

# GENETIC ANALYSIS OF SEMEN FROM DIFFERENT ORIGINS AND THEIR IMPACT ON PRODUCTION TRAITS: A SINGLE AND MULTIPLE TRAIT APPROACH

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Original scientific paper

**Abstract:** This study aims to evaluate the genetics of imported semen and assess the genetic trend of production traits in Holstein cows during their first lactation in Iran. The data was collected from 28 different herds in Isfahan province between 2011 and 2020. Variance-covariance components were estimated by the restricted maximum likelihood method and the single and multi-trait animal model. The correlation between breeding values for bulls reported in catalogs and estimated in this was calculated. The mean of the least squares by countries shows that the daughters of Spanish bulls have the highest average for milk production, and the daughters of German, French, Spanish, and American bulls have the highest average percentage of fat and protein and the amount of fat and protein, respectively. Estimated heritability for milk production, fat, and protein percentage, and the amount of fat and protein were  $0.34 \pm 0.011$ ,  $0.48 \pm 0.021$ ,  $0.41 \pm 0.016$ ,  $0.40 \pm 0.090$ , and  $0.39 \pm 0.010$  respectively. The mean genetic trend of milk production, fat percentage, protein percentage, fat content, and protein content were 92, 0.010, 0.004, 1.73, and 2.52, respectively. The correlation between the estimated and reported breeding value of bulls for milk production trait, percentage of fat and protein, and the amount of fat and protein was estimated at 0.48, 0.67, 0.69, 0.14, and 0.26, and all of the estimated correlations are statistically significant at the level of 0.05. Based on the results for the most critical production trait in Isfahan herds, milk production, American bulls have the best performance and genetic trend.

**Key words:** production traits, variance components, genetic evaluation, animal model, genetic trend

## Introduction

The aim of implementing a breeding program is to simultaneously enhance multiple traits in a herd. This genetic improvement in productive traits results in a higher herd income (*Matthews et al., 2019*). Breeding companies estimate the amount of genetic progress and the efficiency of the breeding program implemented at the herd level during a certain period (*Kavosi et al., 2016*). The genetic progress of a population is a combination of the rate of improvement in different paths of selection, which are associated with varying intensities and accuracies (*Abdollahi-Arpanahi et al., 2021*). Choosing the bulls based on the breeding goals of a dairy herd is one of the most effective ways to improve its genetic level. To achieve this, it is necessary to estimate the genetic value of female livestock and select appropriate semen accordingly. However, accurate information on female livestock is often lacking, leading semen importing companies to estimate breeding values using the pedigree method. The science of breeding relies on genetic trends to observe changes in average traits over several years, which are based on shifts in breeding values (*Strabel and Jamrozik, 2006*). Therefore, estimating and examining genetic trends is one of the key tools for evaluating the effectiveness of breeding projects. This enables the comparison of different breeding programs, allowing for a better understanding of their performance (*Hanford et al., 2003*). Procurement of semen from foreign countries has led to changes in the genetic makeup of Iran's Holstein cows in recent years. In selecting semen for the herd, one of the crucial criteria is the transferable genetic potential of the bulls to the offspring. Previous studies have investigated the genetic trend of productive traits in herds in Markazi Province, with *Kavosi et al. (2016)* being one of them. A study by *Kavosi et al. (2016)* revealed a significant difference in estimated genetic trend for reproductive traits between daughters born from semen imported from America and those born from semen imported from other countries such as Canada, Dutch, France, New Zealand, and Italy. Another study by *Razavi et al. (2007)* estimated the genetic, phenotypic, and environmental trends of production traits in Holstein cows in Markazi Province. Their report indicated that phenotypic trends for milk production (22.79 kg), milk amount (0.23 kg), and fat percentage (0.05%) increased. The genetic trends for these traits were even higher, with increases of 3.75 kg in milk production, 0.06 kg in milk amount, and -0.02% in fat percentage. For the same traits, the environmental trend was 19.79 kg, 0.21 kg, and 0.07%, respectively. In contrast, *Shirmoradi et al. (2012)* reported a positive genetic trend for production traits such as milk production, fat, and protein percentage, but a negative genetic trend for first calving age and calving interval traits. *Naeemipour Younesi and Shariati (2016)* conducted another study in which they estimated the genetic parameters and genetic and phenotypic trends of some productive and reproductive traits of dairy cows in Yazd province using a multi-trait livestock model.

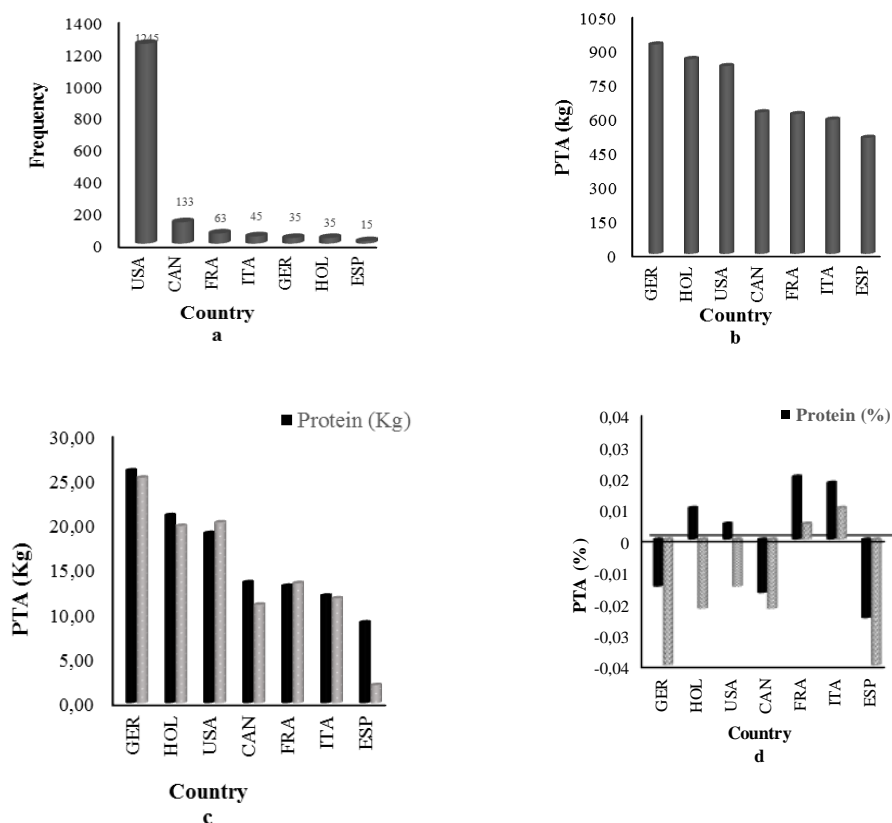
They found that heritability values for milk production traits, fat amount and percentage, protein amount and percentage, length of the dry season, first calving age, and calving interval were 0.23, 0.27, 0.39, 0.28, 0.41, 0.03, 25.0, and 0.05, respectively. The authors also reported significant phenotypic trends for all production and reproduction traits and significant genetic trends for milk production traits and protein amounts. However, due to the negative genetic correlation between the investigated production and reproduction traits, selecting to increase productive traits may lead to reduced efficiency of reproductive performance. In *Seyedsharifi et al. (2018)* study, the estimated heritability for milk production, fat production, and protein production traits was 0.28, 0.49, and 0.42, respectively. For open days and first calving age, the estimated heritability was 69.0, and 0.07, respectively. The estimated heritability for type traits such as body depth, angularity, and udder depth were 0.27, 0.25, and 0.36, respectively. They also reported genetic correlations between milk production and fat production at 0.12 and between milk production and protein production at 0.05. Given that Isfahan province is one of the leaders in the dairy cattle industry and extensively uses imported semen in their herds, it is crucial to perform genetic evaluations and investigate genetic trends and progress obtained from imported semen. This study aims to compare the genetic effects of semen from different origins and estimate the genetic parameters and trends of production traits such as milk production, fat and protein percentage, and the amount of fat and protein belonging to first-parity Holstein cows from the imported semen used in a herd of dairy cows in Isfahan province.

## Material and Methods

This research utilized production data from 71,479 first-parity cows in 28 herds located in Isfahan province from 2011 to 2020 to obtain production traits, fat and protein percentages, and amounts. Pedigree data was reviewed and edited using CFC (*Sargolzaei et al., 2006*) software. Table 1 provides descriptive statistics of the examined and compared traits of the daughters of different groups of bulls. Additionally, Figure 1a depicts the frequency of consumed semen by country. The highest frequency of consumed semen was from American bulls, while the lowest was from Spanish bulls, as shown in Figure 1a. Data editing was performed using Visual Fox Pro version 9. Researchers calculated the animals' total production during the first lactation period using test day records. To calculate the total production, a minimum of 210 lactation days and at least seven records of test days with a recording interval between two consecutive records of 25 to 35 days were considered. The researchers then converted the total production records to the standard 305-day record and used these standardized records to evaluate and estimate parameters and genetic trends. Software airemlf90 (*Misztal et al., 2002*) was utilized to estimate variance-covariance components and genetic parameters.

**Table 1. Descriptive statistics of the studied traits**

Traits	Number of Records	Mean $\pm$ SD	Minimum	Maximum	CV%
Milk (Kg)	71479	11540.00 $\pm$ 1755	5200.00	16545.00	15.00
Fat (%)	69415	3.33 $\pm$ 0.45	2.00	5.90	13.60
Fat (Kg)	68573	384.00 $\pm$ 58.28	229.00	512.00	15.18
Protein (%)	69124	2.95 $\pm$ 0.16	2.25	5.45	5.32
Protein (Kg)	68745	344.00 $\pm$ 52.83	208.00	464.00	15.21



**Figure 1. Distribution and frequency of bull semen originated from different countries (a), Graph for mean breeding value for bull semen originated from different countries (b), Graph for mean breeding value for bull semen originated from different countries for protein and fat contents(c) and graph for mean breeding value for bull semen's originated from different countries (d)**

The genetic trends of traits were calculated in two ways: expected (using pedigree based breeding values) and observed (using estimated breeding values). To estimate the pedigree breeding value, the basis of calculations is as follows:

$$PTA_O = \left(\frac{1}{2} \times PTA_{Sire}\right) + \left(\frac{1}{4} \times PTA_{MGS}\right) + \left(\frac{1}{8} \times PTA_{MMGS}\right)$$

In this regard, PTA Sire, PTA maternal grandsire, and PTA maternal great-grandsire, respectively, include the reported breeding value of the sire, maternal grandsire, and maternal great-grandsire of the cow in question. In the following, single-trait and multi-trait models were used for the production traits to estimate the breeding values.

$$Y_{ij} = \mu + HYS_i + C_k + b(Age - \overline{Age_{ij}}) + a_j + e_{ij}$$

In this model,  $Y_{ij}$  is the record of the  $j^{th}$  cow in the herd, the  $i^{th}$  year and calving season for each trait,  $\mu$  = the average population,  $HYS_i$  = the fixed effect of the herd, the calving year and season,  $C_k$  = the fixed effect of the semen's origin country,  $b(Age)_j$  = the effect of the animal's calving age as a covariate,  $a_j$  = the random additive genetics effect of  $j^{th}$  animal, and  $e_{ij}$  = the random residual effect. The model mentioned above in the matrix notation is as follows.

$$y = Xb + Za + e$$

Where  $y$  = vector ( $n \times 1$ ) of observations,  $b$  = vector ( $p \times 1$ ) of fixed effects,  $a$  = vector ( $q \times 1$ ) of random animal effects,  $e$  = residual vector, and  $X$  and  $Z$  are the design matrices that relate records to fixed and random effects, respectively. In the end, after estimating the variance and covariance components through the AI-REML Algorithm, predicted breeding values. Finally, genetic trends were fitted as a function of the breeding values in the calving year. In the multi-trait model (5 traits), the structure of the variance-covariance matrix was as follows.

$$\text{var} \begin{bmatrix} a \\ e \end{bmatrix} = \begin{bmatrix} A \otimes G & 0 \\ 0 & I \otimes R \end{bmatrix}$$

Where  $A$  = numerator relationship matrix,  $\otimes$  Kronecker's multiplication,  $G$  is the genetic covariance matrix between traits ( $5 \times 5$ ),  $R$  is the residual variance-covariance matrix of the traits, and  $I$  is the identity matrix.  $a$  is the vector of the random additive genetics effect of the animal, and  $e$  is the residual vector.

**Table 2. Comparison of the least square means (LSM) of studied traits between different countries**

Country	Milk (Kg)	Fat (%)	Fat (Kg)	Protein (%)	Protein (Kg)
ESP	11610 <sup>a</sup>	3.30 <sup>ab</sup>	386 <sup>a</sup>	2.97 <sup>c</sup>	350 <sup>a</sup>
ITA	11545 <sup>a</sup>	3.32 <sup>ab</sup>	384 <sup>ab</sup>	2.99 <sup>ab</sup>	348 <sup>ab</sup>
FRA	11423 <sup>a</sup>	3.33 <sup>a</sup>	379 <sup>a</sup>	3.02 <sup>a</sup>	344 <sup>ab</sup>
USA	11392 <sup>a</sup>	3.29 <sup>b</sup>	379 <sup>a</sup>	2.98 <sup>c</sup>	343 <sup>a</sup>
CAN	11342 <sup>a</sup>	3.34 <sup>a</sup>	377 <sup>a</sup>	2.99 <sup>b</sup>	342 <sup>a</sup>
GER	11288 <sup>ab</sup>	3.35 <sup>a</sup>	375 <sup>ac</sup>	2.98 <sup>c</sup>	340 <sup>ac</sup>
HOL	11109 <sup>b</sup>	3.31 <sup>ab</sup>	369 <sup>c</sup>	3.01 <sup>a</sup>	335 <sup>c</sup>

In the columns related to traits, the averages that have at least one letter in common statistically do not have significant differences ( $P < 0.05$ ).

## Results and Discussion

Figure 1 shows the distribution of semen consumed in different countries (a), the average modified value of semen consumed for milk production (b), the amount of fat and protein (p), and the percentage of fat and protein (t) in the studied herds of Isfahan province. According to this graph, it can be seen that America has the largest share in the genetics of the herds of Isfahan province. Regarding the breeding value of milk production, German semen has the highest breeding value. The Netherlands and Germany regarding the amount of fat and protein, and America, Italy, and the Netherlands in terms of the percentage of fat and protein in the semen had the most significant breeding value. Table 1 shows the descriptive statistics of the traits under study. Based on the results of the analysis among the daughters of bulls of different countries, the highest average trait of milk production was related to the daughters of Spanish, Italian, and American bulls, which were equal to 11735, 11690, and 11545, respectively, for the amount of fat. Female American bulls had the highest protein production, 398 and 336 kg, respectively. Daughters of Italian bulls had the highest average for the fat percentage trait (3.98%).

For the protein percentage trait, daughters of French bulls had the highest average (3%), which is consistent with the results of *Kavosi et al. (2016)* and *Nilforooshan and Edriss (2007)*. Table 2 compares the least square-mean of various performance traits of bulls' daughters based on country of origin. According to this table, in terms of amount of milk produced, daughters born of Spanish semen had the highest least square-mean. On the other hand, there was a statistically significant difference at the 0.05 level between the averages obtained from Spain, Italy, France, America, Canada, and Dutch bulls. Still, there was no significant difference between daughters born of German and Dutch semen. Table 3 shows the estimation of genetic and phenotypic variance components and heritability of traits based on single-trait and multi-trait models. In all traits and

models, the residual variance is greater than the additive genetic variance, which indicates the effect of non-additive genetic and environmental factors on different traits. Several factors, such as health, climate and management conditions, recording accuracy, etc., effectively reduce additive genetic variance and increase residual variance (Collier *et al.*, 2006).

**Table 3. Estimated genetic and phenotypic variance components and heritability using univariate and multivariate models**

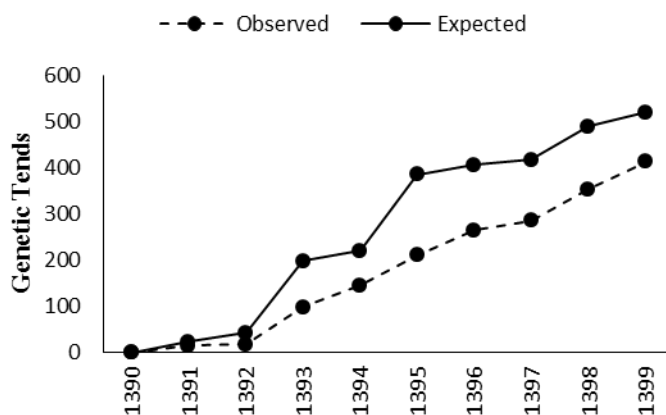
Univariate Model				
Trait	$\sigma_a^2$	$\sigma_e^2$	$\sigma_p^2$	$h^2 \pm SE$
Milk (Kg)	3342300.00	6327400.00	9651700.00	0.35 $\pm$ 0.007
Fat (Kg)	3871.00	6563.00	10434.00	0.38 $\pm$ 0.050
Protein (Kg)	3286.00	6354.00	9740.00	0.35 $\pm$ 0.012
Fat (%)	0.08	0.10	0.18	0.45 $\pm$ 0.020
Protein (%)	0.06	0.09	0.15	0.40 $\pm$ 0.018
Multivariate Model				
Trait	$\sigma_a^2$	$\sigma_e^2$	$\sigma_p^2$	$h^2 \pm SE$
Milk (Kg)	1047800.00	2021000.00	3068800.00	0.34 $\pm$ 0.011
Fat (Kg)	3371.20	5062.60	8433.80	0.40 $\pm$ 0.090
Protein (Kg)	3285.30	5153.80	8439.10	0.39 $\pm$ 0.010
Fat (%)	0.10	0.11	0.21	0.48 $\pm$ 0.021
Protein (%)	0.07	0.10	0.17	0.41 $\pm$ 0.016

The heritability of different traits for single-trait and multi-trait models shows that the highest heritability value is for fat and protein percentage. It can be concluded that these two traits are more affected by the additive effects of genes than other traits. The heritability estimated using the single-trait and multi-trait model for milk production was 0.35 and 0.34, respectively, which was consistent with the results of Moghaddar *et al.* (2001). Still, the current estimates are slightly higher than the report of Shahdadi *et al.* (2017). The heritability rate was 0.45 and 0.48, respectively for the fat percentage trait, which was consistent with the results of Kavosi *et al.* (2016) but was somewhat higher than the rate estimated by Farhangfar and Naeemipour Younesi (2007). Table 4 shows genetic and phenotypic correlations between different traits. The genetic correlation between the trait of milk production with fat and protein percentage was -0.65 and -0.54, respectively, consistent with the results of Toghiani (2012) and Pahlavan and Moghimi Esfandabadi (2010). Still, it was not consistent with the results of Kavosi *et al.* (2016). One of the reasons for the negative correlation was the opposite effect of effective genes on milk production in fat and protein percentage traits. Also, the phenotypic correlation between milk production with fat and protein percentage was -0.11 and -0.30, respectively. Figures 2, 3, and 4 show the studied traits' expected and observed genetic trends (multiple traits). According to the results, for

all studied traits, except fat percentage, the expected genetic trends were more than the observed ones. Among the reasons for this difference, we can mention the interaction of genetics and environment (different performance of bulls). Despite this difference, the direction of the expected and observed trends was positive and increasing. Regarding the fat percentage trait, the observed and expected trends had a slight difference, and the direction of the trends increased from 2010 to 2019. The average genetic trends of milk production obtained from the single-trait and multi-trait models were 93 and 92, respectively, very different from the expected value of 289. For other traits, particularly the amount of fat and protein, the average genetic trends were 1.66, 1.73, 2.42, and 2.52, respectively. This result was not consistent with the results of *Kavosi et al. (2016)* and *Razm Kabir et al. (2009)*. The average genetic trend based on semen type (country) is presented in Table 5.

**Table 4. Estimated genetic (Upper off-diagonal matrix) and phenotypic (Lower off-diagonal matrix) correlation of traits**

Traits	Milk (Kg)	Fat (Kg)	Protein (Kg)	Fat (%)	Protein (%)
Milk (Kg)		0.47	0.33	-0.65	-0.54
Fat (Kg)	0.90		0.21	-0.10	0.08
Protein (Kg)	0.91	0.24		-0.09	-0.12
Fat (%)	-0.11	-0.32	-0.28		0.67
Protein (%)	-0.30	-0.30	-0.31	0.25	



**Figure 2. Genetic trends of milk production in different years of birth**



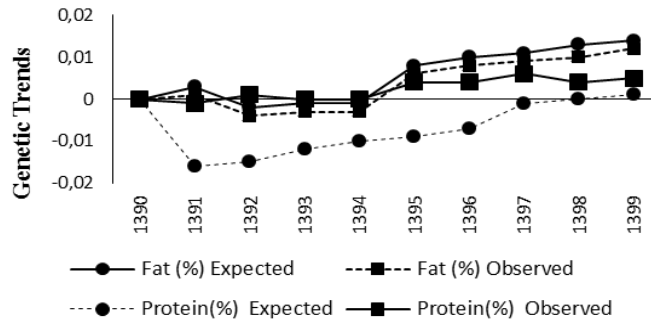


Figure 3. Genetic trends of fat and protein percentage in different years of birth

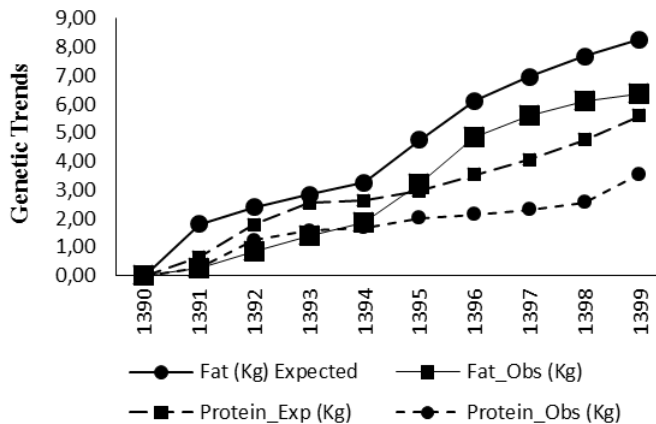


Figure 4. Genetic trends of fat and protein contents in different years of birth

Table 5. The average breeding value of the studied traits of daughter of bulls

Country	Milk (Kg)	Fat (%)	Fat (Kg)	Protein (%)	Protein (Kg)
USA	226	-0.001	2.350	0.007	6.800
CAN	23	0.026	3.490	0.010	2.870
FRA	126	0.001	4.110	0.017	3.770
GER	15	0.023	1.740	0.020	2.570
ITA	9	-0.001	1.380	0.005	-0.310
HOL	17	-0.007	1.600	0.006	-0.560
ESP	158	-0.001	6.200	-0.020	6.300

According to the results of this table, it can be seen that daughters of American bulls had the highest genetic trend in terms of milk production, fat, and protein amounts, and daughters of German bulls had the highest genetic trend for fat and protein percentage traits. These results are consistent with the *Kavosi et al. (2016)* report. Daughters born from American semen have an almost uniform trend with a slight increase from 2011 to 2020, and only a slight decrease can be seen in 2017. The genetic trend of daughters born from French semen fluctuated the most, but since 2017, it has increased with a suitable slope.

The correlation between the reported and estimated breeding values (catalog) in this research, milk production, fat and protein percentage, and fat and protein amounts in bulls were equal to 0.48, 0.67, 0.69, 0.14, and 0.26, respectively. The highest correlation was for protein percentage and the lowest for fat amount trait. The correlation of all breeding values of traits was statistically significant at 0.05 level.

The average breeding values estimated for the bulls of different countries using the records of Isfahan's daughters are shown in Table 6. According to the results of this table, the daughters of American bulls for milk production trait are in the first rank, and for fat and protein amounts traits, they are in the second rank after the daughters of Spanish bulls. Daughters of Canadian and Italian bulls had the highest heredity value for fat percentage traits, and daughters of French bulls ranked first for protein percentage traits.

According to the results of the present research, it can be concluded that the semen of bulls originating from different countries has different functions in terms of diverse traits. Generally, the best genetic performance belongs to American bulls in the herds of Isfahan province. The reason can be due to the vast geographical extent of America and different climatic conditions; therefore, bulls are evaluated based on different geographical regions, and their hereditary value is corrected according to those environmental conditions.

**Table 6. Average of estimated breeding values for bulls using the records of Isfahan's daughters**

Country	Milk (Kg)	Fat (%)	Fat (Kg)	Protein (%)	Protein (Kg)
USA	265	0.009	2.480	0.002	4.210
CAN	35	0.020	-0.680	0.003	-0.650
FRA	26	-0.001	0.850	0.020	0.830
GER	73	0.010	2.470	0.003	2.020
ITA	210	-0.001	6.990	-0.020	6.310
HOL	-9	0.020	-0.390	0.005	-0.310
ESP	23	-0.007	-0.610	0.007	-0.550

## Conclusion

The results of this study show that the genetic trend of the studied traits was upward in the Isfahan herds, and the expected and observed genetic trends were parallel. American, Spanish and Italian bulls had the highest average milk production. The least-squares mean by country shows that daughters of Spanish bulls have the highest average milk yield, while German, French, Spanish and American bulls have the highest average fat and protein percentages and fat and protein content. Predicted genetic trends were greater than those for all traits examined except for fat percentage. American bulls had the greatest genetic tendency for milk production, fat and protein content and had the best genetic performance in the herds of Esfahan province. On the basis of our results, it can be concluded that the use of American bulls in Iranian herds gives better results.

## Genetska analiza semena različitog porekla i uticaj na proizvodne osobine: model sa jednom i više osobina

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## Rezime

Cilj studije je procena genetike uvezene sperme i procena genetskog trenda proizvodnih osobina kod holštajn krava tokom prve laktacije, u odgajivačkim objektima u Iranu. Podaci su prikupljeni iz 28 različitih zapata u provinciji Isfahan između 2011. i 2020. Komponente varijanse i kovarijanse su procenjene metodom ograničene maksimalne verovatnoće i animal modelom sa jednom i više osobina. Izračunata je korelacija između priplodnih vrednosti bikova navedenih u katalozima i procena dobijenih u ovom istraživanju. Srednja vrednost najmanjih kvadrata po zemljama pokazuje da ćerke španskih bikova imaju najveći prosek proizvodnje mleka, a ćerke nemačkih, francuskih, španskih i američkih bikova imaju najveći prosečni procenat masti i proteina, kao i količinu masti i proteina, respektivno. Procenjene vrednosti heritabiliteta za proizvodnju mleka, procenat masti i proteina, kao i količinu masti i proteina su  $0,34 \pm 0,011$ ,  $0,48 \pm 0,021$ ,  $0,41 \pm 0,016$ ,  $0,40 \pm 0,090$  i  $0,39 \pm 0,010$  respektivno. Srednji genetski trend za proizvodnju mleka, procenat masti, procenat proteina, sadržaj masti i sadržaj proteina bio je 92, 0,010, 0,004, 1,73 i 2,52, respektivno. Korelacija između procenjene i priplodne vrednosti bikova koja je dobijena u istraživanju, za mlečne osobine, procenat masti i proteina, kao i količine masti i proteina, procenjena je na 0,48, 0,67, 0,69, 0,14 i 0,26, respektivno, a sve procenjene korelacije su statistički

značajne na nivou od 0,05. Na osnovu rezultata za najkritičniju proizvodnu osobinu u stadima Isfahana, proizvodnju mleka, američki bikovi imaju najbolje performanse i genetski trend.

**Ključne reči:** proizvodne osobine, komponente varijanse, genetska evaluacija, animal model, genetski trend

### **Acknowledgments**

We appreciate the sincere cooperation of the respected management of Arin Delta Gene International Company, Mr. Mehran Rahimi, for preparing the present research data and his unwavering support.

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