of individual within sex (6 levels). At the experimental ages, differences were observed for weight (kg 159.77a, 177.00b, 187.61c, resp.), thoracic circumference (cm 135.88a, 142.25b, 146.00c, resp.) and backfat thickness (mm 21.25a, 36.75b, 45.50c, resp.) ($P<0.05$) but not in ADG ($P>0.05$). The fatty acid composition showed a generalized reduction of short and medium-long chain (up to C18) and an increase of long-chain (> C18) fatty acids. At 641 and 683 d of age SFA decreased ($P<0.05$) and PUFA increased ($P<0.05$), as compared to 570 d. The atherogenic index [(4*C14+C12+C16)/(MUFA+PUFA)] decreased from the first to the last sampling (0.489c, 0.459b, 0.420a, resp.) ($P<0.05$). An age related increase ($P<0.05$) in the area of adipocytes (μ2 17387a, 16566a, 22837b, resp.) as well as in the measures of the major axis (μ 153a, 143a, 173b, resp.) was showed as the minor axis was not different among ages ($P>0.05$) and the perimeter (μ 567b, 481a, 566b, resp.) was lower at 641 d ($P<0.05$). In conclusion, in *Nero di Parma* pig, similarly to what occurs in other pig breeds, adipose tissue has a dynamic in its quantitative and qualitative composition, characterized not only by an increase in the thickness of the subcutaneous fat but also by changes in adipocytes morphology and fatty acid composition.

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Probiotics in milk replacer influence lamb meat quality

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In specialized dairy sheep flocks, lambs are separated at an early age from their dams and fed commercial milk replacer to increase the volume of milk destined for the production of cheese. Artificial rearing can lead to a less favorable profile of intramuscular fatty acid in lamb meat when compared with dam suckling. The use of milk replacer containing microbial cells could represent a suitable feeding strategy to reduce negative effects of artificial rearing system on nutritional feature of lamb meat. In fact, microbial feed additives facilitate the establishment and maintenance of suitable microbial flora in the gastrointestinal tract. This study was undertaken to assess the effect of milk replacer containing Lactobacillus acidophilus and a mix of Bifidobacterium animalis subsp. lactis and B. longum subsp. longum on lamb meat quality, with particular respect to fatty acid profile of lamb meat. A 6-week-trial was conducted on 40 male Comisana lambs, divided into four groups, fed maternal milk (MM), milk replacer (MR), MR with L. acidophilus supplementation (MRL) and MR with a mix of B. animalis subsp. lactis and B. longum subsp. longum supplementations (MRB). Cholesterol level in blood was the lowest in MRL, intermediate in MRB and the highest in MM and MR lambs, whereas meat cholesterol levels was not changed by diet. Meat from artificially reared lambs with and without probiotics was characterized by the highest level of trans-11 18:1; whereas total conjugated 18:2n-6 were the highest in MRL and MRB meat. Meat from the dam-suckled lambs was characterized by 14:0, cis-9 14:1 and 16:0. Polyunsaturated to saturated fatty acid ratio was higher in meat of MR, MRL and MRB than in MM lambs. Meat from lamb fed MR containing probiotics showed an improved fatty acid profile for human diet in terms of CLA content.

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Production of heavy pigs in Friuli Venezia Giulia: a case study

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The limitation of using the tight of pigs slaughtered in Friuli Venezia Giulia Region only for the production of the San Daniele ham DOP and the concomitant ban to export from the Region these tights for the other Italian hams DOP forced the Region and the Regional breeder association (AAFVG) to find new strategy to ensure the economic sustainability of regional pig farms. A 5 year research program was founded to evaluate the reproductive and productive performances and the carcass and seasoned ham quality of Italian Duroc X Italian Large White F1 crosses (DU). This hybrids were compared to a Commercial hybrid X Italian Large White F1 crosses (GO) in 3 commercial farms in Friuli Venezia Giulia. 330 Italian Large White sows were mated with 13 Italian Duroc or 13 Commercial hybrids boars during a 3 year span times and at born a radio frequency identification chip was inserted on left leg of piglets and used. Of the 30000 pigs born, 4784 were individually monitored for live weight at 4 and 11 weeks of age and at the end of fattening (around 280 day of age), carcass weight and quality (Fat-O-Meter), weight of fresh thighs and fat composition. The DU pigs had significantly higher ($P<0.01$) final live weight (173.7 kg±0.5 s.e. *vs* 167.4 kg±0.5 s.e.), carcass weight (141.0 kg±0.4 s.e. *vs* 138.7 kg±0.4 s.e.), fresh thigh weights (17.1 kg±0.1 s.e. *vs* 16.3 kg±0.1 s.e.) and lower monounsaturated fatty acid percentage in subcutaneous fat of thigh (47.9%± 1.5 s.e. *vs* 49.2%±0.9 s.e.) than the GO hybrids. The fat percentage measured by FOM was significantly higher ($P<0.01$) in DU pigs (48.7%±0.1 s.e. *vs* 49.2%±0.1 s.e.) and the percentage of pig carcasses classified as R in the EUROP grid were significantly ($P<0.05$) higher in DU (57.6%) compared to GO (55.6%). According to these results, the DU are proposed as a new brand of *Tipicamente Friulano* for the Friuli Venezia Giulia pig industry.

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Influence of two breeding techniques on Podolian beef productive performances

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The aim of this study was to evaluate the productive performance (*intra-vitam* and *post-mortem*) of castrated and whole Podolian calves finishing on pasture or in shed. The trial was carried out on 19 Podolian male calves naturally weaned between 6 and 8 months of age. Nine calves were castrated at 6 months of age. During the winter the animals were maintained in paddocks and received hay and straw *ad libitum*, and a concentrate mixture (15% corn, 15% barley, 15% middling, 5% faba beans and 50% dehydrated alfalfa) in variable amount according to the weight. After the fattening period, 7 calves (4 castrated – Group A and 3 whole – Group B) were maintained on a *Hedysarum coronarium* pasture, while the remaining 12 calves (7 whole – Group C and 5 castrated – Group D) received a ration constituted by hay of spontaneous essences *ad libitum* and 7 kg of mixture (4 kg concentrate, 0.750 kg whole faba bean, 0.750 kg corn, 1.5 kg wheat bran) in shed. The finishing period lasted 4 months. After that, the animals were slaughtered at a mean of 575 kg (body live weight) and the following parameters were recorded: carcass weight after 24 hours after slaughter (CW24), hanging period (HP) and carcass weight after hanging period (CWahp). Statistical analysis was carried out by ANOVA. Mean daily weight gain (DWG) was higher ($P<0.01$) in Groups B and C *vs* Groups A and D. Slaughtering outcome was similar in Groups A, C and D but tendentially higher than that obtained in Group B. Age at slaughtering was higher ($P<0.01$) in Groups A and D (29.1 months) than in Groups B and C (21.7 months). No differences were observed for CW24, MP and CWahp. Similarly, no differences were observed in hindquarter and forequarter weights among Groups, although the weight of both quarters were tendentially higher in Group C than in the others. From the results of this study it seems that castration does not affect the productive performance of Podolian calves, whereas the breeding technique strongly influence the growth and the age at slaughtering.

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Effect of dietary Verbascoside-based food supplement on some blood parameters and productive performances in intensively reared Italica hare (*Lepus corsicanus*)

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Within the activities of game-hunting of wild animals, kept in breeding to be allocated to restocking areas, the effect of antioxidant-activity of dietary supplement was assessed, on a limited number of Italica hares, being "vulnerable species", on some blood parameters, on slaughter performance and on meat quality. The test lasted 240 days and was conducted on 12 hares (210 days of age) divided into 4 groups, homogeneous by age and body weight. One group was control (CON) without any supplementation in feed and the other three groups received a dietary supplement titrated at 0.5% verbascoside: 100 g/q of feed in LVB, 150g/q in MVB and 200g/q in HVB group. All animals, at the beginning (210d) and at the end of the test (450d), were subjected to the registration of body weight and blood sampling. At the slaughter, gross body weight, hot weight carcass and dressing percentage were determin, as well as some post-slaughter parameters on Semimembranosus muscle: water holding capacity, pH and color (0, 24h and 48h). Moreover, a sample of *Longissimus lumborum* were collected for the following determinations: fatty acid profile, TBARS, vitamin A and E. The dietary verbascoside supplementation resulted in a significant decrease in triglycerides ($P<0.01$), LDL cholesterol and bilirubin ($P<0.05$) and an increase in HDL cholesterol ($P<0.05$) in experimental LVB, MVB and HVB groups. In addition, an improvement of oxidative blood stability was highlighted with a significant decrease in plasma concentration of ROMs ($P<0.05$) and TBARS ($P<0.01$) and increased content of vitamin A and E ($P<0.05$). Verbascoside supplementation produced a significant reduction in saturated fatty acids ($P<0.05$) on hare meat of experimental groups compared to control group. Even the total of mono-unsaturated and poly-unsaturated fatty acids was significantly higher ($P<0.05$) in experimental groups compared to CON group. Dietary treatment resulted in a decrease of the $\omega6/\omega3$ ratio ($P<0.05$) in three experimental groups. The value of TBARS in meat was significantly lower ($P<0.01$) in LVB, MVB and HVB groups compared to CON. The vitamin E content of meat was significantly higher ($P<0.01$) in experimental groups while the vitamin A content reached statistical significance ($P<0.05$) only between CON and HVB group. The present results have shown that dietary verbascoside supplement induced both a blood parameters and meat quality improvement.

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Proximate composition and physical traits of fallow deer and wild boar meat as affected by different hunting methods

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Post-killing conditions may imply lowering of quality standard due to uncertainty in post-mortem meat processing. As the number of wild ungulates has increased in the last decades, a consequent increase in culling rate and venison availability for domestic consumption and for the market was observed. Aim of this study was to evaluate the influence of hunting technique on some quality traits of meat in wild boar (*Sus scrofa*) and fallow deer (*Dama dama*). Wild boars and fallow deer were hunted according to Italian rules. The techniques were use of dogs to drive the animals toward the hunters (D), track down the quarry or wait for game in favourable site (S), implying a stressful and often long lasting escape of animals and the unconsciousness of shooting, respectively. 25 (15 males, 10 females) wild boars and 14 (3 males, 11 females) fallow deer (with and without dog drive stress before still hunting) were used. Animals were grouped into two classes according to the age: young (≤ 15 months and adults (20-40 months). Animals were hunted in the Province of Rome and Viterbo, Central Italy. A portion of Longissimus dorsi muscle (LD) was vacuum packed and frozen at -20°C after having been chilled at +2°C. Water holding capacity of wild boar meat was strongly affected by the hunting technique. Thawing loss was higher in S than in D animals (14.07 *vs* 7.25% $P<0.01$). A similar trend was observed for cooking loss ($p=0.054$) The hunting technique had also effect on colour features. Dog drive hunted wild boars showed a paler and yellower meat than the others (L^*: 43.25 *vs* 33.74 $P<0.01$; b^*: 13.55 *vs* 9.89 $P<0.05$). This disagrees with findings for deer. In fallow deer the main meat quality factor related to the hunting technique was meat toughness. Instrumental shear force was significantly higher in animals from group D (5.06 *vs* 3.68 kg $P<0.05$). Outcomes confirm stress as source of abnormalities in meat quality. These anomalies might result discordant depending on the species taken into account. Further investigations are necessary to evaluate the stress response of the different species.

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A transcriptomic analysis of gut response to stress induced by weaning in piglets

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Weaning is a critical period for piglets, typically accompanied by low feed intake, weight loss and increased mortality. In this period the gut undergoes morphological and histological changes that include reduction of villous height and increase in crypt depth. Antibiotics used in the past to mitigate weaning problems have been recently replaced by organic acids, to avoid the selection and spread of antibiotic-resistant pathogenic strain. The aim of this study was to investigate changes in gene expression induced by the stress of weaning and by the inclusion of sorbic acid in the diet during this critical period. Sixteen female piglets were sacrificed during the experiment: 4 before weaning (T0) and 12 five days after weaning (T5). The T5 group was split in two subgroups, one receiving a standard diet containing 5 g/kg of sorbic acid, and the other receiving the same diet in which organic acid was replaced by barley flour. Gene expression was measured in the ileum using Combimatrix whole transcriptome microarrays. No significant difference was found between diets in term of gene expression, metabolism and histology. This probably as a consequence of the short period of treatment and of the severe stress provoked by weaning that likely masked the effect of the different diets. Conversely, 205 genes were found differentially expressed between T0 and T5. These mediate piglet stress response and metabolic adaptation to a new diet and environment during weaning.

Gene ontology (Blast2Go software), pathway and network analyses (IPA software) of differentially expressed genes indicated the activation of several important pathways related to immune and inflammatory response. Transcriptome analysis well described the biological response to stress of piglets in this critical life stage and will be useful to test the effects of specific nutrients or additives (including pre- and pro-biotics) designed to mitigate the stress-induced inflammatory response in the gut and to improve animal welfare.

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Characterization of gastric microbiota of the young pig

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In mammals, the gastric mucosa is divided in many compartments, each with specific functional characteristics that could create niches for the settlement of resident microbiota. In the last years, the study of the microbiota profile took advantage from the next generation sequencing (NGS). Here we present a pilot trial aimed at studying the composition of gastric microbiota in three different portions of the gastric mucosa of pig, using the NGS based on Ion Torrent technology. Four healthy pigs of 15 kg of body weight were killed. For each subject, three samples, of well characterized gastric mucosa, were taken: from oxyntic area (acid production), pylorus (gastrin secretion), and gastric groove in the small curvature, close to cardias, (immunity). Samples of gastric content were also collected. Total bacterial DNA was extracted and V6 region of the 16S rRNA gene amplified. All the 16 samples were sequenced in the same run with Ion 314™ Chip on Ion PGM™ Sequencer. Quality controlled, chimera checked sequences were taxonomically assigned to operational taxonomic units (OTU) and species richness determined processing data with Ribosomal Database Project pipeline. Bacterial strains were identified from individual OTU using a 97% cutoff value. Sequencing yielded 305,442 total reads; after quality control, 80,103 reads were utilized for analysis. The results showed that in the stomach the dominant phylum is Proteobacteria (70%), followed by Firmicutes (21%), independently from the site. Globally, diversity indexes calculated on OTUs abundances were homogeneous for all samples (mean H'=3.56; mean E=0.57). Principal Coordinate Analysis (PCoA) of OTUs abundances did not show cluster by gastric region; however, 9 out of 12 samples of gastric mucosa clustered by pig, indicating more similarity in the relative abundance and taxonomic composition of the microbiota of different stomach regions within an individual pig than between different pigs; moreover 3 out of 4 samples of gastric contents clustered together. In conclusion, our results show that the gastric microbiota does not differ by sites, but there is a strong subject effect. Apparently there are more differences between the microbiota adhering to the mucosa than in the gastric content.

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Effect of different dietary aromatic essences on development of intestinal lymphoid tissues and villus height in rabbit

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In the last decade, considerable interest has arisen in the use of natural antioxidant to improve meat quality and intestinal health. In this paper we analysed some nutritional strategies to improve the intestinal health of growing rabbits in the framework of aromatic essences integration. Two aromatic essences alone or combined were added to the diet and some characteristics of gut barrier (villus height, crypts depth, area and perimeter of Peyer's patch), morbidity and mortality have been analyzed. Five groups of twenty New Zealand White weaned rabbits were submitted to the following 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). Each diet contained integrations of: 50 ppm vitamin E, CLA 0.5% (from soy oil), 3% Omega Lin® (Mignini&Petrini) and 0.5% mixed vitamins. Eight rabbits per diet were slaughtered at 80 d of age; the Peyer's patch was removed from caudal ileum and a 6 cm sample was excised from the middle part of the jejunum to determine mucosal histology. No morbidity was recorded and only one case of mortality has been registered in O diet. No significant effect of dietary treatments was detected on villus height (627 μm; $P=0.27$) and crypts depth (88 μm; $P<0.76$). The area and the perimeter of Peyer's patch were lightly influenced by diet; S diet showed lower values of area (305,957 μm^2; $P<0.15$) and perimeter (2,246 μm; $P<0.12$). It should be noted that Peyer's patch has a major role in the interaction of the host with gut antigens and regulate IgA production. In conclusion, these preliminary data showed that oregano, rosemary and their combination did not exert any significant effect on gut barrier characteristics of rabbits. Further studies with artificial infections should be performed.

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Effect of LNA dietary integration on endocrine and metabolic parameters in dairy cattle

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In a field trial the effect of long term supplementation of different levels of extruded linseed and energy levels on milk yield and plasma concentration of insulin, T3, cortisol, leptin, glucose and non esterified fatty acids (NEFA) in dairy cows in early lactation, were evaluated. Fifty seven Italian Holstein Frisian cows, reared in *Parmigiano Reggiano* cheese area, homogeneous for lactation number, days in milking (DIM) (41±12 d), BCS (3.17±0.14) and productive level in the previous lactations were allotted into three experimental groups, in different farms, with similar management systems (control group –C- 1.45 Mcal NEl/kg DM, 280 g/d Omega-Lin® - Mignini&Petrini O280: 1.51 Mcal NEl/kg DM, 450 g/d Omega Lin® - Mignini&Petrini O450: 1.56 Mcal NEl/kg DM) and fed isonitrogenous TMR diets. Omega Lin® contained 70% of extruded linseed. Blood samples were taken by coccygeal vein and blood parameters evaluated. Fixed effect of diet, parity and herd within diet were tested. Parity showed a significant effect only for milk yield and T3, while diet significantly influenced milk yield, T3, leptin and NEFA. Milk yield was higher in O450 group compared to the control one (43.6l *vs* 35.5l: $P<0.01$). T3 showed the highest level in O450 group if compared with C group (1.32 nmol/l *vs* 1.07 nmol/l: $P<0.01$) while O280 group showed an intermediate level. NEFA as indicators of body lipids mobilization showed the highest levels in C group (0.50 mmol/l) and lower in O280 group (0.29 mmol/l) ($P<0.01$) while O450 showed an intermediated level. Opposite results were found for leptin which showed the highest level in O280 group and the lowest in C group (4.08 ng/mL *vs* 3.08 ng/mL: $P<0.05$) while O450 group showed an intermediated level. No significant differences were found in the other parameters although cortisol and insulin are key players in the control of intermediary metabolism, and glucose as indicator of energy balance. In conclusion, leptin, T3, NEFA levels in O450 group put in evidence a better energy utilization for milk yield although the trend of these parameters showed in O280 group should be explained by a probably different genetic value of the cows among farms. Further analyses will be desirable.

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Differential gene expression in the oxyntic and pyloric mucosa of the young pig

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In animal nutrition the stomach is often considered a single compartment, however morphological differences among different areas are well known. Oxyntic mucosa is primarily equipped for acid secretion, while it is not enough clear if gastric functional control are shared with other areas. Here the differential gene expression between oxyntic (OX) and pyloric (PY) mucosa is assessed in 7 starter pigs. On quality-tested mRNA, the analysis of whole transcript expression was done by Affymetrix©Porcine Gene 1.1 ST array strips. A Robust Multichip Analysis was performed on the CEL files by Affymetrix Expression Console. Affymetrix Transcripts ID's, in general characterized by several exonic sequences, were associated to 13,406 Human gene names, based on Sus scrofa Ensemble. On processed gene expression values, exploratory functional analysis was performed with Gene Set Enrichment Analysis using the C5.BP catalog of gene sets from Molecular Signatures Database v3.1 (http://www.broadinstitute.org/gsea/msigdb/Index.jsp), comparing OX and PY. Normalized enrichment score (NES) was calculated for each gene set, and statistical significance was defined when False Discovery Rate%<25 and P-values of NES<0.05.

In the oxyntic mucosa HYDROGEN ION TRANSMEMBRANE TRANSPORTER ACTIVITY was the gene set most differentially enriched; this includes the two genes for H+-K+-ATPase, fundamental enzyme for acid secretion; pathways related to mitochondrial activity and feeding behavior were also enriched (the last involving primarily cholecystokinin receptors, ghrelin, and the anorexigenic neuropeptide W). Aquaporin 4, water-selective channel protein present in the plasma membranes was the top ranking gene. In the pyloric mucosa only two gene sets were significantly enriched, compared with oxyntic: LYMPHOCYTE ACTIVATION, with interleukin 7 ranking first, and LIPID RAFT, a gene set involved in specialized membrane domains composed mainly of cholesterol and sphingolipids. The single gene most differentially expressed was gastrin, the peptide hormone produced in pylorus by G cells. The 2nd was SCGB1A1, secretoglobin, family 1A, member 1, presumably located in the epithelial line, to inactivate inflammatory mediators. The data indicate that oxyntic mucosa has a specialization higher than the pyloric mucosa. Nevertheless, the pylorus presents some peculiarities for some gene transcripts that could worth further studies.

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In vitro assessment of immuno-modulating effects of different iodine and selenium compounds on dairy goat lymphocytes

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Dairy farm animals may experience under-nutritionally intake of trace elements such as iodine (I) and selenium (Se). This may be due to poor nutritional management, especially during the transition phase or under the regimen of feeding practices compliant to the organic farming rules. This study was aimed at assessing the effect of selenium (different chemical species) and iodine on proliferative response of dairy goat peripheral blood mononuclear cells (PBMC) to mitogens. Eight healthy lactating Saanen goats were used as blood donors. After isolation, the PBMC were incubated in triple at 39°C (5% of CO_2) in the presence of 0, 50, 100, 150 and 200 ppb of Se (as selenate, selenite, Se-Met) or 0, 1, 2, 4 ans 8 ppb of I (as iodide). Proliferative response of PBMC to concanavalin A (ConA) and pokeweed mitogen (PWM) was evaluated after 48 h of incubation. Data were analyzed by full-factorial ANOVA and differences were declared significant at $P<0.05$. Compared to control, Se at 150 ppb induced a higher proliferative response of PBMC stimulated by ConA without significant differences among the chemical species tested. The PBMC stimulated by PWM showed a significant higher response after exposure to selenate then selenite or Se-Met. In PBMC treated with inorganic iodine, a significant higher response compared to the control was observed above 2 ppb with a maximum (+100% with respect to the proliferation of control) recorded at 4 ppb after stimulation with PWM. These results suggest that Se and/or I levels may affect the proliferative response of goat PBMC thereby conditioning the immune response of the animal. Further studies are needed to verify *in vivo* the relevance of these findings.

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Winter forage resources for dairy sheep farms: chemical and nutritional assessment of fresh olive foliage used in *Comisana* sheep feeding

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The nutritive values of olive leaves (OLs) vary according to several factors, including the variety and phenological stage of the plant.

Some antioxidant effects exerted by OLs extracts may be explained by the high polyphenolic content. The aim of this work was to study the suitability of OLs as an alternative forage source for ewes during the early lactation stage. A group of 15 Comisana ewes in early lactation were fed *ad libitum* with fresh olive leaves plus concentrates for a month. A control group was fed with clover hay (CH). Samples of OLs and CH were submitted to proximate analysis and total polyphenolic (TP) and condensed tannin (CT) were quantitated on OLs. During the trial, the ewes were monitored as far as the nutritional and oxidative status by the BCS and by weekly analysis for the ROS content of blood. The feeding behavior of the ewes was also monitored but milk production was not recorded. Olive leaves showed an higher lipid (4.3% DM) and crude protein content (10.3% DM) if compared to clover hay (1.2% and 9.2% DM, respectively). The OLs Van Soest's fiber analysis showed lower NDF (-11%) and ADF (-5%) values than CH but the lignin content (ADL) was rather high (16.6% DM). The high polyphenolic content (15.4 mg CE/g DM) confirms their anti-oxidant value for the animal as showed by a significant decrease of the ROS blood content observed during the study period (from 390 to 270 U Carr) for the OLs fed group. The mean BCS for the treated group did not show any significant variation throughout the study. Nutritive values and metabolic response of ewes fed fresh OLs are promising even though further studies are in progress to fully verify whether this by-product of the olive oil industry may be an interesting alternative to traditional forages.

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Herbage intake and milk production of Comisana ewes as affected by daily time spent on pasture

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The aim of the current research was to study the influence of daily time at pasture of lactating ewes on intake and milk production. A trial was carried out during spring (T°max, 25.9±3.12°C). Twenty-four Comisana lactating ewes, homogeneous for milk production (837±179 g/d), day of lactation (105±8 d) and body weight (62.5±3.4 kg), were divided into four experimental groups and housed in individual pens, where two groups received 500 g/d of a pelleted concentrate (crude protein 15% DM) supplied during milkings. No supplement was supplied to the other two groups. For 4 weeks the groups were left to graze separately in four fenced areas on a natural pasture (dry matter, 30.0%; crude protein, 10.1% DM; NDF, 52.2% DM). The experimental design was: 4N group, no supplement and pasture for 4 hours, from 11.00 to 15.00; 7N group, no supplement and pasture for 7 hours, from 10.00 to 17.00; 4S group, supplement and pasture for 4 hours; 7S group, supplement and pasture for 7 hours. Hours of grazing and supplement significantly affected pasture DM intake (weighting method with bags for feaces and urine collection), on average, higher in groups grazing 7 hours (1.053 *vs* 0.749 kg DM/d; P<0.001) and in unsupplemented groups (0.953 *vs* 0.849 kg DM/d; P=0.047). Despite the differences in pasture intake, no variations were found between groups in milk production (respectively in 4N, 4S, 7N, 7S: 511, 558, 598, 655 g milk/d), whereas, on average, significantly lower body weight losses were reached by animals from the start to the end of the trial in groups grazing 7 hours, compared to 4 hours (-0.52 kg *vs* -1.47; P=0.047) and in supplemented, compared to unsupplemented groups(-0.333 *vs* -1.65; P=0.008). Results seem to demonstrate that the higher nutrient input reached by ewes grazing 7 hours and eating a supplement was adressed in reducing weight losses more than increasing milk production.

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Evaluation of feed digestibility in Alpine goats in the first sixty days of lactation by using the Small Ruminant Nutrition System

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Feed digestibility measurement is important to estimate energy intake of animals. No evaluations of the Small Ruminant Nutrition System (SRNS; Tedeschi, L.O., Cannas, A., Fox D.G. 2010. Small Rumin. Res., 89, 174) have been carried out to test its predictions of digestibility in goats. Thus, a digestibility trial was conducted in Viçosa, (Minas Gerais, Brazil) by using six Oberhasli Alpine goats in early lactation in a completely randomized design with six replications and one diet (DM basis: corn silage 41.5%, ground corn 25.7%, soybean meal 15.6%, wheat meal 12.4%, oil 2.2%, calcitic limestone 1.1%, sodium bicarbonate 1.0%, salt 0.4%, CP: 14.0% and NDF: 28.5%). *In vivo* digestibility coefficients and total digestible nutrients (TDN) were compared with the values predicted by the SRNS. A comparison was also made on ME and NE intake estimated based either on measured TDN applied to the equations of NRC (2001) for dairy cows or by the SRNS. The results showed that the SRNS accurately predicted dry matter (Predicted – Observed, P-O= –1.39 units, NS; root of mean square error, RMSE= 2.6 units) and organic matter (P-O=1.49 units, NS; RMSE= 2.6) apparent digestibility, total digestible nutrient (P – O=–0.85, NS; RMSE= 1.6), ME intake (P-O= –0.67, NS; RMSE= 0.7), and NE intake (P-O= 0.0, NS; RMSE= 0.003). The SRNS underestimated fat (P-O= –18.04, P<0.001; RMSE= 18.1) and CP (P-O= –12.24, P<0.001; RMSE=12.4) apparent digestibility and numerically overestimated NDF true digestibility (P-O= 4.2, NS; RMSE= 5.9). The underestimation of fat and CP digestibility by the SRNS was due to an overestimation of the endogenous component. The numerical

overestimation of NDF by the SNRS might be due to the fact that the NDF degradations rates of the SRNS feed library are probably too high, being based on corn silage grown in temperate regions and not, as in the case of the experiment, in tropical regions, where climatic conditions may reduce them.

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Growth performances and meat quality of *Maremmana* growing bulls fed grain legumes

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The effects of dietary inclusion of lupine and faba bean throughout the growing and finishing periods were evaluated in *Maremmana* bulls raised in an extensive organic farm (Bracciano, Roma). Twelve young bulls (7.7±0.29 months; 241±13.8 kg BW) were allotted into two groups. The control group was fed farmer's diets based on meadow hay, straw, maize silage and a mixture (50/50) of barley and maize meal. The experimental group was fed diets in which barley/maize meal was substituted by growing and finishing concentrates containing lupine and faba bean (on average, 7.5 and 30.5%, respectively). The average forage/concentrate ratio were, respectively for control and experimental diets, 61/39 *vs* 60/40, Meat FU 0.75 *vs* 0.69/kg DM, CP 8.7 *vs* 12.8% DM. Animals were weighed at the beginning of the trial and thereafter every 2 months until prefixed slaughtering age (21 months). Metabolic profile was measured at month 9 and 12 of experimental period. Meat quality assessment (*i.e.* chemical composition and collagen content, colour, drip and cooking loss, shear force on raw and cooked meat) was performed on 7-day aged *Longissimus thoraci*. ADG was analysed by a mixed model for repeated measures including the effects of diet, time and the interaction diet*time. The effect of diet on metabolic profile, carcass traits and meat quality were analysed by one-way ANOVA. Carcass score was compared by the Kruskal-Wallis test. Legume fed animals showed higher ($P<0.05$) ADG (960 *vs* 1230 g/d, SE 0.44), slaughtering weight (637 *vs* 746 kg, SE 22.4) and carcass weight (310 *vs* 395 kg, SE 22.8) than control animals. No differences were observed for metabolic profile, slaughtering traits and carcass scores. Meat quality was not affected by diet, except drip loss (0.9 *vs* 1.2; SE 0.06; $P<0.01$). Results suggest that use of legumes in diets for *Maremmana* growing bulls may be an advantageous choice for animal performance, without detrimental effect on meat quality.

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Relationships among diet, plasma and milk total polyphenol content in milking goats

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Some Mediterranean forages are interesting for their polyphenol content, which may perform beneficial functions to animal and nutraceutical effects on consumer. In ruminants, the fraction transferred into the milk may reflect the feeding regime and thus used as biomarker for the diet traceability. In order to highlight the relationships among polyphenols in feed intake, plasma and milk, total polyphenols were measured in the diets fed to goats and in animal plasma and milk. During March-May, twelve Girgentana goats, averaging 37.2 kg live weight, 86 days in milk and 2.0 kg milk yield were allocated to three groups and, according to a 3 x 3 Latin square design with three periods of 21 days (14 days for adaptation and 7 days for measuring and sampling), fed three diets in succession: sulla fresh forage (SFF), sulla fresh forage plus 800 g/d barley meal (SFB), mixed hay plus 800 g/d barley meal (MHB). During each period, offered and refused feed of each goat were weighed daily, sampled twice and disaggregated into botanical parts to estimate the mean amount and the quality of feed intake; the individual milk yield was recorded daily and samples of milk and blood were collected at the end of each period. During each period, means of total polyphenols intake (ItP) and total polyphenols in plasma (PtP) and milk (MtP) were used for Pearson correlation. Due to the polyphenols content of sulla forage, such as condensed tannins, the SFF and SFB diets increased ItP (9.21 and 9.29 *vs* 2.84 g GAE/d; $P<0.001$), PtP (304 and 309 *vs* 294 microg/mL GAE; $P<0.05$) and MtP (0.96 and 1.08 *vs* 0.8 g GAE/d; $P<0.05$). During the first period of experiment (March), a positive correlation between ItP and MtP was detected ($r=0.60$; $P=0.040$). This relationship might depend on a high percentage of leaf blade in the fresh forage ingested in this period compared to the others. Also, ItP and PtP content showed a positive correlation ($r=0.61$; $P=0.038$). Regarding all experimental periods, a positive correlation between plasma free polyphenol content and MtP content was found ($r=0.38$; $P=0.031$). Our results suggest that polyphenol content in animal diets is reflected in milk.

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Use of multivariate image analysis for the evaluation of total mixed rations in dairy cow feeding

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Multivariate Image Analysis (MIA) techniques are becoming very successful in food analysis, thanks to the wide diffusion of instrumentation for acquisition of digital images (*e.g.*, photo cameras, scanners, webcams) available at low costs. In this context, colourgrams (Ulrici *et al.,* 2012, Innov. Food Sci. Emerg. Technol. 6: 417-426) are signals that codify the colour-related information content of a digital image, and which can be elaborated by means of proper multivariate analysis techniques to extract those features that are useful to solve a specific problem. Colourgrams can be considered as an inexpensive way to obtain useful information about colour-related properties of inhomogeneous samples, such as the total mixed rations (TMR) used in dairy cow feeding, since all different colours of the image can be reproduced by one or more pixel (picture element). In this work, images of TMR samples both with and without silage (65 for dairy cows; 4, as a comparison, for steers) were acquired using a common flatbed scanner. Replicate images of the TMR samples were acquired to estimate the reproducibility of the method. Each one of the 168 images was converted into the corresponding colourgram, and the resulting dataset was used to calculate both classification models using Partial Least Squares-Discriminant Analysis (PLS-DA) and regression models using Partial Least Squares (PLS). Both the classification and the regression models were properly validated by cross-validation and using an external test set. Classification models showed excellent capability to discriminate between dairy and meat TMR and good capability to discriminate between with- and without-silage TMR. Regression models were calculated to predict 15 nutritional traits: crude protein, crude fat, crude fiber, ash, starch, NDF, ADF, ADL, dry matter, physical effectiveness factor (pef), physically effective NDF (peNDF), hemicellulose, non-fibrous extracts, non-NDF carbohydrates, and UFL. The best results were obtained using the images of without-silage TMR. In particular, for 9 nutritional traits we obtained satisfactory regression models, *i.e.* with R^2 values for the prediction of the external test set falling in the range between 0.70 and 0.88.

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The effects of maternal linseed supplementation on muscle fiber characteristics in suckling lambs

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This study investigated the effects of linseed supplementation to dairy ewes during late-pregnancy and early lactation on distribution of muscle fiber types in suckling lambs. From the last 8 weeks of gestation to the first 4 weeks of lactation, pregnant *Sarda* dairy ewes were fed two isoenergetic diets (1.45 Mcal/kg DM), a control diet (CTL) and an ALA-enriched diet by adding linseed (LIN), in a 2×2 factorial design. During gestation, ewes were divided into two groups of 12 animals: one fed CTL and the other LIN. After lambing, both CTL and LIN groups were divided into two subgroups of 6 animals fed CTL and LIN diets. Twenty-four suckling lambs were slaughtered at 28 d of age. Internal organs and brain were weighed, and the *Semitendinosus* and the *Longissimus dorsi* muscles were collected. Histological analyses were performed using myofibrillar ATPase staining at different pH to determine the type 1 (slow and oxidative; T1) and type 2 (fast and glycolitic; T2) fibers. Fiber type distribution was expressed as the percentage of fiber type out of the total fibers present in each muscle. Data were analyzed separately for each muscle with a model that included fixed effects of ewe gestation diet, ewe's lactation diet and their interaction. In *Semitendinosus* muscle of suckling lambs, LIN supplementation during gestation did not affect muscle fiber proportion, whereas LIN supplementation during lactation decreased the T2 proportion and the T2-to-T1 fiber ratio. In *Longissimus dorsi* muscle, the maternal treatments during gestation or lactation did not influence the proportion of fiber types. Overall, the results evidenced that the maternal linseed supplementation during lactation affected the distribution of fiber types, differently in the *Semitendinosus* and the *Longissimus dorsi* muscles. Acknowledgements: research supported by Cargill - Animal Nutrition Division, Milan, Italy.

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Blood parameters in fattening pigs fed whole ear corn silage

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The increases in corn grain prices and grain drying costs have stimulated the interest in silage corn products in pig feeding. The high moisture shelled corn is largely used in pig feeding as alternative feed to dry corn grain, whilst whole ear corn silage (WECS) is less used because it is considered too high in fiber despite it might increase the DM crop yield by 15-20%. Aim of this work was to evaluate the effects of the inclusion of WECS in diets for fattening heavy pigs in substitution of part of dry corn grain and wheat bran on the main blood parameters. Three experimental diets were fed to 30 barrows (initial average BW of 95 kg) divided into 3 groups: a control diet (C), containing cereal meals (corn, barley and wheat, 85% on DM), wheat bran (8% on DM), minerals and supplements and two diets containing WECS (15 or 30% on DM, 15ES and 30ES, respectively) in partial or complete substitution of wheat bran and corn meal. Diets had similar NDF contents (on average 15.5% DM); the CP content was: 14.0, 13.7 and 13.6% DM for C, 15ES and 30ES, respectively. The pigs were fed restricted: daily DMI ranged from 7.8 to 6.7% $BW^{0.75}$ from 95 to 143 kg BW. Blood was drawn from jugular vein, at 14d intervals, before feeding in the morning. Plasma metabolites related to energy and lipid (glucose, total cholesterol, triglycerides, NEFA, BHB), nitrogen (urea, creatinine, total protein, albumin), mineral metabolism (Ca, inorganic P, Mg, Na, K, Cl, Fe), enzyme activities (amylase, alkaline-phosphatase, tartrate-resistant acid-phosphatase, alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transferase) and total bilirubin, were analysed by an automatic analyser for biochemical chemistry. Data were statistically analysed by a mixed model, considering the repeated measurement in time for each animal and testing the orthogonal contrasts between control diet and WECS diets. The main result was the higher NEFA concentration in groups fed WECS, probably as a signal of an increased availability of intestinal digestible fibre.

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Effect of dietary inclusion of whole ear corn silage on stomach development and gastric mucosa integrity of heavy pigs at slaughter

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The effect of dietary inclusion of whole ear corn silage (WECS) on the stomach (S) development and the incidence of gastric lesions was studied in heavy pigs. Three experimental diets were fed to groups of 14 barrows from 90 to 170 kg of BW : a control diet (C), based on cereal meals, soy-bean meal and wheat bran and two diets containing WECS (15 or 30% DM, 15WECS and 30WECS, respectively) in partly or complete substitution of corn meal and wheat bran. Diets had similar fiber contents (NDF: 15.2-15.8% DM), but differed in terms of average size of feed particles, because the forage was chopped at harvesting and not milled before administration to the pigs. The S of each pig was removed at slaughter, opened along the greater curvature and the content collected. The DM of S contents was higher in pigs fed 30WECS than in control animals (331 *vs* 233 g, $P<0.05$), with animals receiving 15WECS at an intermediate level (276 g). In addition, pigs fed WECS diets showed a selective retention of more fibrous particles, as the NDF of S content (35-36% DM) resulted higher than that of C (23% DM). The dietary inclusion of silage caused ($P<0.05$) an increased weight of the stomach (0.38, 0.40 and 0.42% BW, for diets C, 15WECS and 30WECS, respectively, $P<0.05$), while there were no differences among diets in the S internal surface. Moreover, the surface of the pyloric region, which represents the gastric area responsible for bolus retention, was higher ($P<0.05$) in pigs fed 30WECS (20.4% of total S surface) than in animals receiving 15WECS and C (21.8 an 20.4%, respectively). The incidence of follicular gastritis and the overall gastritis score were significantly lower ($P<0.01$ and $P<0.05$, respectively) in pigs receiving WECS than in control animals. WECS diets influenced the development of stomach and maintained the gastric mucosa integrity with a lower incidence of gastritis in heavy pigs.

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In vitro evaluation of several additives on rumen methanogenesis

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The aim of this study was to evaluate *in vitro* the effects of some additives (essential oils, saponin, live yeasts and quebracho tannins) on gas and methane (CH_4) rumen productions. A standard total mixed ration for lactating cows (200 mg DM) was incubated *in vitro* with 30 mL of cow rumen inoculum for 24 h. Gas Production (GP) was determined using a digital manometer and CH_4 was determined through gas-cromatography. Each additive was tested in duplicate in three incubation runs. Essential oils were dissolved in ethanol. Additives were added at the following concentrations: essential oils= 500 mg/L inoculum; quebracho tannins= 1.0% DM incubated; live yeasts= 0.21 mg/L inoculum (corresponding approximately to an *in vivo* dose of 0.5 g head/d) and saponin (300 mg/L inoculum). There were differences among additives for GP: lemongrass, estragole, eugenol, geraniol, limonen, thyme oil and thymol decreased GP as compared to control with ethanol (33.9 *vs* 43.7 mL/200 mg DM; $P<0.05$). Methane production (mL/200 mg DM) was lower for cinnamic alcohol (10.1), guaiacol (9.8), lemon grass (9.2), thyme oil (10.5) and thymol (2.0) in comparison with control (12.1) ($P<0.05$). Methane proportion (CH_4 production/total GP) was lower ($P<0.05$) for cinnamic alcohol (0.23), guaiacol (0.23) and thymol (0.18) than control (0.28). On the contrary, geraniol significantly increased CH_4 proportion (0.33; $P<0.05$). Live yeasts increased both CH_4 production and proportion in comparison with a negative control without ethanol (13.0 *vs* 10.2 mL CH4/200 mg DM, and 0.30 *vs* 0.25 CH_4 production/total GP, respectively; $P<0.05$). In short time incubations some additives determined a lower methane production; however, for some essential oils the GP was negatively affected. Cinnamic alcohol and guiacol decreased CH_4 production without any negative effect on GP.

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Effect of free thymol on the differential gene expression in gastric mucosa of the young pig

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Thymol is the most representative molecule of thyme and is proposed as oral alternative to antibiotics. Knowledge on physiological effects of thymol on tissues is scarce, particularly for its impact on gastric mucosa, where it is largely absorbed when it is orally supplied. Thymol (TH, 0.5 g) or Placebo (CO) were inoculated only once in the stomach of eight weaned pigs, that were slaughtered after 12 hours and sampled for oxyntic and pyloric mucosa. On quality-tested mRNA, the analysis of whole transcript expression was done by Affymetrix©Porcine Gene 1.1ST array strips. Affymetrix Transcripts ID's were associated to 13,406 Human gene names, based on Sus scrofa Ensemble. Gene Set Enrichment Analysis was done using C5.BP catalog of gene sets (Molecular Signatures Database v3.1), comparing TH and CO. For each set, normalized enrichment score (NES) was defined significant when False Discovery Rate<25 and P-values of NES<0.05. With TH 72 and 19 gene sets were significantly enriched in oxyntic and pyloric mucosa. Several gene sets involved in mitosis and in its regulation ranked the top, primarily in the oxyntic mucosa; the set DIGESTION ranked first and ninth in pyloric and oxyntic mucosa, respectively. Inside this group, somatostatin (SST), SST receptors, peptide transporter 1 (SLC15A1), calpain 9 (a calpain specific for gastrointestinal tract) were the most affected genes. Thymol reduced the enrichment in 120 and 59 gene sets, for oxyntic and pyloric mucosa. Several gene sets related to ion transports and channeling, and aqueous pores across membranes, were less enriched. Among these, there were short transient receptor potential channel 4 (TRPC4), potassium voltage-gated channel members 1&2 (KCNA1&KCNA2) and ryanodine receptors 2&3 (RYR2&RYR3). Data seem conflicting with some physiological evidences about thymol activation of calcium effluxes and on some transient receptor potential proteins. Our observations could depend on the dose of thymol and on the contact with gastric tissues that caused an adaptive response with the reduced activation of genes sensitive to thymol. On the whole, these data are particularly useful as starting point for studies related to the functional impact of thymol on the gut activity.

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In vitro antinflammatory activity of plant extracts

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The chronic inflammatory condition may be involved in the pathogenesis of aging diseases such as obesity, atherosclerosis, arthritis/osteoporosis, cancer and cardiovascular disorders. Murine macrophages Raw 264.7 were pre-stimulated for 1h with H_2O_2 (200 µM) to mimic the oxidative stress condition, which is involved in aging and cellular stress mechanisms. The anti-inflammatory effect of five different plant extracts, *Camellia sinensis, Echinacea angustifolia, Eleutherococcus senticosus, Panax, Vaccinium myrtillus* was analyzed *in vitro*. Cells were preliminary treated with every compound at 1-10-100-200 µg/mL for 24h and cytotoxicity was measured with a cell viability assay, using the 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide reagent (MTT). All compounds showed a dose-dependent effect. In particular, cells showed a low toxicity with 60% of cell viability up to 100 µg/mL of all compounds, except for *C. sinensis* (49%) and *Panax* (54%). For bioactivity analysis, the least cytotoxic dose of 10 µg/mL was used for all the compounds. After 1h stimulation with 200 µM of H_2O_2, cells were incubated for 24 h with 10 µg/mL of the compounds and at the end of the incubation the RNA was extracted and the expression of COX2, Il1β, NFE2, Nfkb1, Nfkb2, Nos2, PPARγ and Tnfα genes was quantified using relative qRT-PCR. The qRT-PCR analysis *vs* H_2O_2 treated cells showed that Il1β was significantly down-regulated after exposure with *C. sinensis* and *V. myrtillus*. COX2, Nos2, Nfkb1/2 were significantly down-regulated by *Panax* and *V. myrtillus*. Only *C. sinensis* decreased significantly Tnfα. PPARy was significantly up-regulated by *C. sinensis* and *Panax*. All the plant extracts significantly up-regulated the expression of NFE2 gene. According to the results, anti-inflammatory activity of *C. sinensis, V. myrtillus* and *Panax* was observed, each one with specific features. This screening suggests that these compounds are potential candidates for further *in vivo* studies.

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In vitro and *in vivo* selection of bioactives enabling the stimulation of chicken microbiome

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The single *in ovo* injection of the lupin raffinose family oligosaccharides (RFO) leads to long term maintenance of a high level of intestinal Bifidobacteria and suggests that the application of antibiotics growth promoters to the chicken diet can be successfully replaced by injecting these compounds *in ovo*. It was hypothesized that combinations of prebiotic and probiotic (synbiotic) may exhibit a synergistic effect. Therefore, the aim of the present study was to evaluate the effect of *in ovo* administration of the selected *in vitro* combinations of prebiotic/probiotics (synbiotics) on hatchability and bacteriological status of chickens. Five lactic acid bacteria (LAB) strains were subjected to five trials in order to evaluate their (i) survival in presence of five prebiotic candidates, (ii) carbohydrate catabolic potential, (iii) enzymatic potential, (iv) global phenotype and (v) resistance to antibiotics used in animal medical treatments. The best two compositions of probiotic (Lactococcus lactis subsp. cremoris IBB477) and prebiotic (RFO or Inulin-S) to formulate the synbiotic preparations were selected for *in vivo* testing. Chicken embryos were treated with two prebiotics and two selected synbiotics. The basic dose (0.1763 mg/egg) and multiple amount (3x, 10x and 20x) of each bioactives, and physiological salt (Control) were injected *in ovo*. The hatchability of the eggs was not significantly affected by the treatment. LAB combined with both oligosaccharides RFO and Inulin S, injected *in ovo*, were able to survive and proliferate in the chicken gastrointestinal tract.

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Cryosurvival of turkey semen at different freezing rates

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Optimizing poultry cryopreservation involving freezing rate and the use of straws for semen packaging (better identification and safety of frozen semen) should therefore be a goal. The aim of this paper was to freeze the turkey semen in straws by exposure to liquid nitrogen vapor using different heights above the nitrogen vapor. Seven pools of semen (9-12 ejaculates/pool) were collected from Hybrid Large White toms, an aliquot from each pool was taken for the analysis of fresh semen, the remaining part of pooled semen was cooled at 5°C for 25 minutes. Each pool was diluted 1:1 (v:v) with the freezing medium composed by Tselutin extender containing DMA (final concentration of 8% DMA). Thus the semen diluted was aspirated into 0,25 mL plastic straws, equilibrated at 5°C for 20 min and then frozen by exposure to liquid nitrogen vapor for 10 minutes using three different heights above the liquid nitrogen surface: 1(-140°C), 5 (-120°C) and finally 10 cm (-90°C) respectively. The straws were plunged into liquid nitrogen for storage (-196°C). The samples were thawed at 50°C for 10 seconds. Sperm mobility (phase contrast microscopy), viability and osmotic-resistance (SyBr-PI staining) were examined on fresh and post-thawed spermatozoa. The results obtained showed that the cryopreservation deteriorated the post-thaw quality of turkey spermatozoa respect to fresh semen. However, the quality the frozen semen was affected from different freezing rates. Better results of motility and viability were obtained utilizing the height of 1 cm resulting significantly respect to the 5 cm. This research showed a better cryosurvival of frozen turkey sperm when DMA at concentration of 8% and the height of 1 cm above the nitrogen vapor were used. Further studies are needed to evaluate the effects between concentrations and type of cryoprotectant and different heights of freezing on quality of frozen turkey semen.

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The occurrence of white striping in chicken breast meat

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White striping defect is an emerging poultry quality issue which has been described as the appearance of white striation parallel to muscle fibres on surface of pectoralis major muscles. Currently, there are no published references concerning the occurrence of this defect under commercial conditions. The purpose of this study was to determine the incidence of white striping defect in commercial strains of meat-type chickens reared and processed under intensive conditions. A survey was conducted in a major commercial processing plant on 28,000 broiler breast fillets (*pectoralis major* muscles) which were collected at random from 56 flocks during a 4-mo period (from June to September 2012). Flocks consisted of meat-type chickens of both sexes belonging to some of the main commercial hybrids which were reared under intensive conditions and slaughtered from 45 to 54 d of age (average live weight: 2.75 kg). The evaluation of white striping occurrence (absence=normal, presence classified in 2 levels as moderate and severe) was performed on the processing line at 3 h post-mortem after the breast-deboning area. The total occurrence of white striped breast fillets was 12.0% (8.9 and 3.1% in moderate and severe degree, respectively). The range in the incidence of total white striping was fairly large and varied from 2.4 to 26.2% among flocks. Considerable variations were also observed for moderate (range: 2.4 to 18.6%) and severe (range: 0 to 8.8%) white striping degrees. When considering the influence exerted by genotype, high breast-yield hybrids had a higher overall incidence of white striping if compared with standard breast-yield strains (15.2 *vs* 10.0%; $P<0.001$). This result was due to the higher incidence of white striping for both degrees of moderate (3.9 *vs* 2.6%; $P<0.05$) and severe (11.3 *vs* 7.4%; $P<0.01$) in high breast-yield birds. In conclusion, this study revealed the importance of white striping defect in the commercial production of broiler breast meat. Moreover, it is most likely that ever-increasing genetic pressure to improve breast yield in broiler chickens is involved in the emergence of this defect.

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