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# The estimation of genetic parameters of somatic cell scores in Murciano-Granadina goats using random regression models

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Abstract In the present study, 7,089 test-day records on milk somatic cell counts (SCC) belonging to 2,432 first-parity Murciana-Granadina does in Kerman province were used. Test-day SCC records were transformed into milk somatic cell scores (SCS) and analyzed by applying random regression models (RRM) with the Legendre polynomials (LEG) function with order of 2 to 5 for additive genetic and permanent environmental effects and heterogeneous residual variances. The models were compared using the Akaike's information (AIC) and Bayesian information (BIC) criteria. The model with orders 3 and 2 for additive genetic and permanent environmental effects (RRM-LEG32), respectively, was the best for genetic analysis of test-day SCS. The estimates of heritability  $(h^2)$ and the ratio of permanent environmental variance to phenotypic variance  $(pe^2)$ for test-day SCS using the RRM-LEG32 were low. They ranged from 0.03 at days in milk (DIM) 155 and 215 to 0.11 at DIM 35 for  $h^2$  and from 0.01 at DIM 65 to 0.14 at DIM 275 for  $pe^2$  estimates. Genetic correlation estimates among test-day SCS at 5, 70, 137, 203, and 275 DIM were lower than the corresponding phenotypic correlations. Genetic correlation estimates ranged from 0.36 between DIM 137 and DIM 275 to 0.97 between DIM 70 and DIM 137 while the phenotypic correlations ranged from 0.03 between DIM 70 and DIM 203 to 0.09 between DIM 5 and DIM 203. In general, the strongest genetic correlations were found between closely located DIMs, with these correlations decreasing as the interval between DIMs increased. The low heritability estimates for test-day SCS records implied that they are mainly controlled by the non-additive genetic and environmental effects, limiting the efficiency of direct genetic selection for improving the test-day SCS. Therefore, including these effects in designing an appropriate breeding program for improvement in Murciano-Granadina goat udder health is of great importance.

<u>Keywords:</u> animal model, genetic parameters, Legendre polynomials, somatic cell count, test-day record

## Introduction

In most developing countries, goats play an important role in the economy and livelihoods of nomadic farmers (Peacock, 2005). However, goat productivity is often low due to several reasons, including the genetic potential of the native breeds (Jembere et al., 2017). Genetic and nongenetic factors significantly influence milk yield and quality in goats (Selvaggi and Dario, 2015). Knowledge of the



genetic and phenotypic aspects of milk production traits is essential for developing efficient breeding programs (Scholtens et al., 2019).

Milk somatic cell count (SCC) is a reliable indicator for early diagnosis of udder disease and health status of (Shook and mammary glands Schutz. 1994: Olechnowicz and Jaskowski, 2012). SCC mainly reflects the number of neutrophils that migrate from blood to the mammary gland in response to infections (Rupp et al., 2011); therefore, a high SCC often implies mastitis, being the most costly disease in dairy goats and the most frequent cause of culling for sanitary reasons (Marogna et al., 2012). Detecting mastitis early through SCC can help in managing and treating the condition, thereby improving the animal welfare and milk quality (Podhorecka et al., 2021; Smistad et al., 2024). High SCC levels can lead to changes in milk composition, such as increased protein and casein levels, which affect milk taste and processing properties (Rychtarova et al., 2023). Furthermore, maintaining a low SCC is economically beneficial for dairy farmers. Reduced milk yield and quality due to high SCC results in lower milk price and potential penalties from milk processors (Rychtarova et al., 2023). High SCC can also be due to the presence of pathogens, which can pose health risks to consumers (Slyzius et al., 2023). By regularly monitoring SCC, dairy goat farmers can ensure the health of animals, guality of milk, and economic viability of their operations.

Random regression models (RRMs) have several advantages in genetic evaluations of livestock, particularly when analyzing longitudinal data such as milk production traits (Oliveira et al., 2019). These models can accommodate the changing nature of traits over time by fitting random genetic and environmental effects. By accounting for time-course changes in environmental effects and additive genetic values, RRMs provide higher accuracy in estimating genetic parameters compared to traditional models (Oliveira et al., 2019). This is particularly useful for traits that vary significantly with age or stage of lactation (Arnal et al., 2019). Arnal et al. (2019) pointed out that, RRMs are well-suited for analyzing test-day records in dairy goats, as they can model the variability of environmental effects throughout lactation more accurately than fixed lactation curve models. Furthermore, RRMs can manage complex data structures, including repeated measures and unbalanced data, making them ideal for genetic evaluations where data may not be uniformly collected (Silva et al., 2013). These advantages make RRMs a powerful tool in the genetic evaluation and selection of livestock for traits with longitudinal data such as SCC, ultimately contributing to more efficient and effective breeding programs. Orthogonal Legendre polynomials are powerful tools used in RRMs for the genetic evaluation of animals and help in reducing multicollinearity issues in the model, leading to more stable and reliable estimates (Li et al., 2020). They can model complex, nonlinear relationships between traits and time or age, which is crucial for accurately capturing the genetic and environmental effects over an animal's lifespan (Li et al., 2020). Furthermore, the mathematical formulation of Legendre polynomials is relatively straightforward, making them easier to implement and interpret in genetic evaluation models (Li et al., 2020).

Estimating genetic parameters for test-day somatic cell scores (SCS) in dairy goats using RRMs is a crucial aspect of improving udder health and overall milk quality in breeding programs. There are limited published estimates on the genetic parameters of SCS in dairy goat breeds. Jimenez-Granado et al. (2022) reported estimates of genetic parameters for SCS in Florida goats using both single and multiple traits models. They found medium to high heritability estimates for SCS, ranging from 0.245 (in the first parity) to 0.365 (in the third parity), indicating potential for genetic improvement of this trait in the studied population.

The Murciano-Granadina goat breed is one of the most important dairy goat breeds in Spain and is distributed extensively in various countries (Miranda-Alejo et al., 2019). In 2015, the private sector imported the Murciano-Granadina goat breed from Spain to the southern region of Iran, with tropical climatic conditions. This initiative aimed to enhance production efficiency in the native and nomadic goat flocks of the region, primarily maintained under a low-input, low-output production system. The goal was to improve the livelihoods of rural flock holders in the area. To achieve this, purebred Murciano-Granadina does and bucks were either distributed to local flocks or used for crossbreeding with indigenous goat breeds. The Murciano-Granadina goat breed is highly valued for its milk production and adaptability. Understanding the genetic parameters for test-day SCC is essential as it serves as an indicator of udder health and milk quality. There is no information on the genetic parameters for test-day SCC in this population of the Murciano-Granadina goat breed. Therefore, this study aimed to estimate the genetic and phenotypic parameters for SCC in the first lactation of this population using RRMs.

# Materials and methods

# Pedigree, data, and editing protocol

The pedigree information of 33,858 Murciano-Granadina goats originated from 920 sires and 10,769 dams was used in the present study. The CFC program (Sargolzaei et al., 2006) was employed to monitor pedigree errors and prepare the pedigree for genetic analysis. The pedigree structure of the population is presented in Table 1. Among the registered animals, those with both parents known, both parents unknown, and one parent known comprised 89.23%, 9.91%, and 0.86 % of all individuals, respectively. Only 34.52 % of the animals had progeny.

The original dataset, comprised of 7,745 test-day SCC from 2616 first-parity does, had been collected from 2017 to 2024 at a private dairy farm located in Ghale-Ganj city, in the southern region of Kerman province, Iran. Test-day records of SCC were limited to does for which measurements were taken between 5 to 275 days in milk, with consecutive sampling intervals of approximately 30 days. The final dataset included 7,089 test-day records of milk SCC from 2,432 first-parity does. The values of SCC were right-skewed, therefore, to achieve normally distributed values, the SCC values were transformed into SCS values using the following transformation (Valencia-Posadas et al., 2022):

$$SCS = \log_2\left(\frac{SCC}{100000}\right) + 3$$

**Table 1.** Pedigree structure of the population of the Murciano-Granadina goat breed

Item	n
Individuals in total	33858
Inbreds in total	2252
Sires in total	920
Dams in total	10769
Individuals with progeny	11689
Individuals with no progeny	22169
Individuals with both parents known	30213
Individuals with both parents unknown	3358
Individuals with one parent unknown	287
Average inbreeding coefficients (percent)	0.3
Average inbreeding coefficients in the inbreds (percent)	4.3
Maximum of inbreeding coefficients (percent)	31.25
Minimum of inbreeding coefficients (percent)	0.10

#### Statistical analysis

#### Random regression models

The estimation of genetic parameters for test-day SCS records was performed applying a single-trait linear mixed random regression model as follows:

#### y = Xb + Za + Wpe + e

where, y is the vector of test-day SCS records; b is the vector of fixed effects; a and pe are regression coefficients related to the additive genetic and permanent environmental effects, respectively. The X, Z, and W are incidence matrices associated with the fixed, additive genetic, and permanent environmental effects to y. The vector of e denotes random residual effects. Significance testing of the fixed effects and estimation of the least squares means for the test-day SCS records across the levels of fixed effects were performed by using the PROC GLM (SAS, 2010). The Tukey-Kramer test was used to compare the mean of test-day SCS records across the levels of fixed effects. The fixed effects were kidding season (spring, summer, autumn, and winter), kidding year (eight levels from 2017 to 2024), kidding type (single and twin), and age at kidding (2- and 3-year-old). It was assumed that:

$$E\begin{bmatrix}\mathbf{y}\\\mathbf{a}\\\mathbf{pe}\\\mathbf{e}\end{bmatrix} = \begin{bmatrix}\mathbf{Xb}\\0\\0\\0\end{bmatrix}, \quad V(\mathbf{a}) = \mathbf{K}_{\mathbf{A}} \otimes \mathbf{A}, V(\mathbf{pe}) = \mathbf{K}_{\mathbf{pe}} \otimes \mathbf{I}, \text{ and } V(\mathbf{e}) = \mathbf{R}$$

where,  $K_A$  and  $K_{pe}$  are the matrices of (co)variance between the regression coefficients related to additive genetic and permanent environmental effects, respectively; A is the numerator relationship matrix, I is an identity matrix, and R is the residual covariance matrix. Random regression models, extensions of the repeatability models in which the random genetic animal effect can vary for each time period (Lidauer et al., 2003), are implemented by applying different covariance functions. Covariance functions are used to describe covariance among records that are measured at different time points (Meyer and Hill, 1997). The Legendre polynomials (LEG) and B-spline functions, two important covariance functions, are used for fitting RRMs. In the present study, the LEG function was used for fitting the random regression model on test-day SCS records. The following single-trait RRM with LEG function was used for the genetic analysis of test-day SCS:

$$y_{ijk} = FE_k + \sum_{m=0}^{K_{\beta}-1} \beta_m \phi_m(t_{ij}) + \sum_{m=0}^{K_{\alpha}-1} \alpha_{im} \phi_m(t_{ij}) + \sum_{m=0}^{K_{\gamma}-1} \gamma_{im} \phi_m(t_{ij}) + \varepsilon_{ij}$$

where,  $y_{ijk}$  is the test-day SCS on the j<sup>th</sup> days in milk (DIM) of the i<sup>th</sup> goat within fixed effect k;  $FE_k$  is the fixed effect;  $\beta_m$  is the m<sup>th</sup> regression coefficient of the test-day SCS on the LEG function for modeling the average curve of the population;  $\alpha_{im}$  and  $\gamma_{im}$  are the m<sup>th</sup> regression coefficients of the additive genetic and permanent environmental effects, respectively, for the i<sup>th</sup> goat;  $K_{\beta}$ ,  $K_{\alpha}$ , and  $K_{\nu}$  are the degree of LEG;  $t_{ij}$  is the control variable, DIM of the ith goat standardized for -1 to 1 interval, as described by Kirkpatrick et al. (1990);  $\phi_m(t_{ii})$  is LEG function for parameter *m* evaluated at DIM  $t_{ij}$ ; and  $\varepsilon_{ij}$  is the residual effect. Different models with the combinations of second-to fifth-order polynomials were used to model the direct additive genetic and permanent environmental variances. considering heterogeneous residual variance across 6 different classes of DIM including 5-45, 46-90, 91-135, 136-180, 181-225, and 226-275.

#### Model comparisons

The RRMs were compared by applying the Akaike's information criterion (AIC; Akaike, 1974) and Bayesian information criterion (BIC; Schwarz, 1978). The AIC and BIC values were calculated as follows:

$$AIC = -2 \log (L) + 2p$$
  
BIC= -2 log (L) + p Log(N-r)

where, log (L) is the maximized logarithm of likelihood, p is the number of parameters, N is the number of records, and r is the rank of matrix X as the incidence matrix of the fixed effects. The lowest values for AIC and BIC indicate the best model. Variance components and genetic parameters were estimated by applying the WOMBAT program (Meyer, 2013).

#### **Results and discussion**

Descriptive statistics for the test-day SCS records are shown in Table 2. High coefficient of variation was obtained for test-day SCS. Within a population, a high coefficient of variation for a trait shows that there is a large amount of variability in the trait relative to the mean. This could be due to several factors such as genetic diversity, differences in management practices, and environmental conditions.

Table 2. Descriptive statistics for the test-day SCS in Murciano-
Granadina goats

Item	Value
No. of records	7089
No. of animals	2432
Average number of records per animal	2.91
Mean (score)	4.86
Standard deviation (score)	1.92
Coefficient of variation (%)	39.51
Minimum (score)	0.16
Maximum (score)	9.88

#### Non-genetic effects

The least squares means of the test-day SCS of the Murciano-Granadina goat across the levels of fixed effects are presented in Table 3. The kidding year and kidding season significantly affected the test-day SCS (P<0.01). The kidding year can influence the test-day SCS due to variations in environmental conditions, management practices, and herd health status over different years. The season of kidding also plays a significant role in determining the SCS. Seasonal variations affect the nutritional status, environmental stressors, and exposure to pathogens, all of which can influence the somatic cell count in milk. Zamuner et al. (2020) pointed out that Saanen goats kidding in different months showed variations in SCS, with certain seasons associated with higher or lower SCS. This is likely due to differences in temperature, humidity, and feed availability, which can affect the immune response and overall health of the does (Jimenez-Granado et al., 2014). The kidding type and kidding age had no significant effect on the test-day SCS (P>0.05). Contrary to us, Zamuner et al. (2020) reported that single-kidding Saanen does had significantly lower test-day SCC than multiple births does (5.6 vs. 7.5 × 10<sup>5</sup> cells/mL of milk).

**Table 3**. Least squares means (± S.E.) of the fixed effects for test-day SCS in Murciano-Granadina goats

	9
Effect	Significance and estimates
Kidding year	**
Kidding season	**
Spring	5.24±0.06 °
Summer	5.20±0.07 °
Autumn	4.99±0.06 b
Winter	5.17±0.07 <sup>a</sup>
Kidding type	ns
Single	5.20±0.04 °
Twin	5.11±0.06 °
Age at kidding	ns
2-yr	5.16±0.05 °
3-yr	5.15±0.06 ª

<sup>a</sup> Means with similar letters in each sub-class within a column do not differ (P>0.05), ns: not significant. Means with different letters in each sub-class within a column differ from another at \* P<0.05 and \*\* P<0.01.

# Fitting different order RRM

The results of fitting different RRMs with LEG function for test-day SCS, accounting for heterogeneous residual variances, are shown in Table 4; the RRM with degrees

of fit 3 and 2 for the additive genetic and permanent environmental effects (RRM-LEG32), respectively, had the best fit.

Table 4. Comparison of different random regression models of
_egendre polynomials function for test-day SCS in Murciano-
Granadina goats

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Model <sup>a</sup>	NP <sup>b</sup>	AIC °	BIC °
LEG22	12	17100.642	17183.022
LEG23	15	20012.132	20115.106
LEG24	19	15781.276	15911.710
LEG25	24	15784.954	15949.712
LEG32	15	9896.306	9982.480
LEG33	18	15783.422	15906.990
LEG34	22	15786.156	15937.184
LEG35	27	15789.450	15974.802
LEG42	19	15782.382	15912.814
LEG43	22	15786.178	15937.206
LEG44	26	15792.622	15971.108
LEG45	31	15795.956	16008.768
LEG52	24	15786.298	15951.056
LEG53	27	15789.298	15974.650
LEG54	31	9944.588	10157.400
LEG55	36	15802.012	16049.148

<sup>a</sup> LEGxy: Legendre polynomial function, where, x and y are degrees of fit for additive and animal permanent environmental effects, respectively.

<sup>b</sup>NP: number of parameters

 $^{\rm c}{\rm AIC}$  Akaike's information criterion, BIC: Bayesian information criterion The best model is shown in bold text.

The associated eigenvalues (%) for the covariance matrices of random regression coefficients of additive genetic and permanent environmental effects obtained from the RRM-LEG32 are shown in Table 5. The first component accounted for 73.44% of the total additive genetic variability, implying that the majority of the additive genetic variation in the form of the test-day SCS curve is due to the constant random regression coefficients. For the permanent environmental effects, the largest variation was associated with the constant term (93.07%). By applying RRM with the LEG function order 5, Soumri et al. (2020) reported that the first components (constant term) for additive genetic and permanent environmental effects constituted 52% and 51.7% of the variability of the test-day SCS in Tunisian dairy cattle, respectively. As shown in Table 5, the second and the third components explained 20.00% and 6.56% of the total additive genetic variability for the testday SCS in the first-parity Murciano-Granadina goats, respectively. In the case of permanent environmental effects, the second component accounted for 6.56% of the total associated variability. Pool et al. (2000) pointed out that goodness of fit can be notably reduced when the sum of the variability associated with the eigenvalues set to zero is larger than 2 %. In our study, to ensure more than 98 % of the total variability, three components for the additive genetic effects and two components for the permanent environmental effects seem to be appropriate to attain this level.

#### Variance components

The additive genetic variance under the RRM-LEG32 ranged from 0.096 (DIM=215) to 0.440 (DIM=5), with a decreasing trend from the initial phase of lactation until

DIM 155, after which it increased slightly until the end of the period (Figure 1). The permanent environmental variance under RRM-LEG32 varied from 0.093 (DIM=35) to 0.514 (DIM=275). It increased from DIM 35 (0.093) until DIM 185 (0.306) and decreased afterward until DIM 245 (0.143) with an increase until DIM 275 (0.514). As shown in Figure 1, except for the early and late stages of the lactation period, similar trends were observed for the residual and phenotypic variances of the test-day SCS of the first-parity Murciano-Granadina goats. Lopez-Romero and Carabano (2003) explained that it is reasonable to observe large variances in the early and late lactation periods given the exposure of animals to multiple factors that have a greater influence

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on production than during the other periods of lactation. This behavior has been largely attributed to a poor fit of the RRMs at the extreme points (early and late stages) of the curve, probably due to the smaller number of observations during these periods (Meyer, 1999).

**Table 5.** Eigenvalues of estimated covariance matrices of random regression coefficients for test-day SCS in Murciano-Granadina goats

Random effect	Eigenvalues		
	Component 1	Component 2	Component 3
Additive genetic	73.44%	20.00%	6.56%
Permanent	93.07%	6.93%	-
environmental			



Figure 1. Variances obtained for test-day SCS along the first lactation period in the Murciano-Granadina goat breed

#### Heritability estimates

The estimates of heritability  $(h^2)$  and the ratio of permanent environmental variance to phenotypic variance  $(pe^2)$  for test-day SCS using the RRM-LEG32 across different DIM in the first lactation period of the Murciano-Granadina goat breed are shown in Table 6. In general, the corresponding estimates of  $h^2$  and  $pe^2$ were low; ranging from 0.03 (DIM=155 and 215) to 0.11 (DIM=35) for  $h^2$  and from 0.01 (DIM=65) to 0.14 (DIM=275) for  $pe^2$ . As shown in Figure 2, the estimates of  $h^2$  decreased from early lactation (about after one month) until DIM 155 and increased afterward generally with some fluctuations. The low estimates of heritability for test-day SCS in the Murciano-Granadina goats may be explained by the low associated additive genetic and high residual variances for the test-day SCS in the current study. Soumri et al. (2020) also reported low heritability estimates for test-day SCS using RRM with LEG function in Tunisian dairy cattle. Arnal et al. (2020) used a RRM with LEG function for genetic analysis of test-day SCS in the French Alpine goat breed. They used order 2 for additive genetic and permanent environment effects and reported an average heritability estimate for SCS of about 0.15. Jimenez-Granado et al. (2022) studied the test-day SCS in the Florida goat breed and

found that SCS had a medium to high heritability; ranging from 0.245 in the first parity to 0.365 in the third parity.

**Table 6.** The estimates of heritability  $(h^2)$  and the ratio of animal permanent environmental variance to phenotypic variance  $(pe^2)$  for test-day SCS in the first lactation period in Murciano-Granadina goats

DIM <sup>1</sup>	$h^2 \pm S.E.$	$pe^2 \pm S.E.$
5	0.10±0.04	0.08±0.04
35	0.10±0.03	0.03±0.03
65	0.09±0.03	0.01±0.02
95	0.08±0.03	0.04±0.03
125	0.06±0.03	0.04±0.03
155	0.03±0.02	0.08±0.03
185	0.05±0.02	0.10±0.05
215	0.03±0.01	0.05±0.03
245	0.05±0.02	0.04±0.04
275	0.04±0.03	0.14±0.08

<sup>1</sup>DIM: days in milk

#### Genetic correlation

The genetic and phenotypic correlations among some selected DIMs, including 5, 70, 137, 203, and 275, are shown in Table 7. Genetic correlation estimates were lower than the corresponding phenotypic correlations. Genetic correlation estimates ranged from 0.36 between DIM 137 and DIM 275 to 0.97 between DIM 70 and DIM 137 while the phenotypic correlations ranged from 0.03 between DIM 70 and DIM 203 to 0.09 between DIM 5

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and DIM 203. In general, the highest genetic correlations were observed between nearby DIMs and decreased as DIMs got further apart. A similar trend was also reported by Soumri et al. (2020) for genetic correlations among test-day SCS in Tunisian dairy cattle. As shown in Table 7, high genetic correlations, generally higher than 0.80,

among the DIMs of the mid-lactation period (DIMs 70 to 203) were observed. This result is of great importance revealing a possible milk recording simplification during some periods of lactation without greatly affecting the estimation of the genetic parameters using RMMs (Soumri et al., 2020).



**Figure 2.** The estimates of heritability ( $h^2$ ) and the ratio of permanent environmental variance to phenotypic variance ( $pe^2$ ) for test-day SCS during the first lactation period in Murciano-Granadina goats

Table 7. Estimate	s of the additive	genetic (abov	ve diagonal)	) and phenotyp	ic (below	<sup>,</sup> diagonal)	correlations	among	days
in milk for test-day	y SCS in Murcia	no-Granadina	goats						

		and anna go ato			
DIM <sup>1</sup>	5	70	137	203	275
5	-	0.76±0.25	0.62±0.27	0.59±0.21	0.55±0.22
70	0.05±0.03	-	0.97±0.21	0.83±0.25	0.57±0.18
137	0.04±0.03	0.08±0.02	-	0.87±0.31	0.36±0.24
203	0.09±0.04	0.03±0.02	0.06±0.02	-	0.59±0.18
275	0.06±0.01	0.05±0.02	0.08±0.04	0.05±0.04	-

<sup>1</sup> DIM: days in milk

# Conclusion

Knowledge of the genetic parameters for test-day SCS in Murciano-Granadina goats using RRMs provides valuable insights into the role of genetic and non-genetic effects that are important for the expression of this trait. The RRMs offer an appropriate method for analyzing test-day SCS across different lactation stages. The low heritability estimates for test-day SCS records implied that they are mainly controlled by non-additive genetic and environmental effects which limit the efficiency of direct genetic selection for improving them. Therefore, including these effects in designing an appropriate breeding program for improving the test-day SCS as a measure of udder health in the Murciano-Granadina goat breed is of great importance.

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