

## Association of transferrin genotypes and production traits of Holstein-Friesian cows in Vojvodina

*Dragomir Lukač<sup>1\*</sup>, Vitomir Vidović<sup>1</sup>, Nemeš Žolt<sup>2</sup>,  
Attila Zsolnai<sup>3</sup>, Milena Stevanović<sup>4</sup>, Beáta Bán<sup>5</sup>*

<sup>1</sup>University of Novi Sad, Faculty of Agriculture, Department of Animal Sciences, Serbia

<sup>2</sup>PIK "Bečej", Novosadska 2, 21220 Bečej, Serbia

<sup>3</sup>Research Institute for Animal Breeding and Nutrition, Herceghalom, Hungary

<sup>4</sup>Institute of Molecular Genetics and Genetic Engineering, Belgrade, Serbia

<sup>5</sup>Genetic Laboratory National Food Chain Safety Office, Budapest, Hungary

Received - Prispjelo: 24.02.2014.

Accepted - Prihvaćeno: 14.04.2014.

### Abstract

The aim of this study was to identify genotypes of transferrin, evaluate their frequency in population of Holstein-Friesian cows, and to study association of transferrin genotypes with productivity of cows. Blood samples were collected from 249 healthy adult Holstein cows, daughters of 18 sires, reared at farm in Vojvodina. The nine transferrin (Tf) genotypes were identified: three (transferrin AA, D1D1 and D2D2) of these were homozygous and the remaining six (transferrin AD1, AD2, AE, D1D2, D1E and D2E) heterozygous. The frequencies of genotypes AD2, D1D2, D2D2 and AD1 were 0.29, 0.20, 0.17, and 0.11, respectively, while the other genotypes had frequencies below 0.11. The frequency of alleles A, D1, D2 and E, which was derived from the frequency of genotypes, was 0.30 for allele A, 0.19 for allele D1, 0.45 for allele D2 and 0.06 for allele E. In the analyzed population, cows Tf genotype D2E, AD2, D2D2, and D1D2 had the highest average of milk and milk fat yield in the first three standard lactation, while cows genotype AA, AD1 and D1E had the least amount. Analysis of variance showed that Tf genotype had a non-significant effect in the case of milk fat. However, cows which showed the highest performance for milk fat yield in three standard lactations were phenovariants Tf D2E, AD2, D2D2 and D1D2 in the analyzed population. Based on these results, the general conclusion can be made that the cows with Tf genotype AD2 recorded best results in all observed traits, and that cows which were heterozygous for Tf gene had higher milk yield and milk fat than the cows homozygous for Tf gene.

*Key words:* polymorphism, transferrin, milk yield, productive life of cows

### Introduction

Transferrin (Tf) is a glycoprotein responsible for the transport of iron from sites of absorption and heme degradation to those of storage and utilization, by binding two Fe<sup>3+</sup> ions in association with the binding of an anion, usually carbonate (Lukač et al., 2013). Transferrin is synthesized by the liver with a molecular weight of about 80 kDa (Fletcher and Huehns, 1968). It contributes to innate immunity to microbial pathogens, primarily by limiting

microbial access to iron. Transferrins have a substantial degree of conservation in amino acid sequence (>70 %); thus, they are predicted to have a very similar overall tertiary structure (Retzer et al., 1996).

The Tf gene is located at 125 cM (centimorgan) on chromosome 1; suggestive quantitative trait loci (QTL) have been detected for fat yield at 50 cM, for retail product yield at 53 cM, and for USDA yield grade at 63 cM (Casas et al., 2004). However, the location of this gene is outside the limit of the

\*Corresponding author/Dopisni autor: E-mail: dragomir.lukac@stocarstvo.edu.rs

support interval of the QTL on this chromosome (Casas and Stone, 2006). The Tf gene has been located in several QTL regions, and some Tf classes have been associated with fat and milk yields (Sanz et al. 2010).

Considerable polymorphism and various biological functions of Tf have resulted in a situation whereby it may be used as genetic markers of reproduction and production traits in cattle. Many polymorphisms have been found in the bovine Tf gene (Ashton et al., 1964; Zhang et al., 2010; Sanz et al., 2010; Lukač et al., 2013). It is highly polymorphic in many species, and 10 variants have been detected in cattle, where the major variants described are A, D1, D2, and E (Gahne et al., 1977).

The interest in the detection and characterization of markers associated with fat and milk production traits has increased in recent years, and there are numerous studies that have focused on this topic (Bagnato et al., 2008; Milanese et al., 2008). Polymorphisms present in the bovine Tf gene could potentially underlie inherited differences in mastitis resistance and milk production traits (Ju et al., 2011). The health status of the mammary gland greatly affects the biological value of collected milk (Sevi et al., 2001). In animals with diagnosed mastitis, the Tf concentration in milk is higher than that in healthy animals (Kmiec, 1998). These results suggest a possible relationship between the Tf gene and mastitis in dairy cattle.

Breed improvement programs have been proven useful for the selection of positive milk production traits, but are expensive and time-consuming. Marker-assisted selection that supports fast and low-cost genetic progress and improves the accuracy of selection is desirable (Vidović et al., 2013). In this regard, it is useful to study the genetic variations of candidate genes and their associations with milk production and somatic cell count (Khatib et al., 2007; Huang et al., 2010), which have a high genetic positive correlation with mastitis (with an estimated average coefficient of 0.7, Heringstad et al., 2000).

The aim of this study was to identify alleles and genotypes of Tf and evaluate their frequency in selected population of Holstein-Friesian cows, and to study association of Tf genotypes with productivity of Holstein-Friesian cows in Vojvodina.

## Material and methods

Blood samples were collected from 249 Holstein-Friesian cows, from the farm in Vojvodina. Transferrin types of the sera were determined by a modification of the zone-starch-gel electrophoresis method developed by Smithies (1959). The gels were prepared by dissolving 168 g of hydrolyzed starch in 1,000 ml of a buffer solution composed of 0.0193 M Tris (hydroxymethyl) aminomethane and 0.0178 M cacodylic acid (pH=7.3 to 7.35). Other procedures for electrophoresis of sera were the same as described by Kristjansson and Hickman (1965).

Direct counting was used to estimate phenotype and allele frequencies of Tf genetic variants. The chi-square test ( $\chi^2$ ) was used to check whether the populations were in Hardy-Weinberg equilibrium. Allele frequencies and mean expected heterozygosities per locus and population were calculated using Arlequin ver. 3.11 package program (Excoffier and Heckel, 2006). For investigated traits (age at first calving, number of lactations, productive life of cows, milk yield, milk fat yield and milk fat percentage, during three standard lactation of 305 days) adjusted mean (LSM - Least Square Means) were calculated, and Least significant difference (LSD post-hoc) test for significance determination between the genotypes of Tf.

## Results and discussion

The genotypic frequencies and allelic frequencies of Tf phenotypes found in the studied population of cows are presented in Table 1. Among 249 cows, 9 different genotypes of Tf were identified. Three (Tf AA, D1D1 and D2D2) of these were homozygous and the remaining six (Tf AD1, AD2, AE, D1D2, D1E and D2E) heterozygous. The frequencies of genotypes AD2, D1D2, D2D2 and AD1 were 0.29, 0.20, 0.17, and 0.11, respectively, while the other genotypes had frequencies below 0.11. In the current study, Tf genotype distribution for the studied population, fitted with Hardy-Weinberg equilibrium, was similar to that demonstrated by Ju et al. (2011) in Chinese Holstein cattle.

The frequency of alleles A, D1, D2 and E, which was derived from the frequency of genotypes, was 0.30 for allele A, 0.19 for allele D1, 0.45 for

Table 1. The distribution of transferrin and allele frequencies in Holstein cattle (Lukač et al., 2013), and Hardy-Weinberg equilibrium

Frequencies	Transferrin genotypes									
	AA	AD1	AD2	AE	D1D1	D1D2	D1E	D2D2	D2E	EE
Observed	20	28	72	10	6	50	4	42	17	0
Genotype	0.08	0.11	0.29	0.04	0.02	0.20	0.02	0.17	0.07	0.00
Allelic frequency	A = 0.30			D1 = 0.19			D2 = 0.45		E = 0.06	
Expected	22.4	28.4	67.3	9.0	9.0	42.3	5.5	50.3	13.5	0.9
Genotype	0.09	0.11	0.27	0.03	0.03	0.17	0.02	0.20	0.05	0.004
	$\chi^2 = 1.11$			df = 9			p value = 0.999			

Table 2. LSM and  $SE_{Lsm}$  of age at first calving, number of lactations and productive life of cows

Genotype transferrin	Age at first calving, days		Number of lactations during the life a cow		Productive life of cows, days	
	LSM	$SE_{Lsm}$	LSM	$SE_{Lsm}$	LSM	$SE_{Lsm}$
AA	746.36	48.52	4.85 <sup>A</sup>	1.78	2010.47	820.73
AD1	752.85	36.79	4.64 <sup>B</sup>	1.59	2001.39 <sup>A</sup>	684.92
AD2	757.06	46.41	4.38	1.94	2040.77 <sup>b</sup>	881.86
AE	767.00	71.18	4.20	1.81	1875.00	830.69
D1D1	736.50	11.94	4.33	1.96	1878.50	959.61
D1D2	749.32	33.49	3.78 <sup>AB</sup>	1.77	1607.67 <sup>ACb</sup>	808.04
D1E	760.66	56.51	3.00	1.73	1363.66	964.79
D2D2	748.33	34.69	4.50	1.91	1987.77 <sup>C</sup>	851.53
D2E	771.29	60.46	4.58	1.54	1972.81	749.24

p<0.01 - the same small letters; p<0.05 - the same capital letters

allele D2 and 0.06 for allele E. This ratio expresses preliminary information about the presence of different genotypes of Tf in the Holstein cows analyzed population. The most famous Tf alleles in European cattle breeds are A, D1, D2 and E and these alleles were found in Friesian cattle in Egypt (Giblett et al. 1959). The closer connection between Friesian breeds in Europe and Egypt is due to the genomes of the breeds and the difference in frequencies of some alleles may be due to drift of gene and/or effect of environment on gene expression. In the research of White et al. (1967), the gene frequency distribution among nine herds (1102 dairy cows) of predominantly Friesian type cattle was Tf A 40.7 %, Tf D 55.9 %, and Tf E 5.4 %. In one herd of 31 Red Poll cattle the distribution was Tf A 32.3 %, Tf D 33.8 %, and Tf E 33.8 % (Ashton et al., 1964).

In tables 2, 3, 4, 5 and 6 are presented the least-squares means (LSM), errors of the means ( $SE_{Lsm}$ ) and analysis of investigated traits.

Table 2 shows the effect of the Tf gene on the age at first calving, number of lactations during productive life and length of productive life in the cows studied. Many authors reported a positive correlation of age at first calving with milk yield, milk composition and milk component (Vilho-Arvizu et al., 2008; Ben Jemaa et al., 2008). In our study, there was no statistically significant difference in age at first calving between different Tf genotypes. There were statistically significant (P<0.05) differences in the number of lactations between cows of the AA, AD1 and D1D2 genotypes, and length of productive life between cows of the AA, D2D2 and D1D2 genotypes, AD2 and D1D2 genotypes (P<0.01). Cows

Table 3. Milk yield during three standard lactations of 305 days

Genotype transferrin	First lactation, kg		Second lactation, kg		Third lactation, kg	
	LSM	SE <sub>Lsm</sub>	LSM	SE <sub>Lsm</sub>	LSM	SE <sub>Lsm</sub>
AA	7398.42 <sup>AB</sup>	1271.00	8584.58	1854.52	8140.25 <sup>aA</sup>	1961.66
AD1	7600.38 <sup>C</sup>	1115.00	8423.50 <sup>AB</sup>	1048.53	9645.14 <sup>a</sup>	1354.27
AD2	8101.66	1142.51	9004.46	1086.37	9276.53 <sup>A</sup>	1221.93
AE	7942.85	1113.42	8899.57	1335.76	8874.33	851.94
D1D1	7772.83	659.01	8221.25	1183.74	9606.33	1202.60
D1D2	8375.00 <sup>AC</sup>	1371.17	8823.80	1690.31	9096.50	1404.30
D1E	7822.00	96.16	8824.13	857.25	8601.12	886.52
D2D2	8065.00	1081.36	9348.12 <sup>A</sup>	1184.30	8971.66	1456.69
D2E	8414.76 <sup>B</sup>	1494.17	9520.50 <sup>B</sup>	1650.10	8951.11	1563.04

p<0.01 - the same small letters; p<0.05 - the same capital letters

Table 4. Milk fat yield in three standard lactations of 305 days

Genotype transferrin	First lactation, kg		Second lactation, kg		Third lactation, kg	
	LSM	SE <sub>Lsm</sub>	LSM	SE <sub>Lsm</sub>	LSM	SE <sub>Lsm</sub>
AA	240.78 <sup>AB</sup>	35.38	273.75	48.24	262.83 <sup>ABa</sup>	51.20
AD1	245.19 <sup>C</sup>	35.47	275.72	38.05	303.05 <sup>A</sup>	40.51
AD2	261.62	34.44	288.96	33.00	299.80 <sup>a</sup>	34.95
AE	252.57	35.77	283.85	45.99	293.00	36.59
D1D1	254.00	24.44	271.00	75.06	310.33	55.80
D1D2	267.77 <sup>AC</sup>	40.51	283.35	49.09	293.80 <sup>B</sup>	44.80
D1E	246.50	4.94	280.00	29.32	314.12	32.11
D2D2	257.50	30.69	298.06	33.51	287.70	41.17
D2E	269.76 <sup>B</sup>	41.16	300.57	48.07	292.55	48.07

p<0.01- the same small letters; p<0.05 - the same capital letters

with Tf AA and AD1 genotypes had about one more lactation and a longer productive life by one year (AD1 and AD2 genotype) compared to cows of Tf D1D2 genotype. The longest productive life of 2040 days was observed in a cow of AD2 genotype, which was also the most frequent genotype from (0.29). This fact is very important from the aspect of overhaul, longevity and herd farm management.

Table 3 presents milk yield in the first three standard lactations of 305 days Holstein cows with different Tf genotypes. The analysis of data revealed that Holstein cows on this farm produced, overall, 7969.60 kg of milk in the first lactation, 8985.66

kg of milk in the second lactation and 9002.51 kg of milk in the third lactation. There were statistically significant ( $P<0.05$ ) differences in the milk yield in first lactation between cows of the AA, AD1 and D1D2 genotypes, and cows of the AA and D2E genotypes. In the second lactation, there were statistically significant ( $P<0.05$ ) differences between cows of the D2D2, D2E and AD1 genotypes. In the third lactation, there were statistically significant ( $P<0.01$ ) differences between cows of the AA and AD1 genotypes, and cows of the AA and AD2 genotypes ( $P<0.05$ ). Cows which showed the best performance in the three standard lactations were phe-

Table 5. Milk fat percentage in three standard lactations of 305 days

Genotype transferrin	First lactation		Second lactation		Third lactation	
	LSM	SE <sub>Lsm</sub>	LSM	SE <sub>Lsm</sub>	LSM	SE <sub>Lsm</sub>
AA	3.26	0.15	3.18	0.29	3.22	0.27
AD1	3.23	0.14	3.27	0.26	3.14	0.08
AD2	3.23	0.17	3.20	0.14	3.23	0.25
AE	3.18	0.12	3.18	0.18	3.30	0.37
D1D1	3.27	0.18	3.29	0.15	3.23	0.16
D1D2	3.20	0.17	3.21	0.18	3.22	0.18
D1E	3.15	0.18	3.17	0.19	3.65	0.18
D2D2	3.20	0.14	3.18	0.16	3.20	0.15
D2E	3.20	0.14	3.15	0.10	3.26	0.13

Table 6. LSM and SE<sub>Lsm</sub> of the observed traits in the investigated population of cows with respect to homozygous and heterozygous genotypes

Traits	Homozygous (AA, D1D1, D2D2)		Heterozygous (AD1, AD2, AE, D1D2, D1E, D2E)		
	LSM	SE <sub>Lsm</sub>	LSM	SE <sub>Lsm</sub>	
	Age at first calving, days	746.70	37,76	759.69	44.80
Number of lactation in the life a cow	4.58	1.86	4.09	1.80	
Productive life of cows, days	1984.32	839.35	1810.21	825.63	
Milk yield	first lactation	7843.30	1119.26	8042.77	1236.18
	second lactation	9063.33	1503.95	8915.99	1339.53
	third lactation	8779.45	1625.59	9074.12	1291.83
Milk fat yield	first lactation	252.40	31.71	257.23	37.02
	second lactation	289.72	6.10	285.40	40.32
	third lactation	282.21	46.02	299.38	38.56
Milk fat %	first lactation	3.21	0.15	3.19	0.16
	second lactation	3.19	0.20	3.20	0.17
	third lactation	3.21	0.19	3.29	0.21

novariants Tf D2E (8961 kg milk), AD2 and D2D2 (8794 kg milk) and D1D2 (8765 kg milk), while types Tf D1E (8415 kg milk) phenovariants were the least productive in the analyzed population.

Table 4 characterizes milk fat yield in the three standard lactations of Holstein cows with different Tf genotypes. There were statistically significant ( $P < 0.05$ ) differences in the milk fat yield in first lactation between cows of the D1D2, D2E and AA genotypes, and cows of the AD1 and D1D2 genotypes.

In the second lactation, analysis of variance showed that Tf genotype had no significant effects, while, in the third lactation there were statistically significant ( $P < 0.01$ ) differences between cows of the AA and AD2 genotypes, and cows of the AD1, D1D2 and AA genotypes ( $P < 0.05$ ). In the analyzed population, cows which showed the best performance for milk fat yield in the three standard lactations were phenovariants Tf D2E (287 kg milk fat), AD2 (284 kg milk fat), D2D2 and D1D2 (281 kg milk fat).

Sanzet al. (2010) found significant differences in genotypic frequencies between their population of Holstein cows and also concluded that the Tf genes had an effect on milk fat yield.

Table 5 shows milk fat percentage in the three standard lactations of Holstein cows studied. Analysis of variance showed that Tf genotype had a no significant effect on the percentage of milk fat, produced by the Holstein dairy cattle. The analysis of data revealed that Holstein cows on this farm produced, overall 3.21% milk fat in first lactation, 3.22 % milk fat in second lactation and 3.27 % milk fat in third lactation.

From the results in table 6, it was observed that on average, the heterozygous cows at the Tf locus (Tf AD1, Tf AD2, Tf AE, Tf D1D2, Tf D1E and Tf D2E) produced more milk and milk fat in the first, second and third lactations (averages 8743.22 kg milk) and more milk than the homozygous cows (Tf AA, Tf D1D1 and Tf D2D2; average 8562 kg milk). Homozygous and heterozygous Tf genotypes had some effect on production of milk and milk fat in the first three lactations in the Holstein-Friesian dairy cattle. In research White et al. (1967), cows of genotype DD produced significantly more milk than those of type AA, but their milk fat test was lower and the average milk fat production of the two groups was identical.

## Conclusions

Studies of polymorphic protein systems are increasingly directed toward establishing connection among the genes controlling protein polymorphisms which control polygenic traits related to the productive traits of domestic animals. Determination of this connection has great economic importance for selection and can increase productivity in livestock. Age at first calving, greater number of lactations and longer productive life of cows are very important economic factors which should be considered in rearing and breeding cows.

Transferrin can be used as a supplement to other genetic markers used generally in milk production, i.e. for milk traits and mastitis-related traits in animal breeding and genetics. The use of polymorphic genes as a supplement for a genetic molecular markers is a promising surrogate to the current methods of selec-

tion once these genes are proven to be associated with traits of interest in animals. However, selection effectiveness depends on allelic frequencies in the breeds and on the effect of these polymorphisms on the selected traits.

Based on these results, it can be concluded that the cows with transferrin genotype D2E, AD2, D2D2, and D1D2 had the highest average milk and milk fat yield in the first three standard lactation, and that the cows with transferrin genotype AD2 had recorded the best results in all observed traits, while cows which were heterozygous for transferrin gene had higher milk yield and milk fat than the cows homozygous for transferrin gene.

## *Povezanost genotipova transferina i proizvodnih osobina holštajn frizijskih krava u Vojvodini*

### Sažetak

Cilj ovog rada bio je identificirati genotipove transferina, procijeniti njihovu učestalost u populaciji holštajn-frizijskih krava, te utvrditi njihovu povezanost s proizvodnim osobinama krava. Uzorci krvi za ovo istraživanje uzeti su od 249 zdrave odrasle krave holštajn pasmine, kćeri 18 bikova, uzgojenih na farmi u Vojvodini. Identificirano je devet genotipova transferina, od kojih su tri (AA, D1D1, D2D2) bili homozigoti, a preostalih šest (AD1, AD2, AE, D1D2, D1E, D2E) heterozigoti. Frekvencije genotipova AD2, D1D2, D2D2 i AD1 bile su 0,29, 0,20, 0,17 i 0,11, dok su ostali genotipovi imali frekvencije ispod 1,00. Učestalost alela A, D1, D2 i E, koji su dobiveni iz učestalosti genotipova, iznosila je 0,30 za alel A, 0,19 za alel D1, 0,45 za alel D2 i 0,06 za alel E. U analiziranoj populaciji, krave transferinskog genotipa D2E, AD2, D2D2 i D1D2 imale su prosječno najveću proizvodnju mlijeka i mliječne masti u prve tri standardne laktacije, dok su krave genotipa AA, AD1 i D1E imale najmanju količinu mlijeka i mliječne masti. Analiza varijance pokazala je da genotipovi transferina nisu imali značajnog utjecaja na postotak mliječne masti. Međutim, u analiziranoj populaciji krave transferinskog genotipa D2E, AD2, D2D2 i D1D2 proizvele su mlijeko s najviše mliječne masti. Na temelju ovih istraživanja možemo primijetiti da su krave transferinskog genotipa AD2 imale najbolje rezultate za

sve promatrane osobine, odnosno da su krave heterozigotne za transferin gen imale veći prinos mlijeka i mliječne masti u odnosu na krave homozigotnog genotipa za transferin.

**Ključne riječi:** polimorfizam, transferin, prinos mlijeka, produktivni vijek krave

## References

- Ashton, G.C., Fallon, G.R., Suthcrland, D.N. (1964): Transferrin ( $\beta$ -globulin) type and milk and butterfat production in dairy cows. *Journal of Agricultural Science* 62, 27-34.
- Bagnato, A., Schiavini, F., Rossoni, A., Maltecca C., Dolezal, M., Medugorac, I., Sölkner, J., Russo, V., Fontanesi, L., Friedmann, A., Wolker, M., Lipkin, E. (2008): Quantitative trait loci affecting milk yield and protein percentage in a three-country Brown Swiss population. *Journal of Dairy Science* 91, 767-783.
- Ben Jemaa, S., Fritz, S., Guillaume, F., Druet, T., Denis, C., Eggen, A., Gautier, M. (2008): Detection of quantitative trait loci affecting non-return rate in french dairy cattle. *Journal of Animal Breeding and Genetics* 125, 280-288.
- Casas, E., Keele, J.W., Shackelford, S.D., Koohmaraie, M., Smith, T.P.L., Stone, R.T. (2004): Identification of quantitative trait loci for growth and carcass composition in cattle. *Animal Genetics* 35, 2-6.
- Casas, E., Stone, R.T. (2006): Putative quantitative trait loci associated with the probability of contracting infectious bovine keratoconjunctivitis. *Journal of Animal Science* 84, 3180-3184.
- Excoffier, L., Heckel, G. (2006): Computer programs for population genetics data analysis: a survival guide. *Nature Reviews Genetics* 7, 745-758.
- Fletcher, J., Huehns, E.R. (1968): Function of transferrin. *Proceedings of the National Academy of Sciences of the United States of America* 218, 1211-1214.
- Gahne, B., Juneja, R.K., Grolmus, J. (1977): Horizontal polyacrylamide gradient gel electrophoresis for the simultaneous phenotyping of transferrin, post-transferrin, albumin and post-albumin in the blood plasma of cattle. *Animal Blood Groups and Biochemical Genetics* 8, 127-137.
- Giblett, E.R., Hiekmann, C.C., Smithies, O. (1959): Serum Transferrin. *Proceedings of the National Academy of Sciences of the United States of America* 183, 1581.
- Heringstad, B., Klemetsdal G., Ruane, J. (2000): Selection for mastitis resistance in dairy cattle: a review with focus on the situation in the Nordic countries. *Livestock Production Science* 64, 95-106.
- Huang, J., Wang, H., Wang, C., Li, J. (2010): Single nucleotide polymorphisms, haplotypes and combined genotypes of lactoferrin gene and their associations with mastitis in Chinese Holstein cattle. *Molecular Biology Reports* 37, 477- 483.
- Ju, Z.H., Li, Q.L., Huang, J.M., Hou, M.H., Li, R.L., Li, J.B., Zhong, J.F., Wang, C.F. (2011): Three novel SNPs of the bovine *Tf* gene in Chinese native cattle and their associations with milk production traits. *Genetics and Molecular Research* 10, 340-352.
- Khatib, H., Zaitoun, I., Chang, Y.M., Maltecca, C., Boettcher, P. (2007): Evaluation of association between polymorphism within the thyroglobulin gene and milk production traits in dairy cattle. *Journal of Animal Breeding and Genetics* 124, 26-28.
- Kmiec, M. (1998): Transferyna-biakopeniacewielerołw-organizmie. *Przegląd Hodowlany* 1, 8-9.
- Kristjansson, F.K., Hickman, C.G. (1965): Subdivision of the allele *Tf* for transferrins in Holstein and Ayrshire cattle. *Genetics* 52, 627.
- Milanesi, E., Negrini, R., Schiavini, F., Nicoloso, L., Mazza, R., Canavesi, F., Miglior, F., Valentini, A., Bagnato, A., Ajmone-Marsan, P. (2008): Detection of QTL for milk protein percentage in Italian Friesian cattle by AFLP markers and selective genotyping. *Journal of Dairy Research* 75, 430-438.
- Sanz, A., Ordovás, L., Serrano, C., Zaragoza, P., Altarriba, J., Rodellar, C. (2010): A single nucleotide polymorphism in the coding region of bovine transferrin is associated with milk fat yield. *Genetics and Molecular Research* 9, 843-848.
- Sevi, A., Taibi, L., Albenzio, M., Annicchiarico, G., Muscio, H. (2001): Airspace effects on the yield and quality of ewe milk. *Journal of Dairy Science* 84, 2632-2640.
- Smithies, O. (1959): An improved procedure for starch gel electrophoresis. *Biochemical Journal* 71, 585.
- Vidović, V., Žolt, N., Popović-Vranješ, A., Lukač, D., Cvetanović, D., Štrbac, Lj., Stupar, M. (2013): Heritability and correlations of milk traits in the view of kappa-casein genotypes in Vojvodina Holstein-Friesian dairy cattle. *Mljekarstvo* 63, 91-97.
- Vlloa-Arvizu, R., Gayosso-Vazquez, A., Ramos-Kuri, M., Estada, F.J., Montano, M., Aonso, T.R.A. (2008): Genetic analysis of Mexican Criollo cattle population. *Journal of Animal Breeding and Genetics* 125, 351-359.
- White, M.B., Banfield, J.C. (1967): The distribution of serum transferrin types in dairy cattle and their relationship to milk and butterfat production. *Australian Journal of Experimental Agriculture Animal Husbandry* 7, 396-399.
- Zhang, F., Huang, J., Li, Q., Ju, Z., Li, J., Shi, F., Zhong J., Wang, C. (2010): Novel single nucleotide polymorphisms (SNPs) of the bovine STAT4 gene and their associations with production traits in Chinese Holstein cattle. *African Journal Biotechnology* 9, 4003-4008.