Factors affecting ewe's milk fat and protein content and relationships between milk yield and milk components

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ABSTRACT: Test-day records of purebred Tsigai, Improved Valachian and Lacaune ewes were used to analyse the effect of environmental factors on milk fat and protein content. There were 121 424 and 121 158 measurements of fat and protein content for Tsigai, 247 742 and 247 606 measurements of fat and protein content for Improved Valachian and 2 194 measurements of fat and protein content for Lacaune ewes lambing between 1995 and 2005. Overall means and standard deviations for fat and protein content were 7.77 ± 1.606% and 5.94 ± 0.690% for Tsigai, 7.48 ± 1.446% and 5.82 ± 0.620% for Improved Valachian, and 6.97 ± 1.514% and 5.62 ± 0.692% for Lacaune. For fat content, analyses showed a highly significant ($P < 0.01$) effect of flock-test day and a highly significant ($P < 0.01$) or significant ($P < 0.05$) effect of the month of lambing, with the only exception of the month of lambing in Lacaune. The effect of litter size was highly significant ($P < 0.01$) or significant ($P < 0.05$) in Improved Valachian and Lacaune. For protein content, analyses showed a highly significant ($P < 0.01$) effect of flock-test day and a highly significant ($P < 0.01$) or significant ($P < 0.05$) effect of the month of lambing. The effect of litter size was highly significant ($P < 0.01$) in Tsigai and Improved Valachian. Covariates of days in milk which modelled the shape of lactation curves were insignificant, except for Improved Valachian fat content (Ali-Schaeffer regression adopted for sheep). The model explained about 50% of fat and protein variation in the breeds, with coefficients of determination between 0.517 and 0.587 for fat content and between 0.495 and 0.527 for protein content. Fat and protein content were almost equally correlated with milk yield in the three breeds. Lactation curves were constructed on the basis of solutions of a statistical model employed in the analyses.

Keywords: sheep; milk composition; environmental effects; correlations; lactation curves

Similarly like in Mediterranean and Balkan countries, ewe's milk is used for making the cheese in Slovakia. Although the milk composition is not considered a factor influencing the price for milk, it is an important factor determining its yield and quality of the final product. A milk recording scheme initialised in 1995, in addition to data on ewe's milk yield, includes also data on milk fat and protein content, so enabling to analyse ewe's populations with respect to milk composition traits. At present, investigations aimed at traits linked with technological aspects of milking, quality of production, functional longevity and animal welfare draw attention of sheep research in Slovakia (Milerski et al., 2006). However, only breeding values for milk yield based on test-day measurements have been considered a selection criterion until now.

Milk composition and factors affecting their variation throughout the lactation and relationships between milk yield and milk composition were studied in dairy cattle (Ng-Kwai-Hang et al., 1984; Schutz et al., 1990; Stanton et al., 1992),

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goats (Ciappesonni et al., 2004) and sheep (Cappio-Borlino et al., 1997; Othmane et al., 2002; Pulina et al., 2005). In Slovakia, analyses of milk quality traits were done, based on data of the individual flock level (Čapistrák et al., 1995, 2002; Špánik et al., 1996) and population level over the years 1995 and 1996 (Margetín et al., 1998). Taking into account the availability of complex information on ewe's milk and its composition across recorded flocks, selection for both milk yield and milk components may have an impact on the further improvement of Slovakian sheep populations, so that they will become capable to compete with breeds of more favourable milk traits. This is an important point mainly for breeds of local origin traditionally kept in Slovakia, i.e. Tsigai and Improved Valachian.

The aim of the present study was: (a) to study main factors which affect milk fat and protein content of Tsigai, Improved Valachian and Lacaune ewes, (b) to estimate breed phenotypic correlations between ewe's milk yield and fat and protein content, and (c) to fit milk composition lactation curves.

MATERIAL AND METHODS

Test-day records of ewe's milk performance testing (gathered by the State Breeding Institute of the Slovak Republic) which contained information on milk fat and protein content were used in the analyses. The AC method was used to take ewe's milk samples (ICAR, 2003). Fat and protein content were determined by routine laboratory procedures using the automated infrared method (STN 57 0536, 1995) and apparatus calibrated for ovine milk. There were 121 424 and 121 158 daily measurements of fat and protein content for Tsigai

Table 1. Structure of data in terms of the proportions of individual test-day measurements over months in lactation

Tsigai DIM	Proportion of test-days (%)			Lacaune DIM	Proportion of test-days (%)		
	1st lactation n = 49 491	2nd lactation n = 43 163	3rd lactation n = 28 320		1st lactation n = 1 192	2nd lactation n = 607	3rd lactation n = 395
1–30	0.4	0.1	0.1	1–30	0.6	0.8	1.0
31–60	3.2	2.5	2.7	31–60	7.2	2.7	2.3
61–90	18.9	19	18.7	61–90	22.1	22.2	20.7
91–120	23.4	23.7	23.4	91–120	24.4	24.1	24.2
120–150	22.9	23.1	23.0	120–150	24.5	23.6	22.7
150–180	21.1	21.5	21.8	150–180	18.1	22.2	22.7
180–210	9.2	9.3	9.5	180–210	2.2	3.5	6.0
Above 210	0.9	0.8	0.8	Above 210	0.9	0.9	0.4

Improved Valachian DIM	1st lactation n = 10 4516	2nd lactation n = 90 923	3rd lactation n = 52 303
1–30	0.4	0.3	0.3
31–60	6.2	6.1	5.9
61–90	22.2	22.6	22.2
91–120	23.8	23.8	23.6
120–150	23.4	23.6	23.5
150–180	18.7	19	19.1
180–210	4.8	4.3	4.9
Above 210	0.5	0.3	0.4

n = number of observations for fat content; DIM = days in milk; differences in the proportion of measurements for fat and protein content were negligible ewes, 247 742 and 247 606 daily measurements of fat and protein content for Improved Valachian ewes lambing between 1995 and 2005, and 2 194 daily measurements of fat and protein content for Lacaune ewes lambing between 1999 and 2005. The structure of data in terms of the proportion of individual test-day measurements over days in milk throughout the individual lactations, which was almost the same for both milk fat and protein content, is given in Table 1. For more details on the characteristics of data see Oravcová et al. (2006).

With the three breeds and both traits under study, the identical statistical model (general linear model), which included Ali-Schaeffer lactation sub-model adopted for sheep (Ali and Schaeffer, 1987), was considered (GLM procedure; SAS/STAT 9.1, 2002–2003):

$$y_{ijklm} = \mu + L_i + S_j + M_k + FTD_l + b_{1i}\left(\frac{\mathrm{DIM}_{ijklm}}{C}\right) + b_{2i}\left(\frac{\mathrm{DIM}_{ijklm}}{C}\right)^2 + b_{3i}\ln\left(\frac{C}{\mathrm{DIM}_{ijklm}}\right) + b_{4i}\ln^2\left(\frac{C}{\mathrm{DIM}_{ijklm}}\right) + e_{ijklm}$$

where:

y_{ijklm} = individual observation of fat or protein content (%)

μ = intercept

L_i = fixed effect of lactation number (i =1, 2 and 3)

S_j = fixed effect of litter size (j = 1 and 2+)

M_k = fixed effect of the month of lambing January, February and March (k = 1, 2 and 3)

FTD_l = fixed effect of flock-test day (l = 1 to 1 034 for Tsigai, 1 to 1 647 for Improved Valachian and 1 to 96 for Lacaune)

b_{1i}, b_{2i}, b_{3i}, b_{4i} = regression coefficients associated with *DIM* (days in milk nested within lactation)

C = constant associated with standardised length of milking period in Slovakian sheep (150 days)

e_{ijklm} = random error, $N(0, \sigma_e^2)$

DIM = days in milk

Breed lactation curves for fat and protein content were constructed on the basis of solutions of the statistical model. Pearson phenotypic and residual phenotypic correlations based on the above model were calculated (CORR procedure; SAS/STAT 9.1, 2002–2003) between fat and protein content. To calculate the correlations between milk yield and fat and protein content, data sets of individual daily milk yield measurements as referred to in Oravcová et al. (2006) were added to the analyses.

RESULTS AND DISCUSSION

The respective overall means of fat and protein content (Table 4) were 7.77 ± 1.606% and 5.94 ± 0.690% for Tsigai, 7.48 ± 1.446% and 5.82 ± 0.620% for Improved Valachian and 6.97 ± 1.514% and 5.62 ± 0.692% for Lacaune. Fat and protein contents for Improved Valachian and Tsigai were higher than the values given in Table 2 for various European breeds and reflect the fact that local Slovakian sheep breeds produce less milk of higher fat and protein content than most breeds reported in the literature (Ploumi et al., 1998; Ligda et al., 2002; Komprej et al., 2003; Hamman et al., 2004). A comparison with recent analyses (Oravcová et al., 2005) done for Tsigai and Improved Valachian showed the stability of fat and protein content (difference of 3 to 4% for fat content, hardly any difference for protein content), so reflecting the stability of milk yield (Oravcová et al., 2006). A comparison with analyses done earlier (Špánik et al., 1996 and Margetín et al., 1998) documented a decrease in fat and protein content (up to 15%), which is probably a result of selection aimed at an increase in milk yield before 2000. Fat and protein content for Lacaune kept in Slovakia was in the range of values reported for breeds of comparable milk yield (Valle del Belice, Sarda and Churra breeds; see Cappio-Borlino et al., 1997; Sanna et al., 1997; Fuertes et al., 1998).

Flock-test day, month of lambing and litter size contributed significantly ($P < 0.05$) or highly significantly ($P < 0.01$) to variation in both fat and protein content (Table 3). The only exceptions were effects of litter size for Tsigai fat and Lacaune protein content and month of lambing for Lacaune fat content. No significant influence of lactation number was found either for fat or for protein content ($P > 0.05$). No clear tendency of the significance of covariates modelling the shape of lactation curves over days in milk (Ali-Schaeffer regression adopted for sheep; Ali and Schaeffer, 1987) was proved. Only covariates associated with the ascending slope were significant for Improved Valachian fat content.

The highest part of variance of fat and protein content was explained by the flock-test day effect. The only exception was observed for Tsigai protein

Table 2. Literature review of fat and protein content in various sheep breeds

Source	Breed	Fat content (%)	Protein content (%)
Špánik et al. (1996)	Tsigai	8.72	5.97
	Improved Valachian	8.70	6.15
Margetín et al. (1998)	Tsigai	8.46	5.70
	Improved Valachian	8.15	5.73
Oravcová et al. (2005)	Tsigai	8.00	6.00
	Improved Valachian	7.70	5.80
Barro et al. (1994)	Churra	–	6.37
El-Saied et al. (1998)	Churra	–	5.62
Fuertes et al. (1998)	Churra	6.54	5.70
Komprej et al. (2003)	Istrian Pramenka	7.23	5.65
	Bovec	6.57	5.53
Hamann et al. (2004)	East Friesian	5.81	4.98
de la Fuente et al. (2006)	Assaf	6.82 and 7.20	5.50 and 5.43
Ligda et al. (2002)	Chios	6.60	5.80
Ploumi et al. (1998)	Chios	6.77	5.45
Cappio-Borlino et al. (1997)	Valle del Belice	6.90	5.18
Sanna et al. (1997)	Sarda	6.45	5.71

The (%)-unit is to be the equivalent to g/100g

content with about the same proportion of variance explained by the effects of flock-test day and litter size. These findings correspond with findings of Othmane et al. (2002) for Churra ewes and demonstrate the importance of flock-test day effect as it is associated with actual circumstances of the flock on the day of testing, i.e. feed, environment, management, etc. In contrast, the importance of flock-test day effect on daily milk yield in the breeds studied was less clear (Oravcová et al., 2006).

As given in Table 4, adjustments for considered effects diminished standard deviations of fat content from 1.606 to 1.008, from 1.446 to 1.009 and from 1.514 to 1.003% for Tsigai, Improved Valachian and Lacaune, respectively, and those of protein content from 0.690 to 0.477, from 0.620 to 0.442 and from 0.692 to 0.502% for Tsigai, Improved Valachian and Lacaune, respectively. The lower values for protein content correspond with the lower variation of protein which was twice lower than the variation of fat content. Coefficients of determination R^2s were almost identical in the three breeds: from 0.517 to 0.587 for fat content and from 0.495 to 0.527 for protein content. The values agreed with findings of Komprej et al. (2003) for Slovenian sheep, who reported R^2s from 0.415 to 0.545 for fat and protein content.

Least-squares means and standard errors, estimated within the effects of lactation number, litter size and month of lambing for fat and protein content according to breeds, are given in Tables 5 and 6. The pattern showing an increase or stability of fat and protein content in successive lactations corresponded with findings of Cappio-Borlino et al. (1997), Fuertes et al. (1998) and Othmane et al. (2002) for Valle del Belice and Churra ewes, although no significant differences between successive lactations were found in Slovakian ewes. The lower protein content in the earlier lactations was explained by Cappio-Borlino et al. (1997) by reduced rumen functionality, reduced synthesis efficiency of the mammary gland and preferential utilisation of available amino acids in the growing tissues of younger animals. The difference in milk composition was, however, so limited that, for practical purposes, these authors recommended milk composition to be considered the same for all lactations.

Table 3. General linear model for fat and protein content according to breeds

Effects	Tsigai		Improved Valachian		Lacaune	
	DF	MS	DF	MS	DF	MS
Fat content						
Lactation No. (L)	2	3.51	2	1.17	2	1.10
Litter size (S)	1	2.07	1	8.04^{++}	1	2.70^{+}
Month of lambing (M)	2	5.52^{+}	2	2.48^{+}	2	1.48
Flock-test day (FTD)	1 033	96.85^{++}	1 646	114.40^{++}	95	19.64^{++}
DIM/C	3	1.86	3	2.05+	3	1.32
$(DIM/C)^2$	3	2.77	3	1.89+	3	1.54
ln(C/DIM)	3	1.75	3	1.40	3	1.26
$\ln^2(C/DIM)$	3	1.56	3	0.99	3	0.94
Protein content						
Lactation No. (L)	2	0.03	2	0.15	2	0.03
Litter size (S)	1	13.13^{++}	1	5.43^{+}+	1	0.01
Month of lambing (M)	2	3.01^{++}	2	0.95^{++}	2	0.81+
Flock-test day (FTD)	1 033	11.37^{++}	1 646	13.56++	95	1.95^{++}
DIM/C	3	0.05	3	0.03	3	0.02
$(DIM/C)^2$	3	0.14	3	0.07	3	0.05
ln(C/DIM)	3	0.16	3	0.10	3	0.02
$\ln^2(C/DIM)$	3	0.40	3	0.15	3	0.04

DF = degrees of freedom; MS = mean squares; DIM = days in milk; C = const = 150; $^{+}P < 0.05$; $^{++}P < 0.01$ (Fisher's test)

Fat content was found lower in ewes giving birth to multiples, probably as a result of dilution effect (Othmane et al., 2002). In contrast, protein content was found lower in ewes giving birth to singles (Tsigai and Improved Valachian). In Lacaune no difference in protein content between the ewes lambing singles and multiples was found. The findings for both fat and protein content agree with

Table 4. General linear model for fat and protein content according to breeds – cont.

	Tsigai	Improved Valachian	Lacaune
Fat content			
R^2	0.528	0.517	0.589
RSD	1.108	1.009	1.003
Overall mean	7.77	7.48	6.97
SD	1.606	1.446	1.514
Protein content			
R^2	0.527	0.495	0.498
RSD	0.477	0.442	0.502
Overall mean	5.94	5.82	5.62
SD	0.690	0.620	0.692

R^2 = coefficient of determination; RSD = residual standard deviation; SD = standard deviation

Table 5. Least-squares means and standard errors for fat content according to breeds

Effect	Tsigai		Improved Valachian		Lacaune	
	n	$\mu \pm s_\eta$ (%)	*n*	$\mu \pm s_\eta$ (%)	*n*	$\mu \pm s_\eta$ (%)
Lactation						
First (1)	49 941	7.83 ± 0.007	104 516	7.58 ± 0.004	1 192	7.08 ± 0.040
Second (2)	43 163	7.81 ± 0.007	90 923	7.58 ± 0.004	607	7.05 ± 0.051
Third (3)	28 320	7.83 ± 0.008	52 303	7.59 ± 0.006	395	7.22 ± 0.063
Multiple-range tests		–		–		–
Litter size						
Single birth (1)	94 029	7.83 ± 0.006	20 0493	7.58 ± 0.003	1 540	7.16 ± 0.042
Multiple birth (2)	27 395	7.82 ± 0.008	47 249	7.57 ± 0.005	680	7.07 ± 0.044
Multiple-range tests		–		$1{:}2^{++}$		$1{:}2^{+}$
Month of lambing						
January (1)	37 546	7.84 ± 0.010	34 948	7.57 ± 0.009	361	7.02 ± 0.098
February (2)	73 425	7.82 ± 0.005	175 553	7.57 ± 0.003	1 273	7.17 ± 0.041
March (3)	10 453	7.84 ± 0.020	37 241	7.59 ± 0.009	562	7.16 ± 0.090
Multiple-range tests		$1{:}2^{+}$		$2{:}3^{+}$		–

$^{+}P < 0.05$; $^{++}P < 0.01$ (Scheffe's test); *n* = number of observations

findings of Cappio-Borlino et al. (1997), Fuertes et al. (1998); Othmane et al. (2002). It is need to stated, however, that milk composition tended to remain constant while milk yield tended to vary more in dependence on litter size (see Oravcová et al., 2006 for comparison).

Table 6. Least-squares means and standard errors for protein content according to breeds

Effect	Tsigai		Improved Valachian		Lacaune	
	n	$\mu \pm s_\eta$ (%)	*n*	$\mu \pm s_\eta$ (%)	*n*	$\mu \pm s_\eta$ (%)
Lactation						
First (1)	49 812	5.94 ± 0.003	10 449	5.83 ± 0.002	1 192	5.58 ± 0.020
Second (2)	43 073	5.94 ± 0.003	90 883	5.83 ± 0.002	607	5.63 ± 0.025
Third (3)	28 273	5.95 ± 0.005	52 274	5.83 ± 0.002	395	5.68 ± 0.040
Multiple-range tests		–		–		–
Litter size						
Single birth (1)	93 807	5.93 ± 0.003	20 0406	5.82 ± 0.002	1 540	5.63 ± 0.021
Multiple birth (2)	27 351	5.95 ± 0.004	47 200	5.84 ± 0.002	680	5.63 ± 0.022
Multiple-range tests		$1{:}2^{++}$		$1{:}2^{++}$		–
Month of lambing						
January (1)	37 433	5.94 ± 0.004	34 926	5.83 ± 0.004	361	5.53 ± 0.049
February (2)	73 302	5.92 ± 0.002	175 458	5.83 ± 0.002	1 273	5.64 ± 0.020
March (3)	10 423	5.95 ± 0.008	37 222	5.84 ± 0.004	562	5.72 ± 0.040
Multiple-range tests		$1{:}2^{+}$, $2{:}3^{+}$		$2{:}3^{+}$		$1{:}2^{+}$, $1{:}3^{+}$

$^{+}P < 0.05$; $^{++}P < 0.01$ (Scheffe's test); *n* = number of observations

Table 7. Phenotypic (above the diagonal) and residual phenotypic (below the diagonal) correlations according to breeds

	Milk yield	Fat content	Protein content
Tsigai			
Milk yield		–0.297	–0.313
Fat content	–0.092		+0.403
Protein content	–0.167	+0.342	
Improved Valachian			
Milk yield		–0.211	–0.169
Fat content	–0.083		+0.389
Protein content	–0.087	+0.331	
Lacaune			
Milk yield		–0.303	–0.370
Fat content	–0.161		+0.455
Protein content	–0.194	+0.381	

The three breeds showed a pattern opposite to that of milk yield (Oravcová et al., 2006) when the effect of the month of lambing on fat and protein content was investigated. The lower fat and protein content was found in ewes lambing in January and February compared to ewes lambing in March. However, the effects of the month of lambing, flock-test day and days in milk remained partially confounded because individuals giving birth in the same time are also measured in the same time (Cappio-Borlino et al., 1997; Ciappessoni et al., 2004).

The phenotypic and residual phenotypic correlations (adjusted for environmental effects included in the model) between milk yield and milk composition are shown in Table 7. The pattern shown is similar to that found for Churra ewes (Fuertes et al., 1998 and Othmane et al., 2002) and the remaining

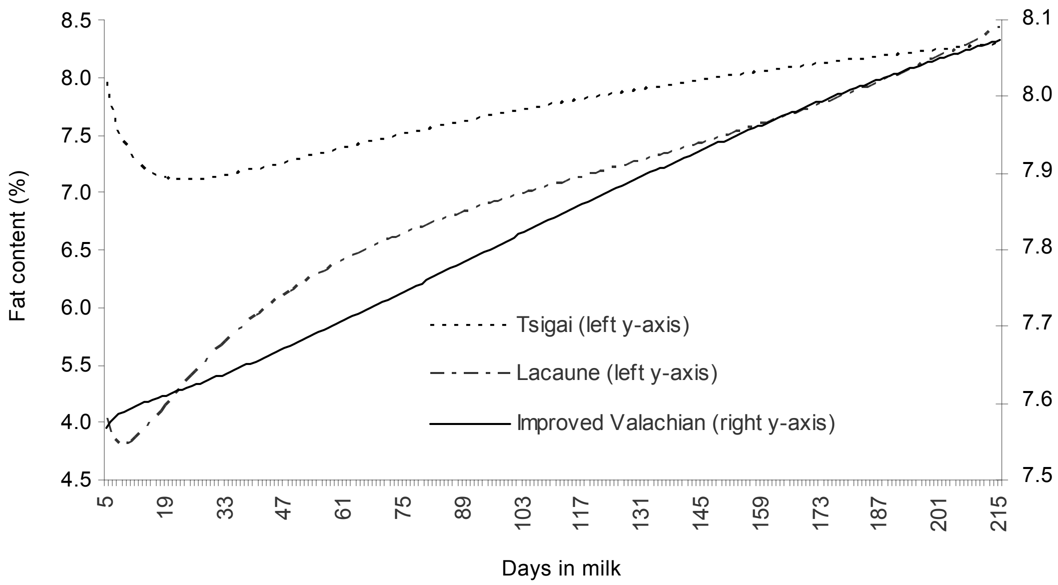

Figure 1. First lactation curves estimated for fat content (according to breeds)

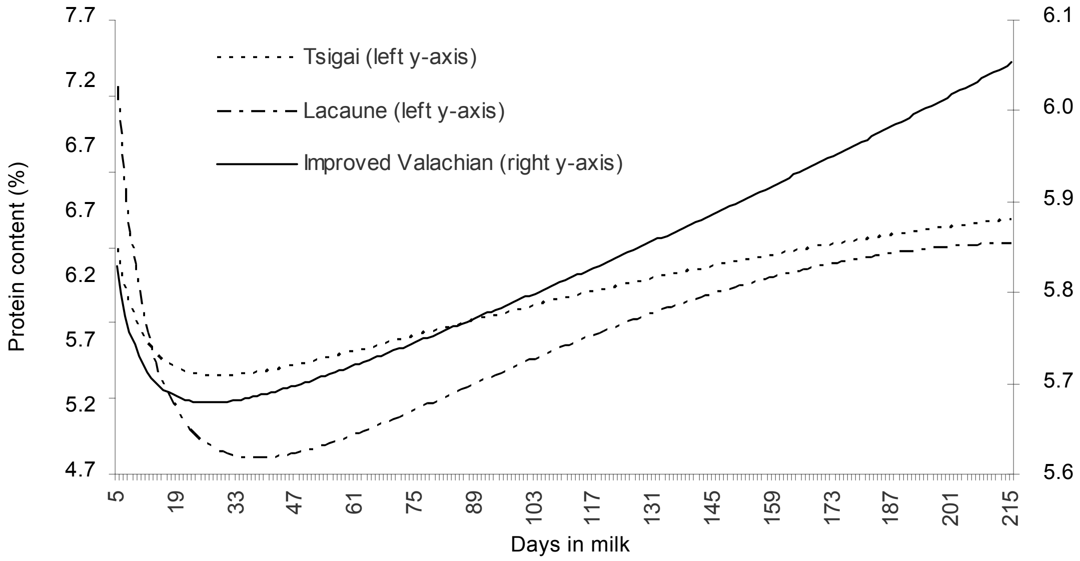

Figure 2. First lactation curves estimated for protein content (according to breeds)

livestock ungulates (Jenness, 1985). The correlations calculated for Tsigai and Improved Valachian were lower than the correlations reported for Churra, the correlations found for Lacaune were almost the same as the correlations reported for Churra by Othmane et al., 2002 (i.e. the residual correlations for Lacaune in comparison with Churra were –0.161 vs. –0.20; –0.194 vs. –0.24; and +0.381 vs. +0.39 between milk yield and fat content, milk yield and protein content, and fat and protein content). According to Fuertes et al. (1998), the residual correlations for Churra ewes were slightly higher: –0.27, –0.31 and +0.57, respectively.

The first lactation curves for fat and protein content estimated for Tsigai, Improved Valachian and Lacaune are given in Figures 1 and 2. The curves differed according to breeds and milk components. Protein content curves (Figure 2) showed higher stability, probably reflecting the lower variation of protein content throughout the lactation. The curves showed the properties of lactation curves observed for the protein content of dairy cows (Schutz et al., 1990 and Stanton et al., 1992) characterized by a descending phase in early lactation, i.e. after reaching the trough, followed by an ascending phase (the trough and peak of protein content and milk yield were reached at about the same day in milk), except for Tsigai, for which less correspondence in the lowest and highest point of protein content and milk yield was observed (see Oravcová et al., 2006 for comparison). Compared to protein content, higher variation in fat content throughout the lactation was observed (Figure 1). For Improved Valachian, only the ascending phase was observed whereas for Lacaune and Tsigai both the descending and ascending phase were observed. Smaller differences in lactation stages found in Improved Valachian could result from the fact that variation associated with days in milk was explained to a greater extent by the flock-test day effect in this breed. However, the dynamics of changes in fat content due to days in milk could progress similarly in the three breeds. There was only scarce information available for the comparison of fat and protein content lactation curves estimated for sheep breeds in the literature. Only the ascending phase was reported for fat content (Cappio-Borlino et al., 1997; Fuertes et al., 1998; Carta et al., 2001; Othmane et al., 2002 and Komprej et al., 2003) although the lactation curves were modelled from the early beginning or two and four weeks after parturition; and for protein content, either only the ascending phase (Carta et al., 2001; Othmane et al., 2002; Komprej et al., 2003) or both the descending and ascending phase were observed (Cappio-Borlino et al., 1997 and Fuertes et al., 1998). Further research and more data (mainly at the early lactation) are needed to assess whether the occurrence of different shapes of lactation curves is conditioned biologically or if it is a result of random variation.

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