

## Genetic Aspects of Persistency of Milk Yield in Boutsico Dairy Sheep

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**ABSTRACT :** Test-day records (n=13677) sampled from 896 ewes in 5-9 ( $\mu=7.5$ ) monthly test-days were used to estimate genetic and phenotypic parameters of test-day yields, lactation milk yield (TMY), length of the milking period (DAYS) and three measures of persistency of milk yield in Boutsico dairy sheep. The measures of persistency were the slope of the regression line ( $\beta$ ), the coefficient of variation (CV) of the test-day milk yields and the maximum to average daily milk yield ratio (MA). The estimates of variance components were obtained under a linear mixed model by restricted maximum likelihood. The heritability of test-day yields ranged from 0.15 to 0.24. DAYS were found to be heritable ( $h^2=0.11$ ). Heritability estimates of  $\beta$ , CV and MA were 0.15, 0.13, 0.10, respectively. Selection for maximum lactation yields is expected to result in prolonged milking periods, high rates of decline of yields after peak production, variable test-day yields and higher litter sizes. Selection for flatter lactation curves would reduce lactation yields, increase slightly the length of the milking period and decrease yield variation as well as litter size. The most accurate prediction of TMY was obtained with a linear regression model with the first five test-day records. (*Asian-Aust. J. Anim. Sci.* 2002. Vol 15, No. 3 : 315-320)

**Key Words :** Dairy Sheep, Milk Yield, Persistency, Genetic Parameters

### INTRODUCTION

Lactation length can be increased in two major ways: i) by shifting the whole curve upwards, i.e. enhanced yield and ii) by changing the shape of the curve to reduce the rate of decline in yield after peak lactation, i.e. enhanced persistency. The main result of selective breeding for many years has been the increase of yield but not of persistency (Knight, 1997). The shape of the lactation curve may be expressed using a measure of persistency and numerous such measures have been used in the literature. Sanders (1930) expressed persistency as the ratio between the total lactation yield and the maximum weekly milk yield. In dairy cattle, Johansson and Hanson (1940) introduced the most common measures, P2:1 and P3:1, i.e. the ratios between the milk yields of the second and the third 100 days of lactation respectively to that of the first 100 days. Later on, Fisher (1958) first fitted linear regressions and used regression coefficients as a measure of persistency of milk yield in dairy cows. Sölkner and Fuchs (1987) proposed a number of measures related to the variation of milk yield during lactation calculated as the standard deviation of test-day milk yields.

In dairy cows, a flat shaped lactation curve is favorable in the sense that the cows are easier to feed, are less troubled by a negative energy balance at peak production and it is possible to increase the proportion of roughage in their feed (Madsen, 1975). In dairy sheep, like the high producing Chios breed, ewes with the highest yields in the first test-days lost weight most quickly in the first phase of

lactation as a consequence of poor nutrition (Bizelis et al., 1993).

Changing the lactation curve in dairy sheep genetically would require selection for some of its characteristics. If the characteristics of the lactation curve are sufficiently heritable and closely correlated with milk yield, they could be used in a selection index for genetic gain of total yield as well as a desirable change of shape of the curve. Studies on the genetic basis of persistency in dairy sheep are, however, scarce in the literature.

The objectives of the present study were thus to estimate genetic parameters of test-day yields, lactation milk yield and three measures of persistency of milk yield in Boutsico dairy sheep.

### MATERIAL AND METHODS

#### Data

Data were available on the Boutsico dairy sheep through the Agricultural Research Station in Katsika Ioannina (western Greece). The data consisted of daily milk records of the first six lactations spanning years 1986 to 1995. Milk recording was applied on an A-type recording scheme (every 4 weeks recording of the two daily milkings). Ewes were lambing each year at the beginning of November and were weaned in-groups from the 10th to the 15th of December. First milk record was taken on the 5th to the 8th of January. Usually, 5 to 9 ( $\mu=7, 5$ ) samples per ewe and milking period were collected. Ewes were therefore on average on 24th, 52nd, 80th, 108th, 136th, 164th, 192nd, 220th and 248th day of the milking period when 9 successive test-days are considered. The initial data set included 15,195 test-day records. Records (n=1,518) were excluded using the following criteria: daily milk yield less than 50 g, unknown dam and inconsistent milk recording i.e.

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minimum of five successive test-day records existent. The final number of test-day records was 13,677 sampled from 896 ewes in 5-9 test-days. The number of sires and dams in the herd with progeny records was 151 and 589, respectively. Figure 1 shows the daily milk yield (average of the morning and evening milkings) of ewes sampled on 5-9 test-days respectively. As shown in figure 1, test-day milk yields were sampled after peak production, implying that a linear regression could sufficiently fit the daily milk yields at the phenotypic level.

### Measures of persistency of milk yield

In this study the three following measures of persistency were used:

$$\hat{\beta}_k = \frac{\sum_{i=1}^n x_{ik} y_{ik} - n \bar{x}_k \bar{y}_k}{\sum_{i=1}^n x_{ik}^2 - n(\bar{x}_k)^2} \quad (1)$$

where

$\hat{\beta}_k$  the slope of the regression line of the kth ewe ( $k=1, \dots, 1,827$ )

$y_{ik}$  the test-day milk yield of kth ewe on the  $x_{ik}$  day in milk ( $i=24, \dots, 248$ )

$\bar{y}_k$  the average test-day milk yield of the kth ewe

$\bar{x}_k$  the average days in milk of the kth ewe

$n_k$  the days in milk of the kth ewe

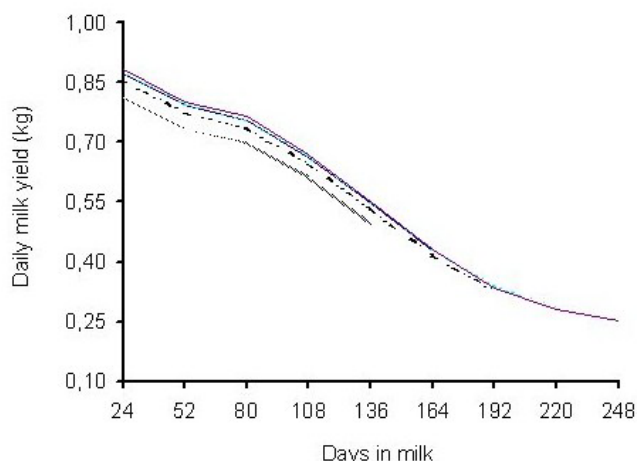
$\hat{\beta}_k$  thus expresses the rate of decline in milk yield after peak yield of the kth ewe

$$CV_k = \frac{\text{Standard deviation of test-day milk yield}}{\text{Average test-day milk yield}} \times 100 \quad (3)$$

$$MA_k = \frac{\text{Maximum test-day milk yield}}{\text{Average test-day milk yield}} \times 100 \quad (4)$$

$CV_k$  is a measure associated with variation of test-day yields while  $MA_k$  is a measure of the decrease of yield relative to the level of yield in early lactation of the kth ewe.

There were 104, 264, 275, 1,008 and 176 linear regression coefficients estimated on 5, 6, 7, 8 and 9 test-day yields. The average coefficient of determination of the fitted regression lines was  $0.72 \pm 0.01$  with a standard deviation of 0.20. For each lactation and ewe with estimated regression line, days in milk (DAYS) and lactation milk yield (TMY) were also available. TMY was calculated using the Fleischman method.



**Figure 1.** Daily milk yield (in kg) per test-day in Boutsico dairy sheep (lines represent average lactation curves on 5-9 test-days respectively)

### Estimation of the genetic parameters, univariate and bivariate analyses

Estimates of variance components were obtained under a general linear mixed model by restricted maximum likelihood using the average information algorithm (Gilmour et al., 1995) and sparse matrix techniques. Fixed effects fitted for test-day yields included year of lambing (1 to 10), lactation number (1 to 6), litter size (1 or 2), the year of lambing by lactation number interaction (1 to 60) and the age (in months) of ewe at lactation as a covariable. Random effects included the additive genetic effect of the animal, the permanent environmental effect and the residual term. All the available pedigree information was included in the analysis in order to minimize bias due to selection and to increase the accuracy of estimation of the parameters. Repeatability ( $r$ ) was calculated as the ratio of the sum of the variances of the additive genetic and the permanent environmental effects to the total phenotypic variance as follows

$$r = (\sigma_a^2 + \sigma_{pe}^2) / \sigma_p^2$$

Genetic as well as phenotypic correlations among the various traits ( $n=12$ ) were obtained by bivariate analyses. In the bivariate analyses, an animal model with animal's additive genetic effect as the only random effect was fitted. The number of records of animals as well as the pedigree information used in this analysis were as in the univariate analysis for each trait. The fixed effects part of the model was identical to that of the univariate analysis for each trait. Additional bivariate analyses were performed in an attempt to obtain correlations between TMY, DAYS, litter size (LS) and the persistency measures. All the calculations were carried out using the ASREML program (Gilmour et al., 1999). In all analyses, convergence was considered to have been reached when the variance of the function values was

**Table 1.** Means ( $\mu$ ) with standard errors ( $s_{\mu}$ ) of test-day milk yields, total milk yield (TMY), days in milk (DAYS) and the measures of persistency

Trait	n	$\mu \pm s_{\mu}$
MY1 (kg)	1,827	0.879 $\pm$ 0.007
MY2 (kg)	1,827	0.797 $\pm$ 0.006
MY3 (kg)	1,827	0.757 $\pm$ 0.006
MY4 (kg)	1,827	0.666 $\pm$ 0.006
MY5 (kg)	1,827	0.549 $\pm$ 0.005
MY6 (kg)	1,723	0.430 $\pm$ 0.005
MY7 (kg)	1,459	0.340 $\pm$ 0.004
MY8 (kg)	1,184	0.288 $\pm$ 0.004
MY9 (kg)	176	0.253 $\pm$ 0.009
TMY (kg)	1,827	138.0 $\pm$ 1.0
LS	1,827	1.20 $\pm$ 0.01
DAYS	1,827	218.4 $\pm$ 0.7
$\beta$ (kg/day)	1,827	-34.3 $\times 10^{-4} \pm 4$
CV (%)	1,827	43.2 $\pm$ 0.3
MA (%)	1,827	159.9 $\pm$ 0.6

MY1-MY9 = Milk yield at the *i*th test-day (*i*=1,...,9).

LS=litter size.

$\beta$ =the slope of the regression line (see text for details).

CV=coefficient of variation of test milk yields.

MA=maximum to average test-day milk yield.

less than  $10^{-8}$ . Furthermore, for each analysis, a restart was performed in an attempt to ensure that a global than a local maximum had been reached.

## RESULTS

### Descriptive statistics

The means of the test-day yields, DAYS, TMY and the measures of persistency are shown in table 1. There was a progressive decline in daily milk yield from 0.88 kg to 0.25 kg at first and last test-day respectively. Average TMY and DAYS were 138.0 kg and 218 days respectively.

Averages of the various measures of persistency were 3.43 g/day, 43.2 and 159.9, for  $\beta$ , CV and MA respectively.

Table 2 presents the least squares means of  $\beta$ , CV and MA per lactation, litter size and production level. By definition of the measurements, high absolute values stand for poor persistency. Two production levels were considered (1 and 2) each one representing one phenotypic standard deviation ( $\pm$ SD) above and below the average lactation milk yield respectively. Ewes at first lactation, compared to later lactations, exhibited lower rates of decline of milk yield after peak production. Furthermore, ewes at first lactation, produced less variable yields, i.e. smaller values of CV with lower peak to average yields. No statistically significant effect of the lactation number on the measures of persistency was detected beyond the first lactation. The litter size had a clear effect on the various measures of persistency resulting in absolute higher values for  $\beta$ , CV and MA in twin litters. Furthermore, the various measures of persistency clearly discriminated between the two levels of milk yield. High producing ewes clearly exhibited higher rates of decline of milk yield after peak production, produced less variable yields, i.e. lower CV and had lower peak to average yields (MA).

### Heritabilities

Estimated variance components as well as heritabilities and repeatabilities of the test-day milk yields, TMY, DAYS and the various persistency measures are presented in table 3. Heritabilities of test-day yields ranged from 0.15 to 0.24 from the first to the 8th-test-day, with the highest value at the 5th test-day ( $h^2=0.24$ ). DAYS was lowly heritable ( $h^2=0.11$ ) as were all measures of persistency. Heritabilities of  $\beta$ , CV and MA were 0.15, 0.13 and 0.10 respectively.

### Correlations

Genetic as well as phenotypic correlations of the various test-day yields and the measures of persistency are

**Table 2.** Least squares means of linear regression coefficients ( $\beta \times 10^{-4}$ ), coefficient of variation (CV) and ratio of maximum to average daily milk yield (MA) per lactation, litter size and production level (1 and 2: one phenotypic standard deviation above and below the average milk yield respectively)

	Lactation number					
	1 (n=517)	2 (n=458)	3 (n=331)	4 (n=253)	5 (n=184)	6 (n=96)
$\beta$ (kg/day)	-32.5 <sup>a</sup> $\pm$ 7	-37.1 <sup>b</sup> $\pm$ 7	-37.8 <sup>b</sup> $\pm$ 7	-37.9 <sup>b</sup> $\pm$ 7	-37.7 <sup>b</sup> $\pm$ 10	-36.0 <sup>b</sup> $\pm$ 14
CV (%)	39.8 <sup>a</sup> $\pm$ 0.7	43.9 <sup>b</sup> $\pm$ 0.7	45.1 <sup>b</sup> $\pm$ 0.7	44.6 <sup>b</sup> $\pm$ 0.8	45.9 <sup>b</sup> $\pm$ 0.9	45.0 <sup>b</sup> $\pm$ 1.3
MA (%)	153.2 <sup>a</sup> $\pm$ 1.3	160.4 <sup>b</sup> $\pm$ 1.2	162.0 <sup>b</sup> $\pm$ 1.4	162.7 <sup>b</sup> $\pm$ 1.6	163.8 <sup>b</sup> $\pm$ 1.8	163.3 <sup>b</sup> $\pm$ 2.5
	Litter size		Production level			
	1 (n=1465)	2 (n=374)	1 (n=298)	2 (n=308)		
$\beta$ (kg/day)	-35.3 <sup>a</sup> $\pm$ 4	-37.9 <sup>b</sup> $\pm$ 7	-44.6 <sup>a</sup> $\pm$ 7	-29.6 <sup>b</sup> $\pm$ 7		
CV (%)	43.1 <sup>a</sup> $\pm$ 0.4	45.06 <sup>b</sup> $\pm$ 0.7	40.8 <sup>a</sup> $\pm$ 0.7	46.6 <sup>b</sup> $\pm$ 0.8		
MA (%)	159.2 <sup>a</sup> $\pm$ 0.8	162.6 <sup>b</sup> $\pm$ 1.3	155.3 <sup>a</sup> $\pm$ 1.5	164.0 <sup>b</sup> $\pm$ 1.5		

<sup>a,b</sup> Means with different letters as superscripts are statistically different ( $p \leq 0.05$ ).

**Table 3.** Variance components, heritabilities and repeatabilities of test day milk yields, total milk yield (TMY), days in milk (DAYS), and the measures of persistency

Trait	$\sigma_a^2$	$\sigma_{pe}^2$	$\sigma_e^2$	$\sigma_p^2$	$h^2$	$r$
MY1 (kg)	0.0097	0.0044	0.0427	0.0567	0.17±0.05	0.25±0.03
MY2 (kg)	0.0094	0.0088	0.0357	0.0539	0.17±0.05	0.34±0.03
MY3 (kg)	0.0120	0.0043	0.0375	0.0538	0.22±0.05	0.30±0.03
MY4 (kg)	0.0089	0.0084	0.0287	0.0460	0.19±0.05	0.37±0.03
MY5 (kg)	0.0094	0.0068	0.0285	0.0387	0.24±0.05	0.42±0.03
MY6 (kg)	0.0055	0.0061	0.0188	0.0304	0.18±0.05	0.38±0.03
MY8 (kg)	0.0024	0.0069	0.0133	0.0165	0.15±0.06	0.19±0.04
TMY (kg)	337.34	329.32	569.29	1263.0	0.27±0.06	0.53±0.04
DAYS	70.81	90.29	470.09	631.2	0.11±0.05	0.26±0.04
$\beta$ (kg/day)	$0.3112 \times 10^{-6}$	$0.2002 \times 10^{-6}$	$1.5586 \times 10^{-6}$	$2.0631 \times 10^{-6}$	0.15±0.05	0.25±0.03
CV (%)	19.03	17.82	113.82	150.7	0.13±0.05	0.25±0.03
MA (%)	57.58	51.17	436.56	545.3	0.10±0.04	0.20±0.03

$\sigma_a^2$  : additive variance,  $\sigma_{pe}^2$  : animal's permanent environmental variance,  $\sigma_e^2$  : residual variance,  $\sigma_p^2$  : phenotypic variance,  $h^2$  : heritability;  $r$  : repeatability

**Table 4.** Genetic (below the diagonal) and phenotypic (above the diagonal) correlations ( $\times 100$ ) of test day yields, total milk yield (TMY), days in milk (DAYS) and the measures of persistency

Trait	1	2	3	4	5	6	7	8	9	10	11	12
MY1		64 <sup>1</sup>	55	48	41	33	20	65	02	-73	27	30
MY2	99 <sup>2</sup>		64	60	52	42	29	73	02	-63	12	-01
MY3	91	97		68	59	48	32	78	10	-45	-02	-13
MY4	79	90	98		74	60	49	80	23	-21	-20	-26
MY5	67	82	92	97		69	39	80	26	-04	-34	-32
MY6	57	72	82	86	93		49	72	34	10	-52	-40
MY8	45	70	74	86	97	75		50	-04	33	-71	-47
TMY	88	95	90	98	95	89	90		42	-31	42	-15
DAYS	39	40	69	59	99	77	69	66		22	-04	08
$\beta$	-77	-72	-57	-35	-12	06	07	-40	19		-65	44
CV	16	-01	-16	-38	-59	-68	-78	66	-48	-67		83
MA	10	-10	-23	-40	-56	-58	-72	-27	-22	46	90	

<sup>1</sup> Standard errors of genetic correlations in the magnitude of 0.08-0.12.

<sup>2</sup> Standard errors of phenotypic correlations in the magnitude of 0.04-0.06.

presented in table 4. Genetic correlations between test-day yields were generally high, ranging from 0.45 to 0.99 and declined as the interval between yields increased. Test-day yields were genetically highly correlated to TMY with genetic correlations in the range of 0.88 (with MY1) to 0.98 (with MY4). A positive, relatively high genetic correlation between TMY and DAYS was also found ( $r_G=0.66$ ). Genetic correlations between  $\beta$  and the first two test-day yields (MY1 and MY2) were found to be negative and high ( $r_G=-0.77$  and  $-0.72$ , respectively). This correlation, however, decreased gradually from  $-0.57$  (MY3) to  $-0.12$  (MY5) and changed to be slightly positive with MY6 and MY8. As an overall result, the genetic correlation between

$\beta$  and TMY was  $-0.40$ . The genetic correlations between CV and MA with test-day yields were positive with MY1 but negative with the successive test-day yields. The overall genetic correlation between CV and TMY was 0.66 and the correlation between MA and TMY was  $-0.27$ . Furthermore, CV and MA showed a negative correlation with DAYS.  $\beta$  was negatively correlated with CV ( $r_G=-0.67$ ) and positively correlated with MA ( $r_G=0.46$ ). CV and MA were highly positively correlated ( $r_G=0.90$ ). The estimates of phenotypic correlations were generally lower than the respective genetic correlations estimates.

The genetic, phenotypic and environmental correlations between TMY, DAYS and the various measures of

**Table 5.** The genetic ( $r_G$ ), phenotypic ( $r_P$ ) and environmental ( $r_E$ ) correlations of total milk yield (TMY) and persistency traits with litter size

	$r_G$	$r_P$	$r_E$
TMY (kg)	0.11±0.09	0.05±0.03	0.03±0.03
DAYS	0	0	0
$\beta$ (kg/day)	-0.19±0.10	-0.08±0.03	-0.05±0.03
CV (%)	0.06±0.11	0.05±0.04	0.04±0.04
MA (%)	0.04±0.11	0.05±0.04	0.05±0.04

persistency with LS are presented in table 5. Genetic correlations between TMY, DAYS,  $\beta$ , CV and MA with LS were 0.11, 0, -0.19, 0.06 and 0.04, respectively.

## DISCUSSION

### Environmental effects

First lactations are more persistent and their peak to average yields lower in comparison to later lactations; this has also been repeatedly found in other studies conducted on dairy cattle (Leukkunen, 1985; Sölkner and Fuchs, 1987). The most conceivable physiological reason for this is that the mammary gland of the ewe is not yet fully developed at the beginning of the first lactation. Besides the number of lactation, litter size was also found to considerably influence persistency, i.e. increasing rate of decline of milk yield after peak yield, the variation of milk yields and the maximum to average ratio. Such a finding was also reported for the Improved Valachian (Texel×Valachian) breed (Louda and Doney, 1976) and it may be associated with increased mammary growth of ewes carrying more than a single lamb. Bizelis et al. (1993) have reported statistically significant correlations between the number of foetuses and mammary growth during late pregnancy in Chios ewes.

### Genetic parameters

The heritability estimates of the measures of persistency in the present study are in the range of those reported for dairy cattle. Madsen (1975) found relatively high heritabilities of 0.40, 0.59, 0.47 and 0.49 for  $\beta$ , P2:1, P3:1 and the ratio of total yield in 305 days after parturition on maximum daily yield, respectively. Gravert and Baptist (1976) estimated heritability of 0.18 for the regression coefficient of daily yield on days in milk. Leukkunen (1985) reported heritabilities in the range of 0.11-0.21 for the P2:1, MA and the  $\beta$  regression coefficient of the Wood's lactation curve. The heritability estimates of P2:1, P3:1 and MA were 0.14, 0.19 and 0.21, respectively, when 305 day first lactations were considered (Sölkner and Fuchs, 1987). In Sfakia dairy sheep, Kominakis et al. (1999) estimated heritabilities of 0.26, 0.16, 0.17, 0.24 and 0.28 for MY2:MY1, MY3:MY1, MY4:MY1, MA and CV,

respectively. The genetic correlations between MY2:MY1, MY3:MY1, MY4:MY1, MA and lactation milk yield in the Sfakia dairy sheep were 0.37, 0.23, 0.25, -0.37 and -0.62, respectively. In dairy cattle, positive genetic correlations of intermediate magnitude (0.39-0.50) between the ratio measures (P2:1 and P3:1) and lactation milk yield had been reported but in contrary, reported genetic correlations between MA and lactation milk yield were negative and of medium magnitude (-0.46 to -0.53) (Leukkunen, 1985; Sölkner and Fuchs, 1987).

The genetic correlations presented in table 4 show that ewes with maximum lactation yields would have prolonged milking periods, high rates of decline of milk yield after peak production and more variable test-day yields. Those ewes are also expected to litter twins (table 5). Furthermore, selection for flatter lactation curves would result in reduced lactation yields, slightly higher length of the milking period and decreased test-day yield variation. These ewes are expected to give less twin births.

The genetic correlations between the measures of persistency and litter size were statistically significant only for  $\beta$  ( $r_G=-0.19$ ). Irrespective of the diet and the number of lambs suckled, ewes tend to lose body weight and body condition during early lactation (Louda and Doney, 1976; Bizelis et al., 2000) implying that the nutrient intake is not sufficient to support milk production without mobilization of the body's reserves. The mobilization of the body's reserves and its impact on the reproductive physiology of the dairy sheep has not yet been studied in detail. Metabolic factors associated with milk yield *per se* may be important determinants of reproductive performance. In high producing cows, peak milk yields and lactation persistency were significantly associated with lowered reproductive performance measured as number of breedings for pregnancy and days open (Lean et al., 1989).

### Prediction of lactation milk yield

Using the linear regression model, lactation milk yield can be written as a function of the intercept ( $\hat{a}$ ) and the slope of the regression line ( $\hat{\beta}$ ) as:

$$\hat{y} = \int_1^x (\hat{a} + \hat{\beta}x) dx = \hat{a}x + \frac{\hat{\beta}x^2}{2} \text{ where } \hat{y} \text{ is the}$$

predicted lactation milk yield and  $x$  is the days in milk. In the present study, the correlation coefficient between the predicted milk yield on five test-days and the sampled milk yield was  $r=0.95$ . An interesting issue arising here is the predictive ability of the regression model including the intercept and the slope terms estimated on early as three, four or five first test-days. In these cases, the respective correlation coefficients were 0.62, 0.81 and 0.90, respectively. At the phenotypic level, the most accurate prediction of the lactation milk yield was obtained when the

regression parameters were estimated on the first five test-day records.

Selection for enhanced TMY or  $\beta$  would result in reduced yield variation. Variable milk yields are undesirable in dairy cattle because of problems with feeding cows and indicate a lack of robustness. In dairy sheep, there is no indication about the relationship between milk yield variability and farm profitability. Except for yield variation, flatter lactation curves may also be desirable in high yielding dairy sheep, because they may be associated with decreased stress and inputs. In this case, selection for flatter lactation curves will do so at the expense of gain of milk yield. Genetic change of TMY and the slope of the lactation curve both in the desired directions could be attained at the expense of gains on both traits.

Most recently, random regression models (RRM) have been recognized as ideally suited to the analysis of longitudinal data in animal breeding. RRM allow the estimation of heritabilities, genetic and phenotypic correlations of test-day yields and the derivation of functions of the breeding values of animals for various parts of lactation that could serve as measures of persistency (Jamrozik et al., 1997). Problems associated with convergence as well as the relatively small number of records per test-day have not allowed the use of RRM in the present study. Kominakis et al. (2001) have used RRM to genetically model test-day records in Sfakia dairy sheep and found discrepancies between estimates of genetic parameters obtained by the RRM and those obtained by univariate and multivariate analyses.

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