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Factors affecting test-day milk composition in dairy ewes, and relationships amongst various milk components

By M. HOUCINE OTHMANE, J. ANTONIO CARRIEDO, L. FERNANDO DE LA FUENTE* and FERMÍN SAN PRIMITIVIO

Departamento de Producción Animal 1, Universidad de León, 24071 León, Spain

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Summary. A total of 7492 test-day observations for mean contents of fat, protein, casein, serum protein and lactose and individual laboratory cheese yield (ILCY) were obtained, at approximately monthly intervals, from 1119 ewes belonging to eight Churra dairy flocks. The effect of various factors on these variables was examined and phenotypic correlations among all traits were estimated. Least squares analyses showed significant effects of flock test-date, stage of lactation, age of ewe, and number of lambs weaned on almost all variables. Protein content and composition were not affected by the number of lambs weaned. ILCY had an unadjusted mean (26·55 kg cheese/100 l milk) close to those reported for real cheese yield in dairy ewes and was affected similarly to the main milk components. Fat, protein, casein, and serum protein contents, and ILCY, showed a generally increasing trend as lactation progressed. These components reached a minimum at 1 month into lactation, when milk yield was highest, and increased for the remainder of the lactation. ILCY depended mainly on fat, protein and casein contents. Protein and casein contents were closely related and equally correlated with ILCY. An increase in somatic cell count (SCC) was associated with decreased milk yield and decreased lactose content.

Keywords: Dairy ewes, individual cheese yield, test-day, milk components

Milk from sheep is widely used in the Mediterranean and Balkan regions for making the cheeses typically consumed in those countries. Therefore, milk composition is important to the cheese manufacturer, because it is a major factor determining the yield and quality of the final product. Some of these countries, where normal husbandry traditionally includes a lamb suckling period of approximately 1 month and a milking period that begins after lambs are weaned, have well-established milk recording schemes.

Milk composition traits and the factors affecting them are well documented in dairy cattle (Ng-Kwai-Hang *et al*. 1985; Bowland & Foegeding, 1999; Fenelon & Guinee, 1999). Several studies show the relationships between milk composition, somatic cell counts (SCC) and cheese yield (Baldwin *et al*. 1986; Banos & Shook, 1990; Barbano *et al*. 1991). However, except for milk fat and protein contents, little is known about factors affecting milk components and their interrelationships in dairy ewes. Milk fat and protein contents have traditionally received most attention.

* For correspondence; e-mail: dp1lfc@unileon.es

However, comprehensive information on factors influencing contents of casein, serum protein and lactose and SCC is limited, and no studies have examined individual cheese yield (ILCY). ILCY is a useful predictor of real cheese yield that can be recorded for each milk recording and which could be of interest in studying its relationship with the main milk components. Lactation curves for these parameters and the effect of such factors as flock nutrition and management, age, and number of lambs weaned are important for dairy ewe testing programmes (Gonzalo *et al*. 1994; Cappio-Borlino *et al*. 1997).

Our objectives were: (a) to study fat, protein, casein, serum protein and lactose contents, SCC, milk yield and ILCY from monthly test-day observations, to provide updated information about Churra dairy sheep; (b) to identify the main factors (flock, stage of lactation, age of ewe and number of lambs weaned) influencing those variables and (c) to estimate phenotypic correlations among all the variables studied.

MATERIALS AND METHODS

Data collection

From April 1997 to July 1999, a total of 7492 test-day observations for milk contents of fat, protein, casein, serum protein and lactose, and ILCY were obtained from 1119 ewes at approximately monthly intervals, following an alternate a.m.-p.m. recording scheme. Milk samples taken from eight Churra dairy flocks in the north of Spain were analysed. A maximum of four milk tests was carried out after weaning of lambs, which occurred about 25–30 d after birth. The mean number of test-days per lactation was 3·75 and each ewe averaged 1·8 lactations. Samples were prepared, within a few hours, for chemical analysis and cheesemaking in the laboratory.

Other pertinent information available, including date of milk sampling, number of lambs weaned, date of birth of ewe and lambing date, were taken from the data file of the National Association of Spanish Churra Breeders (ANCHE, Palencia, Spain). Data for milk yield and SCC were also supplied by ANCHE.

Milk analysis

On arrival at the laboratory, samples were analysed after warming to 40 °C and mixing. Contents of fat, protein, casein, serum protein and lactose were determined by the mid-infrared FTIR (Fourrier Transformed InfraRed) method using an AEGYS MI 200 spectrometer (ANADIS®, Trappes, France), which was calibrated against known standards. SCC was determined using the Fossomatic method as described by Gonzalo *et al*. (1993).

Cheese yield measurement

An ILCY measurement was determined for each test-day milk sample as described by Othmane (2000). All samples were previously equilibrated at 30 °C, a condition which is similar to that of a commercial cheese vat, before the addition of rennet. ILCY was measured as follows: 10 ml of individual, preheated and homogenized milk was curdled for 1 h at 37 °C. The rennet was diluted 10-fold with distilled water before being added to the milk. The coagulum (cottage cheese plus whey) was centrifuged for a standard time of 15 min at 2500 rpm after longitudinal cutting and removal of the whey by draining for 45 min. ILCY was defined as the net weight of the final residue after centrifugation, expressed as kg per 100 l milk.

Statistical analysis

For data analysis, SCC were transformed to their logarithmic form (Baro *et al.* 1994). To account for the effect of the test-day within flock, contemporary groups were formed on the basis of flock test date (FTD). There were 113 levels for FTD. Age of ewe (AE) consisted of four subclasses: 1·5 (between 1 and 2 years old), 2·5 (between 2 and 3 years), 3·5 (between 3 and 4 years), and 4·5 (> 4 years). Stages of lactation (SL), i.e. the intervals elapsing between lambing and sample collection, were classified into five subclasses of 1 month each with the first subclass beginning after the suckling period. There were two subclasses for lambs weaned (NL), single and multiple.

Data were analysed with a test-day model that included these environmental effects and a random residual effect (e) in the general linear model (GLM):

$$y_{ijklm} = \mu + FTD_i + SL_j + AE_k + NL_l + e_{ijklm}$$

Dependent variables (y) were fat, protein, casein, serum protein and lactose contents, SCC, milk yield and ILCY. To obtain residual correlations for these variables, the above model was used. The data were analysed by the Statistical Analysis System program SAS (1992) using GLM and Varcomp procedures.

RESULTS AND DISCUSSION

Means and standard deviations of the test-day variables are shown in Table 1. The laboratory assessment of potential cheese yield is a new variable and so there are no previous studies on dairy ewes or dairy cattle to compare it with. ILCY obtained from this study is within the range reported for real cheese yield in dairy ewes (Anifantakis & Kaminarides, 1983; Pirisi *et al.* 1999), although we are dealing with a different manufacturing process. ILCY, like that of cottage cheese, tends to be higher than industrial cheese yield, perhaps because of the very small amounts of milk used and the forced draining. In real manufacture, draining lasts about 12 h, depending on the type of cheese, whereas whey removal in the laboratory is accelerated by centrifugation.

Means for casein, serum protein and lactose contents were higher than those reported by Pellegrini *et al.* (1997) for the French Lacaune breed because Lacaune ewes produced more, but less rich milk. Mean SCC in the current study was close to recent values reported for Churra ewes (El-Saied *et al.* 1998*b*) and higher than means recorded for the Lacaune breed (Lagriffoul *et al.* 1993; Barillet *et al.* 1999). The means for fat and protein contents were within the range of estimates recorded for dairy ewes (IDF, 1981; Barillet & Boichard, 1987; Manfredini *et al.* 1992; Cappio-Borlino *et al.* 1997; Ploumi *et al.* 1998).

FTD, stage of lactation, and age of ewe contributed significantly ($P < 0·001$) to variations in all variables. The only exceptions were the non-significant effects of stage of lactation and the age of ewe on transformed SCC (lnSCC), which can be explained by the strict mastitis control measures (teat dip after milking, selective dry therapy and culling of ewes with chronic mastitis) and the general high level of husbandry applied to the flocks used in this study. Similar results were obtained by Fuertes *et al.* (1998) for a flock of hygienically improved ewes. The effect of number of lambs weaned was highly significant ($P < 0·001$) for ILCY, fat content, lnSCC and milk yield. However, it had no significant effect on the contents of protein, casein,

Table 1. *Arithmetic means, standard deviations and the statistical significance of the effects of various factors*[1] *on the test-day variables*

Trait	x̄	SD	*F* value FTD	SL	AE	NL
Milk yield, ml/d	956	548	11·8***	10·3***	0·4***	0·5***
Fat, g/l	71·2	19·7	15·6***	12·1***	0·5***	0·1***
Protein, g/l	59·8	8·2	18***	10·8***	0·3***	0^{NS}
Casein, g/l	47·6	7·3	20·5***	10·2***	0·3***	0^{NS}
Serum Protein, g/l	12·0	1·4	15·9***	7·8***	0·4***	0·1**
Lactose, g/l	42·5	6·1	20·8***	7·7***	0·2***	0^{NS}
ILCY[2], kg/100 l	26·5	5·7	39·2***	33***	0·2***	0·2***
LnSCC[3]	12·1	1·5	11***	0	0·1	0·3**

[1] FTD, Flock test date; SL, stage of lactation; AE, age of ewe; NL, number of lambs weaned.
[2] ILCY, individual laboratory cheese yield.
[3] SCC, somatic cell count.
$^{***}P < 0·001$, $^{**}P < 0·01$, $^{NS}P > 0·05$.

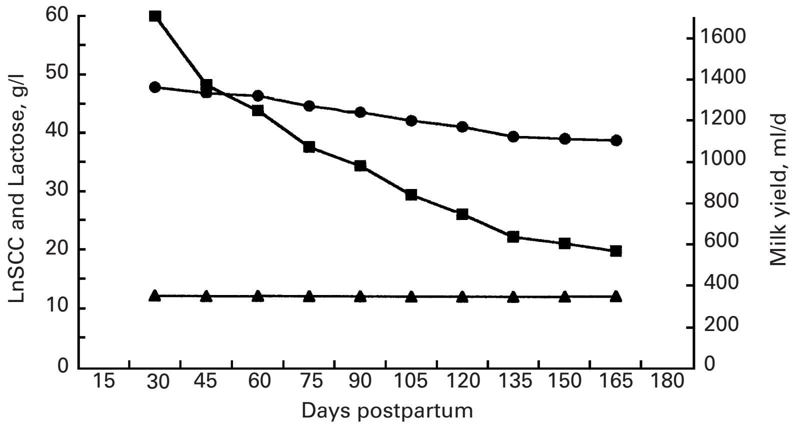

Fig. 1. Changes in milk yield (■), lactose content (●) and somatic cell count (▲) during lactation.

serum protein or lactose. Hence all sources of variation (FTD, stage of lactation, age of ewe, number of lambs weaned) contributed significantly to variations in ILCY.

The proportion of variance explained by FTD was 0·26 for test-day ILCY, 0·14 for fat content, 0·15 for protein content, 0·16 for casein content, 0·14 for serum protein content, 0·21 for lactose content, 0·09 for lnSCC, and 0·11 for milk yield. As expected, FTD becomes the major source of variation. In fact, all variables studied depend mainly on FTD and then on stage of lactation. These results demonstrate the importance of FTD as it is associated with the actual circumstances (feed, environment, management, etc.) of the flock on the day of testing. Similar results for the usual traits (milk yield, fat and protein contents) and SCC were reported for dairy ewes (Gonzalo *et al.* 1994; El-Saied *et al.* 1998*a*). There are no results for the other variables, perhaps because of the complexity and laboriousness of the analysis when dealing with a large number of qualitative traits. For dairy cattle, Ptak & Schaeffer (1993) observed that adjustment for herd test date (HTD) reduced the residual variance considerably, indicating the importance of taking into account effects specific to the day of test within each herd. It would therefore seem necessary to include FTD within the statistical models used for genetic evaluation carried out with test-day records.

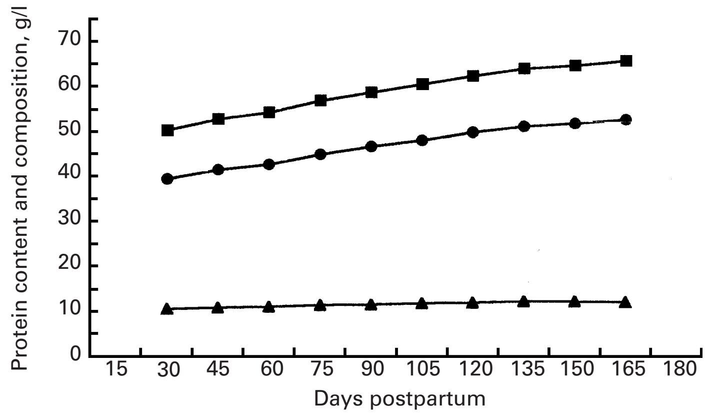

Fig. 2. Changes in the milk contents of total protein (■), casein (●) and serum protein (▲) during lactation.

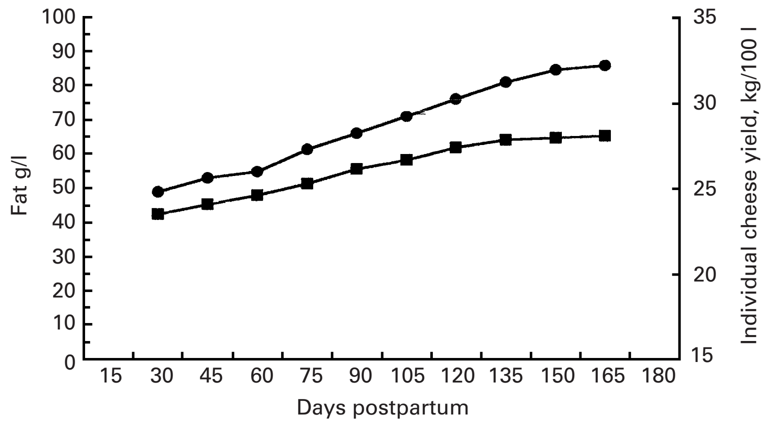

Fig. 3. The effect of stage of lactation on individual laboratory cheese yield (■) and the content of milk fat (●).

Lactation curves were configured by grouping monthly data into 2-week intervals, according to stage of lactation. The 15 least squares means of the variables studied over a milking period are shown in Figs 1–3. Because of the suckling period in dairy ewes, lactation curves for milk yield do not always show the typical pattern seen in dairy cows, which is characterized by an initial phase that increases to a maximum, followed by a decreasing phase. Thus, the lactation curve consists only of a decreasing phase. Milk yield reached its highest point at day in milk (DIM) 30, as did lactose content, which decreased as lactation progressed. However, lnSCC was not affected by the stage of lactation.

As expected, contents of fat, protein, casein and serum protein showed a pattern opposite to that seen for milk yield and lactose content; the lowest point of these curves (Figs 2 and 3) occurred at approximately the same period of peak milk yield. This was followed by a gradual increase in the four components during the latter part of lactation. Milk enrichment was higher for fat than for protein. The fat/protein ratio increased progressively from 0·97 to 1·30 between DIM 30 and DIM 150, which is noteworthy because this criterion is important for cheesemaking, especially for the quality of the final product.

Table 2. *Least squares means of test-day variables according to age of ewe*

	Age of ewe (years)			
	1·5	2·5	3·5	> 4
Milk yield, ml/d	1012[b]	1030[b]	1100[a]	1148[a]
Fat, g/l	62·6[c]	63·6[c]	65·6[b]	67·8[a]
Protein, g/l	56·3[c]	57·9[b]	58·7[a]	58·3[b]
Casein, g/l	44·6[c]	45·9[b]	46·6[a]	46·2[b]
Serum Protein, g/l	11·4[b]	11·8[a]	11·9[a]	11·8[a]
Lactose, g/l	44·4[a]	43·3[b]	43·7[b]	44·1[a]
ILCY[1], kg/100 l	24·1[b]	23·9[b]	25·5[a]	25·5[a]
LnSCC[2]	12·0	12·4	12·2	12·3

[a,b,c] Means in the same row with unlike superscripts differ ($P < 0·05$).
[1] ILCY, individual laboratory cheese yield.
[2] SCC, somatic cell count.

Protein content and composition (protein, casein and serum protein) were affected significantly ($P < 0·001$) by stage of lactation (Fig. 2). They changed in parallel throughout the milking period. This parallelism resulted in an unchanging casein number, which remained close to 80% during the same period. Up until now, as mentioned above, no studies have described the change in potential cheese yield during the course of lactation. Our results show that the lactation curve of ILCY was ascendant (Fig. 3). It went from 23·5 kg/100 l at DIM 30 to 28·1 at DIM 150. The increase in ILCY as lactation progressed (20%) was comparable to that indicated by the main milk components (fat, protein, and casein contents), which supports the hypothetical influence of these components on cheese yield. Furthermore, it is clear that the best ILCY is obtained in late lactation (DIM 150), when milk yield is lower but the milk is richer in fat and protein.

Variations in milk composition depend mainly on the stage of lactation. Lactation curves can also be used to obtain lactational measures of milk composition, adjusting test-day for stage of lactation (Fuertes *et al*. 1997; El-Saied *et al*. 1999). Such measures are also frequently used for SCC in breeding programmes (Wiggans & Shook, 1987; Schutz *et al*. 1990).

Age of ewe had a significant effect on all variables, except for lnSCC, as illustrated in Table 2. Milk yield and fat content increase with age. Total protein, casein and serum protein increase up to age 4 years then decrease slightly. ILCY increases up to age 4 years and then remains constant. The highest milk yield, fat content, and ILCY were obtained from the oldest ewes. The highest values for protein content and composition were for ewes aged from 3 to 4 years. In an experimental flock of the Churra breed, and including the suckling period, the oldest ewes (> 5 years) showed the highest fat and protein contents (Fuertes *et al*. 1998).

Table 3 shows the influence of the number of lambs weaned, which had a highly significant effect ($P < 0·001$) on test-day milk yield, SCC, fat and ILCY. This effect was not significant ($P > 0·05$) for total protein, casein, serum protein and lactose contents. Milk yield and SCC were higher after weaning for ewes that had multiple lambs than for ewes that had given birth to singletons. However, fat content was lower for ewes giving birth to multiple lambs: it decreased by 1·6, probably as a result of a dilution effect. Milks from ewes with single lambs showed higher cheese yield potential than those from ewes with more than one lamb. The difference between both categories reached 2% in favour of ewes with only one lamb. This difference probably derived from the richer milk in ewes that had only one lamb. LnSCC was

Table 3. *Least squares means of test-day variables according to type of birth*

	Type of birth	
	Single	Multiple
Milk yield, ml/d	1032[b]	1112[a]
Fat, g/l	65·7[a]	64·1[b]
Protein, g/l	57·8	57·9
Casein, g/l	45·8	45·9
Serum protein, g/l	11·7	11·8
Lactose, g/l	44·0	43·8
ILCY[1], kg/100 l	25·0[a]	24·5[b]
LnSCC[2]	12·1[b]	12·3[a]

[a,b] Means in the same row with unlike superscripts differ ($P < 0·05$).
[1] ILCY, individual laboratory cheese yield.
[2] SCC, somatic cell count.

Table 4. *Phenotypic (above the diagonal) and residual (below the diagonal) correlations between test-day variables*

Trait (i/j)[1]	1	2	3	4	5	6	7	8
1 Milk yield, ml/d		−0·43	−0·41	−0·39	−0·36	0·42	−0·20	−0·14
2 Fat, g/l	−0·20		0·62	0·62	0·40	−0·45	0·37	0·04
3 Protein, g/l	−0·24	0·39		0·99	0·71	−0·41	0·31	0·13
4 Casein, g/l	−0·23	0·41	0·99		0·62	−0·39	0·31	0·12
5 Serum protein, g/l	−0·21	0·13	0·70	0·60		−0·33	0·16	0·15
6 Lactose, g/l	0·13	−0·36	−0·29	−0·29	−0·24		−0·11	−0·12
7 ILCY[2], kg/100 l	−0·10	0·52	0·38	0·39	0·18	−0·08		0·04
8 LnSCC[3]	−0·16	0·01	0·09	0·08	0·12	−0·14	−0·01	

[1] i/j, trait i and trait j.
[2] ILCY, individual laboratory cheese yield.
[3] SCC, somatic cell count.

higher for multiple birth sheep in spite of their higher milk yield. Gross *et al.* (1978) found that the incidence of a positive California mastitis test increased with the number of lambs born and weaned in Targhee sheep. Therefore, suckling influenced later udder health during the milking period. These results agree with those of Gonzalo *et al.* (1994) in the same breed and Gabiña *et al.* (1993) for milk yield in the Latxa breed.

The phenotypic and residual correlations among the variables studied are shown in Table 4. Both correlations were similar so we refer to the residual correlation values, as these are adjusted for the environmental factors. Correlations were high and negative between milk yield and composition variables ($r = -0·16$ to $-0·24$), except for lactose content, which had a positive correlation with milk yield (0·13), as expected, and negative correlations with the rest of the milk composition traits ($-0·13$ to $-0·36$). The negative correlations between lactose content and protein content and composition ($-0·24$ to $-0·29$) corroborated those of Jenness (1985), who explained this effect by the synthesis of lactose in the Golgi vesicles regulating the amount of water secreted, so diluting the protein to its final concentration. High and positive correlations were also found between the contents of protein and fat (0·39) and casein and fat (0·41). Protein and casein contents were strongly related (0·99) and, accordingly, had similar correlations with the rest of the variables. Fuertes *et al.* (1998) also reported a high correlation between protein and casein (0·97) in the Churra breed.

The positive correlations between SCC and serum protein content (0·12) and between SCC and total protein content (0·09) could be explained, as a high SCC produces changes in the quantity and activity of proteolytic enzymes in milk (Andrews, 1983; Verdi *et al*. 1987). These proteases may degrade the casein, inducing the passage of soluble products of casein proteolysis to the liquid phase (serum). The lower, but positive, correlation between SCC and casein content follows from the correlation between total protein and SCC. The negative correlations between SCC and milk yield (−0·16) and between SCC and lactose content (−0·14) suggested that high SCC in milk, as a result of subclinical mastitis, is associated with low milk yield and low lactose content. Similar results have been found before for dairy ewes (El-Saied *et al*. 1998*b*; Fuertes *et al*. 1998), cows (Ng-Kwai-Hang *et al*. 1984) and goats (Upadhyaya & Rao, 1993).

Our study indicated that test-day laboratory cheese yield, regularly obtained at official milk recording, depends mainly on fat (0·52), casein (0·39) and total protein (0·38) contents (Table 4). Therefore, protein and casein contents were equally related to ILCY, a result that would follow from the strong correlation between both variables. However, ILCY was inversely related to milk yield ($r = -0{\cdot}10$). So, as expected, the lower the yield, and the richer the milk (in fat and protein), the higher the laboratory cheese yield.

In conclusion, flock test-day, stage of lactation, age of ewe and number of lambs weaned were important factors influencing milk yield, SCC, milk composition and ILCY. These factors would be considered as fixed effects in the statistical models used for breeding values and estimation of genetic parameters from test-day records. When lactation measures are used, test-day records would also be adjusted for stage of lactation because of the influence of this factor has on the variables considered. Lactation curves for fat, protein and casein contents and ILCY had a similar pattern of change throughout the milking period. The best ILCY was obtained in later lactation. The milk composition traits most closely correlated to ILCY were fat, total protein and casein contents. The phenotypic correlation between protein and casein contents was very high, close to unity, so both traits may be considered as very similar, with the analytical determination of casein content being more expensive. The negative correlation between milk yield and SCC, close to that reported previously for dairy ewes and cows, indicated that an increase in SCC involved a loss in milk yield.

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