

Italian Journal of Animal Science



ISSN: (Print) 1828-051X (Online) Journal homepage: http://www.tandfonline.com/loi/tjas20

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To cite this article: Massimo Todaro, Maria Luisa Scatassa & Pietro Giaccone (2005) Multivariate factor analysis of Girgentana goat milk composition, Italian Journal of Animal Science, 4:4, 403-410, DOI: 10.4081/ijas.2005.403

To link to this article: https://doi.org/10.4081/ijas.2005.403

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Multivariate factor analysis of Girgentana goat milk composition

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Paper received February 22, 2005; accepted May 15, 2005

ABSTRACT

The interpretation of the several variables that contribute to defining milk quality is difficult due to the high degree of correlation among them. In this case, one of the best methods of statistical processing is factor analysis, which belongs to the multivariate groups; for our study this particular statistical approach was employed.

A total of 1485 individual goat milk samples from 117 Girgentana goats, were collected fortnightly from January to July, and analysed for physical and chemical composition, and clotting properties. Milk pH and tritable acidity were within the normal range for fresh goat milk. Morning milk yield resulted 704 ± 323 g with $3.93 \pm 1.23\%$ and $3.48 \pm 0.38\%$ for fat and protein percentages, respectively. The milk urea content was 43.70 ± 8.28 mg/dl. The clotting ability of Girgentana milk was quite good, with a renneting time equal to 16.96 ± 3.08 minutes, a rate of curd formation of 2.01 ± 1.63 minutes and a curd firmness of 25.08 ± 7.67 millimetres.

Factor analysis was performed by applying axis orthogonal rotation (rotation type VARIMAX); the analysis grouped the milk components into three latent or common factors. The first, which explained 51.2% of the total covariance, was defined as "slow milks", because it was linked to r and pH. The second latent factor, which explained 36.2% of the total covariance, was defined as "milk yield", because it is positively correlated to the morning milk yield and to the urea content, whilst negatively correlated to the fat percentage. The third latent factor, which explained 12.6% of the total covariance, was defined as "curd firmness," because it is linked to protein percentage, a30 and titatrable acidity. With the aim of evaluating the influence of environmental effects (stage of kidding, parity and type of kidding), factor scores were analysed with the mixed linear model. Results showed significant effects of the season of kidding and parity on common factors, while no differences were found between goats with one or more kids. The multivariate factor analysis technique was effective in describing the quality of Girgentana milk with a low number of new latent variables. These new variables have been useful in the study of the effect of some technical factors such as parity and season of kidding on the quantitative and qualitative aspects of milk production in this goat breed.

Key Words: Girgentana goat, Milk composition, Multivariate analysis

RIASSUNTO ANALISI DELLA COMPOSIZIONE CHIMICA DEL LATTE DI CAPRA GIRGENTANA TRAMITE ANALISI MULTIVARIATA

I parametri che definiscono la qualità del latte sono diversi, spesso la loro interpretazione è resa più difficile per l'esistenza di alte correlazioni fra loro. In questi casi, i metodi di analisi statistica che meglio si prestano al trattamento dei dati, sono quelli appartenenti al gruppo dell'analisi multivariata. Fra questi, risultati interessanti vengono forniti dall'analisi dei fattori. Questo tipo di analisi statistica è stata applicata ad un data-set di 1485 campioni individuali di latte appartenenti

a 117 capre di razza Girgentana, che sono stati prelevati quindicinalmente per tutta la lattazione, da gennaio a luglio. I campioni di latte sono stati prelevati soltanto alla mungitura del mattino, rilevando anche la produzione di latte. Le analisi chimiche e fisiche hanno riguardato la determinazione di grasso, proteine, lattosio e cellule somatiche a mezzo Combi-Foss (Foss Italia), del pH tramite pH-metro portatile, dell'acidità titolabile per titolazione con soda N/4, dell'urea tramite pH-metria differenziale, delle proprietà elastometriche del latte (r, a30 e K20) tramite Formagraph (Foss Italia).

La produzione media rilevata al mattino è stata di 704 ± 323 g con un tenore in grasso e proteine rispettivamente del $3,93\pm1,23$ % e $3,48\pm0,38$ %. Il contenuto di urea nel latte è risultato piuttosto alto, pari a $43,70\pm8,28$ mg/dl. L'attitudine alla coagulazione del latte della capra Girgentana ha presentato dei buoni valori elastometrici ($r=16,96\pm3,08$ min; $K_{20}=2,01\pm1,63$ min; $a30=25,08\pm7,67$ mm). L'analisi dei fattori ha dato origine a tre nuove variabili, chiamati fattori latenti. Il primo fattore, definito come "latti lenti", ha spiegato il 51,2% della varianza totale, ad esso sono correlati positivamente r=pH; il secondo fattore, definito "produzione lattea", ha spiegato il 36,2% della varianza totale, ad esso sono risultati correlati positivamente la produzione di latte e l'urea, mentre sono risultati correlati negativamente la percentuale di grasso. Il terzo fattore, che ha spiegato il 12,6% della varianza totale, è stato definito "Fermezza del coagulo", ad esso sono risultati correlati positivamente la percentuale di proteina, la consistenza della cagliata e l'acidità titolabile del latte. L'analisi delle medie stimate degli score dei fattori latenti estratti ha permesso di valutare gli effetti di alcuni fattori ambientali, quali la stagione di parto, l'ordine di parto ed il tipo di parto. E' emerso come la stagione di parto abbia influenzato il fattore 2=3, l'ordine di parto abbia influenzato significativamente tutti e tre i fattori, mentre il tipo di parto non ha influenzato significativamente i fattori.

L'analisi multivariata si è dimostra quindi un approccio statistico efficacie nel descrivere la qualità del latte della razza Girgentana con un numero limitato di nuovi fattori latenti. Questi ultimi si sono dimostrati utili strumenti per lo studio dell'effetto di alcuni fattori tecnici come l'ordine e la stagione di parto sulla produzione quantitativa e qualitative di latte della razza Girgentana.

Parole chiave: Capra Girgentana, Composizione del latte, Analisi Multivariata.

Introduction

The Girgentana goat is an indigenous breed from the area around Agrigento in Sicily (Italy). The breed mostprobably originated in Afghanistan and $_{
m the}$ Himalaya (Portolano, 1987), its particular features being its characteristic, long, corkscrew horns and high milk yield, which, in the past, had helped the breed to spread. Girgentana goat numbers are now so greatly reduced (Giaccone et al., 1994; Portolano et al., 2004) that several research institutes have been proposing further studies aimed at safeguarding and valorising the breed. The Girgentana goat is particularly appreciated by breeders for the quality of its milk, which is often used for cheese making. One interesting aspect that emerges with regard to goat milk is its possible utilization in human and infant nutrition (Birkbeck, 1984; Haenlein, 1992; Pellerin, 2001).

The study of milk quality is a specific and basic factor in evaluating a goat breed (Jenness, 1980). The parameters that contribute to defining milk quality are several (Bencini and Pulina, 1997) and the correlations between them render their interpretation difficult. In this case, one of

the best methods of statistical processing is factor analysis, which belongs to the multivariate groups (Todaro *et al.*, 2001).

Multivariate factor analysis is suitable as a statistical method for reducing a complex system of correlations into one of smaller dimensions through the extraction of a few unobservable latent variables, called common factors, which are able to explain the complex (co)variance structure of the observed variables. A common factor is an unobservable, hypothetical variable that contributes to the variance of at least two of the observed variables. After the factors have been estimated, they must be interpreted, assigning them a name that reflects the importance of the factor in predicting each of the observed variables; this factor interpretation is a subjective process. Factor analysis also enables one to calculate the scores of common factors obtained and to consider them as quantitative measures (Macciotta et al., 2004). In this way, it is possible to evaluate the environmental effects (stage of kidding, parity and type of kidding) on common factors.

The purpose of this study was to evaluate the Girgentana goat milk composition employing this new multivariate approach.

Material and methods

The study was carried out on 117 Girgentana goats reared in a single flock in western Sicily. Does were reared under extensive husbandry conditions; concentrate was provided twice daily during machine milking. The 1,485 samples were collected fortnightly at morning milking, from 30 days after lambing to the end of lactation (from January to July); morning milk yield was also recorded. The average records per goat was 12.7; lactations with fewer than three test day records were discarded. Milk from the first 30 days of lactation was suckled by the kids. Each milk sample was divided into two parts, kept at 5°C without preservatives and brought to the laboratories for analysis of milk composition within 6 h. The pH of milk samples was checked at 20°C, 1 hour after their arrival at the laboratory, in the same way as for titatrable acidity (SH). Milk fat (FAT), protein (PRT), lactose (LAT) and somatic cells (SCC) were determined by Combi-Foss (Foss Electric). Employing a CL10 instrument the Urea content was determined using the pH-differential technique (Luzzana and Giardino, 1999). The renneting parameters, clotting time (r), rate of clot formation (K₂₀) and curd firmness after 30 minutes (a30), were determined using a Formagraph instrument (Foss Electric), adding 10 ml of fresh milk at 35°C, 400 µl of lamb rennet (Hansen) with the title 1:10,000, diluted to 8:103, in line with Zannoni and Annibaldi (1981).

Analysis of descriptive statistics, correlation coefficients and factors was carried out with SAS software, *vs* 8e, procedures MEAN, CORR and FACTOR. An axis orthogonal rotation (type VARIMAX) was applied to the factor analyses.

The scores for common factors for each milk sample were calculated as described by Macciotta *et al.* (2004). In order to evaluate relationships between the common factors and the environmental effects, factor scores were analysed with the following mixed linear model:

$$Y_{ijlmk} = \mu + SEA_i + PAR_j + TK_l + GOAT_m + \epsilon_{ijlmk} \label{eq:equation_equation}$$
 where:

 $Y_{ijlmk} = Factors scores;$

 $\dot{SEA}_i =$ fixed effect of the season of kidding (1 = Nov-Dec; 2 = Jan-Mar); $\begin{array}{ll} PAR_{j} = & \text{fixed effect of parity class } (1, 2, 3, \geq 4); \\ TK_{l} = & \text{fixed effect of the type of kidding } (1 = \text{single; } 2 = \text{twin}); \\ GOAT_{m} = & \text{random effect of the goat } (1.117); \end{array}$

 $\varepsilon_{ijlmk} =$ random residual.

Results and discussion

The descriptive statistics of the milk parameters are reported in Table 1.

Mean morning milk yield resulted 704 ± 323 g, with 3.93 ± 1.23 % and 3.48 ± 0.38 %, respectively, for fat and protein percentages. Milk somatic cells presented a mean value equal to 5.63 ± 0.55 logarithmic points, corresponding to 426,000 cells/ml, which resulted within the range prescribed by Italian law (DPR 54 of 14/01/1997).

The pH value was 6.59 ± 0.12 , in accordance with literature for fresh goat milk (Fantuz *et al.*, 2001; Morgan *et al.*, 2003; Bonanno *et al.*, 2004). Milk acidity was 3.36 ± 0.49 °SH/50 ml, in agreement with results reported by Scatassa *et al.* (2002), but lower than those reported by other authors (Morgan *et al.*, 2003; Bonanno *et al.*, 2004).

Mean milk urea content was 43.70 ± 8.28 mg/dl, higher than the mean produced by goats reared in Sicily (Scatassa *et al.*, 2002), probably because of widespread use of feeds (concentrate with 17% of PG) and pastures with high protein content, and probably with high rumen degradability (Cannas *et al.*, 1998).

The clotting ability of Girgentana milk presented a clotting time (r) equal to 16.96 ± 3.08 minutes and a curd firmness (a_{30}) of 25.0 ± 7.67 millimetres, in accordance with the results reported by Fantuz *et al.* (2001) and Scatassa *et al.* (2002).

The Pearson correlation coefficients between variables considered are shown in Table 2. Morning milk yield had negative correlations with SCC (-0.21; $P \le 0.001$), fat (-0.55; $P \le 0.001$) and protein (-0.32; $P \le 0.001$) percentages; similar values were found by Zeng *et al.* (1997) and Zumbo *et al.* (2004), but with smaller correlation coefficients. A significant and positive correlation was found between milk yield and urea content (0.38; $P \le 0.001$), probably due to the fact that greater milk production is obtained in February and March, when the availability of rich protein pastures is at a maximum (Giaccone *et al.*, 1995).

Table 1.	Descriptive statistics of yield, composition and clotting properties
	of Girgentana goat milk.

Milk components		Mean	SD	Maximum	Minimum
Milk	g	704	323	2271	126
SCC	Log	5.63	0.55	7.39	4.20
Fat	%	3.93	1.23	9.19	1.29
Protein	w	3.48	0.38	5.72	2.53
Lactose	W	4.55	0.30	5.70	1.87
pH		6.59	0.12	7.23	5.46
SH	°SH/50 ml	3.36	0.49	5.67	1.47
Urea	mg/dl	43.70	8.28	70.16	16.71
r	min	16.96	3.08	28.45	8.30
a30	mm	25.08	7.67	41.94	2.80
K ₂₀	min	2.01	1.63	12.15	0

SCC: Somatic Cell Count

Somatic cells were positively correlated with fat $(0.21; P \le 0.001)$ and protein $(0.08; P \le 0.01)$ percentages, in accordance with other authors (Delgado-Pertinez *et al.*, 2003; Sung *et al.*, 1999; Zeng *et al.*, 1997); this correlation could be due to the milk yield effect; in fact, negative correlations were found between milk yield and somatic cells (-0.21; $P \le 0.001$), between milk yield and fat percentage (-0.55; $P \le 0.001$) and between milk yield and protein percentage (-0.32; $P \le 0.001$).

The correlation between fat and protein percentages resulted 0.30 (P≤0.001), in accordance with international literature, which reported coefficients between 0.24 and 0.75 (Park, 1991; Zeng and Escobar, 1995; Zeng *et al.*, 1997; Sung *et al.*, 1999).

The clotting properties of the milk are defined by r (renneting time), K_{20} (rate of curd formation) and a30 (the consistency of the curd 30 min after the addition of rennet). These parameters, in particular r and a30, were significantly correlated with most milk constituents. Milk protein percentages resulted positively correlated with r (0.15; $P \le 0.001$) and a30 (0.47; $P \le 0.001$), showing that milks richer in protein coagulate more slowly, but give more resistant curds. Analogous results are reported by Zumbo *et al.*, (2004) for goat milk and by Bencini (2002) for ewe and cow milks.

Milk pH and acidity have a fundamental role in the coagulation process and, in particular, they influence the renneting time (r). The correlation between r and pH resulted 0.49 (P \leq 0.001); therefore, the more acid the milk the sooner it coagulates. Analogous results are reported by Zumbo *et al.*, (2004) for goat milk and by Bencini (2002) for ewe and cow milks.

Factor analysis split the total covariance into three latent factors (Table 3); the first factor explained 51.2%, the second factor explained 36.2%, while the third factor explained 12.6% of the total covariance. The Kaiser index, which measures the adequacy of data set for factor analysis, was 0.67, a little lower than the threshold value (0.80) indicated by some authors (Cerny *et al.*, 1977).

The first latent factor could be defined as "slow milks", because the high loadings (indicated with an asterisk in Table 3) are linked with pH and r variables. The name given to factor 1 implies that it is linked to qualitative characteristics, typical of milk samples that clot slowly. In fact it is positively correlated to pH (0.852) and to clotting time (0.562).

The second latent factor could be defined as "milk yield." In fact, this factor is positively correlated to the morning milk yield (0.737) and to the urea content (0.469) and negatively correlated to the fat percentage (-0.660). The positive correlation between the second latent factor and the urea content could be explained by a positive correlation between milk yield and urea content (Table 2).

Table 2.	Pearson	Pearson correlation	s among y	ield, compo	among yield, composition and clotting properties of Girgentana goat milk.	clotting pro	operties of	Girgentana	a goat milk		
	Milk	SCC	Fat	Protein	Lactose	Н	SH	Urea	L	a30	K ₂₀
Milk	н	-0.21***	-0.55***	-0.32***	0.25***	0.20**	-0.25***	0.38**	*90.0	-0.02	**80.0
SCC		H	0.21***	**80.0	-0.28***	-0.02	-0.03	-0.18**	-0.02	-0.15***	**80.0-
Fat			Н	0.30***	**80'0-	-0.15***	0.13***	***00'-0	-0.11***	-0.04	-0.07**
Protein				₽	0.13***	0.07**	0.37***	-0.05*	0.15***	0.47***	-0.07**
Lactose					H	0.21***	-0.13***	0.19***	0.10***	0.24***	-0.02
Hd						П	-0.47***	0.19***	0.49***	0.13***	*50'0
SH							П	-0.16***	-0.16***	0.21***	**60.0-
Urea								H	0.07**	0.12***	**60'0
<u>.</u>									H	-0.23***	-0.10***
a30										H	0.16***
K ₂₀											1
*P<0.05 **P<0.01 ***P<0.001	<0.01; ***P.	<0.001									

*P<0.05; **P<0.01; ***P<0.001

Table 3. Rotated F	Factor i	pattern.
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Variables	Factor 1 (Slow milks)		Factor 2 (Milk yield)		Factor 3 (Curd Firmness)		Communality
Milk	0.125		0.737	*	-0.124		0.574
SCC	-0.028		-0.380		-0.039		0.147
Fat	-0.047		-0.660	*	0.096		0.447
Protein	0.190		-0.303		0.934	*	0.999
Lactose	0.384		0.313		0.213		0.291
pH	0.852	*	0.194		0.004		0.764
SH	-0.438		-0.181		0.480	*	0.455
Urea	0.153		0.469	*	0.081		0.250
r	0.562	*	-0.039		0.051		0.320
a30	0.051		0.164		0.559	*	0.342
K ₂₀	0.006		0.177		-0.019		0.032
Variance explained	i 51.2		36.2		12.6		

^{*} high loading values.

The negative correlation between the second latent factor and milk fat percentages confirmed the appropriateness of the name attributed to the factor. In fact, the negative correlation between milk yield and fat percentage is shown above.

The third latent factor could be defined as "curd firmness" because this factor is linked to a30, SH and protein percentage, which give a nice firmness to the curd and, consequently, a good structure to the cheese. The higher correlation between this latent factor and milk protein percentage (0.934) confirms said that which has been stated above. On the other hand, the positive correlation between the third latent factor and SH variable (0.480) showed how the tritable acidity markedly influenced the cheese-making process (Todaro *et al.*, 2001). In fact, 2/5 of SH content is due to the casein and 3/5 to organic acids, such as phosphoric acid (Alais, 1984).

As for the communality (comparable to R^2), a low value was found only for the K20 variable (0.032), demonstrating that the rate of curd formation was not well explained by the three latent factors according to Todaro *et al.* (2001).

Results of mixed-model analysis carried out on

1, 2 and 3 Factors scores are reported in Table 4. Factor 1 was markedly affected by parity (P<0.001); in particular, significantly lower least square means scores for the multiparous goats (3 and ≥4) were observed. This confirms the fact that the milk produced by young goats is less suitable for cheese making because the clotting time is longer (Zumbo et al., 2004). Factor 2, defined as milk yield, was significantly influenced by season of kidding (P<0.001) and parity (P<0.001). The least square means score for season 2 was higher than that for season 1 (0.240 vs 0.008; $P \le 0.01$); this means that the goats kidding in winter (Season 2) produced more milk than goats kidding in autumn (Season 1), as reported in other papers (Giaccone et al., 1995). Moreover, the least square means scores of different parities presented an increasing course, meaning that first and second kidding goats produced less milk than multiparous goats, as widely reported in literature (Giaccone et al., 1995; Zeng and Escobar, 1995; Todaro et al., 1999; Vacca et al., 1999; Zumbo et al., 2004). Factor 3 was significantly influenced by season of kidding (P<0.001) and parity (P<0.001). The milk produced by goats kidding in season 2 resulted significantly more suitable for cheese making, and capable of producing sturdier and more resistant curds. Parity significantly influenced the least square means scores. The first kidding goats, in particular, presented significantly higher values, implying that their milk produces strong curds (Zumbo *et al.*, 2004). The type of kidding did not influence any of the three common factors.

Conclusions

The problems in defining factors that can describe and synthesise the relationships between all parameters defining milk quality could be resolved by Multivariate factor analysis. This statistical approach is a simple and effective method for obtaining the latent factors, F1, F2 and F3, related respectively to the slow milks, milk yield and curd firmness.

The results of the mixed linear model analysis highlight the relationships between F1, F2 and F3 and certain environmental factors, such as season of kidding, parity and type of kidding. Lastly, the results of the current study reveal the marked influence of season of kidding and parity on the principal traits of goat milk, while the type of kidding did not statistically influence the common factors.

Research funded by EU and Sicilian Regional Government (grant POP 1994/99).

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