Original scientific paper - Izvorni znanstveni rad UDK: 636.2.045

Genotypic frequencies of the β-lactoglobulin, κ-casein and transferrin in Serbian Holstein-Friesian dairy cattle

Dragomir Lukač[1], *Vitomir Vidović*[1], *Žolt Nemeš*[2], *Milanko Stupar*[1], *Anka Popović-Vranješ*[1]

[1]University of Novi Sad, Faculty of Agriculture, Department of Animal Sciences, Trg Dositeja Obradovića 8, 21000, Serbia
[2]PIK "Bečej", Novosadska 2, 21220 Bečej, Serbia

Received - Prispjelo: 26.06.2013.
Accepted - Prihvaćeno: 05.11.2013

Summary

The study included 765 Holstein cow for determining of β-lactoglobulin genotypes, 420 cows for determining κ-casein genotypes and 252 cows for determining transferrin genotypes, daughters of 18 sires. Among 765 cows, 172 were of the β-LG AA genotype, 448 were of genotype AB, and 145 were of BB genotype. The frequencies of genotypes AA, AB and BB were 0.23, 0.58 and 0.19, respectively. The frequency of alleles A and B, which was derived from the frequency of genotypes, was 0.52 for allele A and 0.48 for allele B. Among 420 cows, 105 were of the κ-casein AA genotype, 219 were of genotype AB, and 96 were of BB genotype. The frequencies of genotypes AA, AB and BB were 0.25, 0.52 and 0.23, respectively. The frequency of alleles A and B, which derived from the frequency of genotypes, was 0.51 for allele A and 0.49 for allele B. Among 249 cows, was identified 9 different genotypes of transferrin, 72 were of the Tf AD2 genotype, 50 were of genotype D1D2, 42 were of genotype D2D2, 28 were of genotype AD1, 20 were of genotype AA, 17 were of genotype D2E, 10 were of genotype AE and less than ten were of genotype D1D2 and D1E.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 studied population of Holstein Friesian dairy cattle in Serbia, a significant number of heterozygous individual and population variability were found. The large variability gives us the opportunity for further selection, favoring the genotype cows depending on the desired properties of milk(milk yield, content of milk fat and proteins) for further technological processing of milk. According to previous studies, it is obvious, that heterozygous cow tended to have a better production performance than the homozygous cows. This genetic information of polymorphic gene could be useful in marker assisted selection to improve production performance.

Key words: polymorphism, β-lactoglobulin, κ-casein, transferrin

Introduction

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 (Vidović et al., 2013). Prediction of the future performance of farm animals is the most rational point in animal breeding and animals of superior traits and phenotype should be selected to hasten genetic improvement. The use of polymorphic genes as genetic molecular markers is a promising surrogate for the current methods of selection once these genes are proven to be associated with traits of interest in animals. Selection effectiveness depends on allelic

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

frequencies in the breeds and on the effect of these polymorphisms on selected traits.

Selection of dairy sires and cows has been based mostly on quantitative traits such as milk, fat or protein yield, which are assumed to be controlled by multiple *loci*. Genetic improvement of quantitative traits is, therefore, relatively slow, as productive traits can only be measured in one sex, and isaffected by numerous polygene's (each polygene exerting a small effect on trait) and environmental factors have an important influence on their expression. This undoubtedly lowers the accuracy of genetic evaluation of sires and cows. In addition, productive traits can only be measured in adult animals, thereby increasing the generation interval and lowering the genetic progress per year. Knowledge of genetic parameters, e.g., heritability, has an important place in modelling genetic progress and selection efficiency to improve milk traits in dairy cattle.

There are many studies about milk protein polymorphism and its relationship with economic traits in livestock animals. Vidović et al. (2013) recommended that genetic variant of milk protein could be a criteria of selection for the improvement of dairy cattle production.

β-lactoglobulin (β-LG) is the major whey protein in ruminant milk. As one of the important genes that may affect economically important traits in cattle, the β-lactoglobulin locus has been previously studied (Tsiaras et al., 2005). The β-LG gene is situated on bovine chromosome 11, and encodes for a single chain polypeptide of 18 kDa comprising of 162 amino acid residues. The complete amino acid sequence of β-LG has been reported and genetic variation in amino acids sequence has been identified (Rachagani et al., 2006). Polymorphism of this gene was discovered in 1955 (Aschaffenburg and Drewry, 1955) and a total of 15 alleles are known, of which, five common variants; A, B, C, D, and E are well identified (Matejicek et al., 2007). Among these common alleles A and B are the most frequent. These two protein variants have small chemical differences between them, where two amino acids, aspartate-64 and valine-118 in variant A are substituted by glycine and alanine, respectively in the B variant (Rachagani et al., 2006).

Many studies have been performed to investigate the effect of β-LG genotypes on milk production traits, milk composition and quality. They found that the AA genotype of β-LG had a favorable effect on protein yield, and the association of significantly higher fat, protein, casein, true protein, and total solids content with BB variant has also been reported (Matejicek et al., 2007). β-LG AA and β -LG BB phenotypes were found to produce higher milk and fat yields than β-LG AB in the Black and White cattle breed by Mayer et al., (1990).

Kappa-casein (κ-casein) is of special interest for a milk protein polymorphism due to its known relation with milk quality and composition. Kappa-casein constitutes approximately 12 % of the total casein. Normally, cow's milk contains 3 to 4 % protein, of which 80 % is casein and 20 % is whey protein (Azevedo et al., 2008). These whey proteins and the κ-caseins are a source of amino acids for the calves, and they also play a crucial role in the coagulation and curdling of milk. Milk protein polymorphisms attract considerable interest because of their potential use as an aid to genetic selection and to genetic characterization of bovine breeds (Caroli et al. 2004). The κ-casein variants A and B differ at amino acid 136 and 148 (Lin et al., 1992). In position 136, amino acid Thr (ACC) is replaced by Ile (ATC) and in position 148, Asp (GTA) is replaced by Ala (GCT). Genetic variability in the κ-casein locus has been reported for several breeds, with allelic frequencies incorporated into studies on genetic diversity among breeds. Several studies have reported that some bovine protein variants, particularly κ-casein, are associated with lactation performance and have a major influence on milk composition and its processing properties, including production technology and cheese yield (Stojčević-Maletić et al., 2012; Hallen et al., 2008; Alipanah et al., 2005; Antunac et al., 1991), and in physiological process such as cytotoxic and antibacterial effects that enhance immunity (Hamza et. al., 2010; Matinand Otani, 2002). Bovenhuis et al. (1992), suggested that, because of economic interests, the favourable milk protein genotype, κ-casein BB, should be included in the criteria for selection of dairy cattle.

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^{3+} ions in association with the binding of an anion, usually carbonate.

Considerable polymorphism and various biological functions of Tf resulted in a situation when it may be used as genetic markers of reproduction and production traits in cattle. Thus, polymorphisms present in bovine Tf could potentially underlie inherited differences in mastitis resistance and milk production traits (Ju et al., 2011). Bovine Tf is encoded by the transferrin gene (Tf), which is located on bovine chromosome 1. Many polymorphisms have been found in the bovine Tf gene (Zhang et al., 2010; Sanz et al., 2010). 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; Milanesi et al., 2008). The health status of the mammary gland greatly affects the biological value of collected milk (Sevi et al., 2001). Mastitis in dairy cattle is a common and costly inflammatory disease of the mammary gland caused by intramammary infections, and leads to reduced yield, degraded quality, reduced lactation persistency, and early culling of cow (Seegers et al., 2003). Environmental and contagious pathogens including *E. coli*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Streptococcus agalactiae* and *Staphylococcus aureus*, are by far the main causes of mastitis (Mason, 2006). In animals with diagnosed mastitis, the Tf concentration in milk is higher than that in healthy animals (Kmiec, 1998). These facts suggest a possible relationship between the Tf gene and mastitis in dairy cattle. Tf may contribute to innate host defense against bacterial and fungal pathogens by limiting microbial access to iron (Chaneton et al., 2008).

Marker-assisted selection that supports fast and low-cost genetic progress and improves the accuracy of selection is desirable. 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). Also, some transferrins are associated with resistance and susceptibility to disease. Tf can be used as a genetic marker in milk production and mastitis-related traits for animal breeding and genetics (Ju et al., 2011).

The black and white Holstein cattle breed is an excellent population to study the genotypic frequencies of β-lactoglobulin, κ-casein and transferrin, because it is a breed that was created by many years by artificial selection for milk traits. The aim of this study was to identify alleles and genotypes of β-lactoglobulin, κ-casein and transferrin in a population of cows evaluate their frequency in Serbian Holstein-Friesian dairy cattle.

Material and methods

The study included 765 Holstein cow for determining of β-lactoglobulin phenotype, 420 cows for determining κ-casein phenotype and 252 cows for determining transferrin phenotype, daughters of 18 sires. The blood samples were kept at 4 °C until isolation of DNA. Isolation of DNA was performed using standard procedures (Sambrook et al., 1989) which included a lyses protocol with Proteinase K in the presence of detergent, phenol-chloroform extraction and ethanol precipitation. After that, 300 ng of DNA was used in 50 μL PCR reactions to yield a 760 bp fragment. PCR reactions were performed as follows: PCR buffer (10 mM TRIS-HCl pH 8.3, 50 mMKCl); 20 pM of each primer; 2.5 mMdNTP; 200 μM $MgCl_2$; 5 U Taq polymerase (Popovski, 1999). PCR steps were: denaturation at 95 °C for 5 min, 3 steps of denaturation at 94 °C/L min, hybridization at 65 °C/L min and subsequent polymerisation at 72 °C during 2 min, with 35 cycles. Termination was followed by a final extension for 5 min at 72 °C. Primers for PCR were designed based on the Gen Bank β-lactoglobulin genomic DNA sequences DQ489319 (Braunschweig and Leeb, 2006). The primer sequences used for the amplification of β-lactoglobulin were as follows: 5′GAGTTGGGCTTCCAGAGTGA-3′ (forward) and 5′GGAATCAAGCTCCCTGCTC-3′ (reverse). The restriction enzyme Hinf I (recognized location 5′ - GANTC - 3′) was used. The primer sequences used for the amplification of κ-casein were as follows: 5′ ATG AAG TTC TTC ATC TTT ACC TGC-3′ (forward) and 5′ GAA GCA GTT AAT TCC AGA ATC TTA -3′ (reverse). Restriction enzyme Hinf I (recognized location 5′ - GANTC - 3′) was used. Transferrin types of the sera were deter-

mined by a modification of the zone-starch-gel electrophoresis method developed by Smithies (1955, 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 (hy-droxymethyl) amino methane 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 β-lactoglobulin, κ-casein and transferring genetic variants. The chi-square test (χ^2) was used to determine whether the populations were in Hardy-Weinberg equilibrium. Mixed Model Equation (MME) was used to analyze fixed effects including: year-season, lactation and genotype (β-lactoglobulin, κ-casein, transferrin) and sire as random effects. The MME used the following model:

$$Y_{ijklm} = \mu + YS_i + L_{ij} + G_{ijk} + S_{ijkl} + E_{ijklm}$$

Y_{ijklm}= total observed traits;
μ = mean value of observed traits;
YS_i= fixed effect of years and season;
L_{ij}= fixed effect of lactation;
G_{ijk}= fixed genotype effects;
S_{ijkl}= random sire effect;
E_{ijklm}= random error.

Results and discussion

The genotypic frequencies and gene frequencies of β-lactoglobulin phenotypes found in the studied population of cows are presented in Table 1.

Among 765 cows, 172 were of the β-LG AA genotype, 448 were of genotype AB, and 145 were of BB genotype. The frequencies of genotypes AA, AB and BB were 0.23, 0.58 and 0.19, respectively. In the current study, β-LG genotype distribution for the studied population, fitted with Hardy-Weinberg equilibrium ($P<0.05$), was similar to that demonstrated by Gouda et al., (2011) in Egyptian Holstein cattle, and Ren et al. (2011) in Chinese Holstein and Jersey cows. The frequency of alleles A and B, which was derived from the frequency of genotypes, was 0.52 for allele A and 0.48 for allele B. This ratio expresses preliminary information about the presence of different genotypes of β-LG in the black and white Holstein cows in the analyzed population.

Ivanković et al. (2011) reported that the frequencies of genotypes AA, AB and BB were 0.08, 0.67 and 0.24, respectively in Croatia Holstein cattle. Previous studies demonstrated that, the heterozyote AB genotypes were found to be more frequent in Holsteien cows (Oner and Elamci, 2006). Similar results were also reported by Gurcan (2011). Notably, the B allele of β-LG gene was found to be the most frequent variant among dairy breeds worldwide. The genotypic frequencies and gene frequencies of κ-casein phenotypes are presented in Table 2.

Table 1. The distribution of β-lactoglobulin and allele frequencies in Holstein cattle, and Hardy-Weinberg equilibrium

	Phenotype			Allelic frequency	
	AA	AB	BB	A	B
Observed frequencies	172	448	145		
Genotype frequency	0.23	0.58	0.19	0.52	0.48
χ^2		14.78			

Table 2. The distribution of κ-casein and allele frequencies in Holstein cattle, and Hardy-Weinberg equilibrium

	Phenotype			Allelic frequency	
	AA	AB	BB	A	B
Observed frequencies	105	219	96		
Genotype frequency	0.25	0.52	0.23	0.51	0.49
χ^2		1.16			

Table 3. The distribution of transferrin and allele frequencies in Holstein cattle, and Hardy-Weinberg equilibrium

Genotype transferrin	Observed frequencies	Genotype frequency	χ^2	Allelic frequency	
AA	20	0.08	151.22	A	0.30
AD1	28	0.11			
AD2	72	0.29		D1	0.19
AE	10	0.04			
D1D1	6	0.02		D2	0.45
D1D2	50	0.20			
D1E	4	0.02		E	0.06
D2D2	42	0.17			
D2E	17	0.07			

Among 420 cows, 105 were of the κ-casein AA genotype, 219 were of genotype AB, and 96 were of BB genotype. The frequencies of genotypes AA, AB and BB were 0.25, 0.52 and 0.23, respectively. In the current study, κ-casein genotype distribution for the studied population, fitted with Hardy-Weinberg equilibrium ($P>0.05$), was similar to that demonstrated by Ma et al. (2007) and Ju et al. (2008) in southern Chinese Holstein cattle, and with that found by Hanusová et al. (2010) in Slovakia. The frequency of alleles A and B, which derived from the frequency of genotypes, was 0.51 for allele A and 0.49 for allele B. This ratio expresses preliminary information about the presence of different genotypes of κ-casein in black and white Holstein cows in analyzed population. A similar result was found in a Brazilian cattle population by Azevedo et al. (2008). In contrast, in relation to allele frequency presented in this research, Ren et al. (2011) observed a higher frequency of allele A (0.69) and lower frequency of allele B (0.31) in Holstein cows in China. Ivanković et al. (2011) concluded that the κ-casein allelic variant A is dominant in selected cattle breeds (60.7-76.4 %), while the share of B variant is significantly more presented in autochthonous cattle breeds (48.2-84.1 %). The genotypic frequencies and allelic frequencies of transferrin phenotypes found in the studied population of cows are presented in Table 3.

Among 249 cows, was identified 9 different genotypes of transferrin, 72 were of the Tf AD2 genotype, 50 were of genotype D1D2, 42 were of genotype D2D2, 28 were of genotype AD1, 20 were of genotype AA, 17 were of genotype D2E, 10 were of genotype AE and less thanten were of genotype D1D2 and D1E. 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 theother genotypeswith frequencies below 1.00. In the current study, Tf genotype distribution for the studied population, fitted with Hardy-Weinberg equilibrium ($P<0.05$), 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 allele D2 and 0.06 for allele E. This ratio expresses preliminary information about the presence of different genotypes of Tf in the black and white Holstein cows in the analyzed population of Serbia. Inthe research of White and Banfield (1967), the gene frequency distribution among nine herds (1102 dairy cows) of predominantly Friesian type cattle was Tf A 40.7 %, TfD 55.9 %, and TfE 5.4 %.

Ashton et al. (1964) reported for the first time a hereditary polymorphism of the transferrins, presenting a system composed of 3 alleles: TfA, TfD and TfE. These three alleles have been described in the European breeds. Alleles Tf B and TfF have been identified in Zebu and in *Bos indicus* crossbreds. The most famous transferrin alleles in European cattle breeds are A1, D1, D2 and E and these alleles are found in Friesian cattle in Egypt (Giblett

et al., 1959).The closer connection between Friesian breeds in Europe and Egypt is due to the genome of breed and the difference in frequencies of some alleles may be due to drift of gene and/or effect of environment on gene expression. Ashton (1964) identified these alleles in Sindhi, Sahival, Brahman and cattle in their crossbreds (Ashton, 1964). TfG allele was observed in Eastern Africa Boran cattle, while Osterhoff and Van Heerden (cited by Buschmann and Schmid, 1968) reported the presence of TfG allele in South Africa Red Poll and Simmental cattle. Transferrin polymorphism is determined genetically by 6 autosomal alleles that can combine into 21 genotypes with as much phenotypes. In the decreasing order of migration, they were noted: G>A>B>D> F>E. Al polymorphism was first reported by Ashton (1964) and Braend and Efremov (1965), who had identified alleles AlA and AlB. Later, Carr (1965) had identified other variants observed in African cattle, while Spooner and Oliver (cited by Cazacu, 1977) reported that AlA allele is genetically fixed in the European breeds.

Conclusions

Holstein-friesian dairy cattle in Serbia demonstrates a high degree of genetic variability for the β-lactoglobulin, with a frequency of 0.52 for allele A and 0.48 for allele, κ-casein, with a frequency of 0.51 for allele A and 0.49 for allele B and transferring (9 different genotypes - AD2, D1D2, D2D2, AD1, AA, D2E, AE D1D2, D1E; or 4 different alleles with a frequency of 0.30 for allele A, 0.19 for allele D1, 0.45 for allele D2 and 0.06 for allele E). A genetic screening program for breeding dairy cattle should be set up in Serbia to increase possibilities for profit and to show new options for milk processing industry. Prediction of the future performance of farm animals is the most rational point in animal breeding and animals of superior traits and phenotype should be selected to hasten genetic improvement.

Frekvencija genotipova β-laktoglobulina, κ-kazeina i transferina u holštajn-frizijskih mliječnih goveda u Srbiji

Sažetak

Istraživanjem je obuhvaćeno 765 holštajn krava za određivanje genotipova β-laktoglobulina, 420 krava za određivanje κ-kazeinskih genotipova i 252 krave za utvrđivanje genotipova transferina, koje su kćeri 18 bikova. Od 765 krava, 172 su bile AA, 448 bile su AB i 145 krava je bilo BB β-laktoglobulinskog genotipa. Frekvencija genotipova AA bila je 0,23, AB 0,58 i BB genotipova svega 0,19. Frekvencije alela A i B, koje potječu od frekvencije genotipova, za alel A je iznosila 0,52 i za alel B 0,48. Od 420 krava, 105 je bilo AA, 219 AB i 96 BB κ-kazeinskog genotipa. Frekvencija genotipova AA, AB i BB varirala je u granicama 0,25, 0,52 i 0,23. Također, frekvencije alela A i B, koje proizlaze iz frekvencije genotipova, iznosio je 0,51 za alel A i 0,49 za alel B. Kod 249 krava, identificirano je 9 različitih genotipova transferina: 72 su bili AD2 Tf genotipa, 50 D1D2 Tf genotipa, 42 D2D2 Tf genotipa, 28 AD1 Tf genotipa, 20 AA Tf genotipa, 17 D2E Tf genotipa, 10 AE Tf genotipa i manje od deset krava bilo je D1D2 i D1E Tf genotipa. Tri Tf genotipa (AA, D1D1 i D2D2) bili su homozigoti a preostalih šest (Tf AD1, AD2, AE, D1D2, D1E, D2E) heterozigoti. Frekvencija alela A, D1, D2 i E, koji potječu od frekvencije genotipova, bila je 0,30 za alel A, 0,19 za alel D1, 0,45 za alel D2 i 0,06 za E alel. U istraženoj populaciji holštajn-frizijskih krava u Srbiji, utvrđen je značajan broj heterozigotnih individua, odnosno izražena je varijabilnost populacije. Velika varijabilnost daje nam mogućnost daljnje i efikasnije selekcije, favorizirajući one genotipove krava ovisno o postavljenim ciljevima, odnosno željenim osobinama mlijeka (količina mlijeka, sadržaj mliječne masti i proteina) za daljnju tehnološku obradu mlijeka. U literaturi je poznato da heterozigotne krave imaju bolje proizvodne performanse nego homozigotne. Ovakve genetske informacije o polimorfizmu gena mogu biti korištene i u marker asistiranoj selekciji za poboljšanje proizvodnih osobina krava.

Ključne riječi: polimorfizam, β-laktoglobulin, κ-kazein, transferin

References

1. Alipanah, M., Kalashnikova, L., Rodionov, G. (2005): Kappa-kasein genotypic frequencies in Russian breeds Black and Red Pied Cattle. *Iranian Journal Biotechnology 3*,191-194.
2. Antunac, N., Lukač-Havranek, J., Čurik, I., Samaržija, D. (1991): Polimorfizam proteina mlijeka u odnosu na proizvodnju i sastav mlijeka. *Mljekarstvo 41*, 297-302.
3. Aschaffenburg, R., Drewry, J. (1955): Occurrence of different β- lactoglobulin in cow's milk. *Nature 176*, 218-210.
4. Ashton, G.C., Fallon, G.R., Suthcrland, D.N. (1964): Transferrin (β-globulin) type and milk and butterfat production in dairy cows. *Journal of Agricultural Science 62*, 27-34.
5. Azevedo, A.L.S., Nascimento, C.S., Steinberg, R.S., Carvalho, M.R.S., Peixoto, M.G.C.D., Teodoro, R.L., Verneque, R.S., Guimarães, S.E.F., Machado, M.A. (2008): Genetic polymorphism of the kappa-casein gene in Brazilian cattle. *Genetics and Molecular Research 7*, 623-630.
6. Bagnato, A., Schiavini, F., Rossoni A., Maltecca C., (2008): Quantitative trait loci affecting milk yield and protein percentage in a three-country Brown Swiss population. *Journal of Dairy Science 91*, 767-783.
7. Bovenhuis, H., Van Arendonk, J.A.M., Korver, S. (1992): Associations between milk protein polymorphisms and milk production traits. *Journal of Dairy Science 75*, 2549-2559.
8. Braend, M., Efremov, G. (1965): Polymorphism of albumin in farm animals. *5th International Congress in Animal Reproduction 7*, 401-403.
9. Braunschweig, M.H., Lebb, T. (2006): Aberrant low expression level of bovine β-lactoglobulin is associated with a C to A transversion in the BLG promoter region. *Journal of Dairy Science 89*, 4414-4419.
10. Buschmann, H., Schmid, O. (1968):Serogroups in animals, Paul Parcy in Berlin and Hamburg (Serumgruppen bei tieren, Paul Parcy in Berlin und Hamburg), 108, 200.
11. Caroli, A., Chessa, S., Bolla, P., Budelli, E., Gandini, G.C. (2004): Genetic structure of milk protein polymorphisms and effects on milk production traits in a local dairy cattle. *Journal of Animal Breeding and Genetics 121*, 119-127.
12. Carr, W.R. (1965): A new bovine haemoglobin variant. *Anim. Breeding Abs. 34*, 2.
13. Cazacu, O. (1977): Hemoglobin - animal genetic types (Hemoglobina - tipurigenetice la animale), Editura Ceres, Bucuresti.
14. Chaneton, L., Tirante, L., Maito J., Chaves J. (2008): Relationship between milk lactoferrin and etiological agent in the mastitic bovine mammary gland. *Journal of Dairy Science 91*, 1865-1873.
15. 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 Biochemistry Genetics 8*, 127-137.
16. Giblett, E.R., Hiekmann, C.C., Smithies, O. (1959): Serum Trasnferrin. *Nature 183*, 1581.
17. Gouda, E.M., Galal, M.K., Wasfy, M.A., Abdelaziz, S.A. (2011): Phenotypes, Genotypes and Allele Frequencies of β-lactoglobulin in Egyptian Cattle and Buffalo. *Journal of Agricultural Science 3*, 203-210.
18. Gurcan, E.K. (2011): Association between milk protein polymorphism and milk production traits in Black and White dairy cattle in Turkey. *African Journal of Biotechnology 10*, 1044-1048.
19. Hallen, E., Wedholm, A., Andren, A., Lunden, A. (2008): Effect of beta-casein, kappa-casein and beta-lactoglobulin genotypes on concentration of milk protein variants. *Journal of Animal Breeding and Genetics 125*,119-129.
20. Hanusová, E., Hub, J., Oravcová, M., Polák, P., Vrtková, I. (2010): Genetic variants of beta-casein in Holstein dairy cattle in Slovakia. *Slovak Journal of Animal Science 43*, 63-66.
21. Hamza, A.E., Wang, X.L., Yang, Z.P. (2010): Kappa Casein Gene Polymorphism in Holstein Chinese Cattle. *Pakistan Veterinary Journal 30*, 203-206.
22. 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.
23. 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.
24. Ivanković, A., Ramljak, J., Dokso, A., Kelava, N., Konjačić, M., Paprika, S. (2011): Genetski polimorfizam β-laktoglobulina i k-kazeina pasmina goveda u Hrvatskoj. *Mljekarstvo 61* (4), 301-308.
25. Ju, Z., Li, Q., Wang, H., Li, J., An, O., Yang, W., Zhong, J., Wang, F. (2008): Genetic polymorphism of κ-casein gene exon4 and its correlation with milk production traits in Chinese Holsteins. *Hereditos 10*, 1312-1318.
26. 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
27. Khatib, H., Zaitoun, I., Chang Y.M., Maltecca, C., (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.
28. Kmiec, M. (1998): Transferrin and white box full niace many roles in the body (Transferyna- bia ko pe niące wiele ról w organizmie). *Przegląd Hodowlany 1*, 8-9.
29. Kristjansson, F.K., Hickman, C. G. (1965): Subdivision of the allele Tf^D for transferrins in Holstein and Ayrshire cattle. *Genetics 52*, 627.
30. Lin, C.Y., Sabour, M.P., Lee, A.J. (1992): Direct typing to milk proteins as an aid for genetic improvement of

dairy bulls and cows. *Animal Breeding 60*, 1-10.

31. Ma, X., Wang, X., Hu, G., Ma, G., Zhao, J., Peng, C., Chang, G. (2007): Analysis of genetic polymorphisms at κ-CN Exon 4 and Exon 5 in southern Chinese Holstein Cattle. *China Dairy Cattle 2*, 5-8.
32. Matejicek, A., Matejickova, J., Nemcova, E., Frelich, J. (2007): Joint effect of CSN3 and LGB genotypes and their relation to breeding values of milk production parameters in Czech Fleckvieh. *Czech Journal of Animal Science* 52, 83-87.
33. Matin, M.A., Otani, H. (2002): Cytotoxic and antibacterial activities of chemically synthesized κ-casecidin and its partial peptide fragments. *Journal of Dairy Research 69*, 329-334.
34. Mason, C. (2006): Basic mastitis bacteriology: untangling the pathogens. *Irish Veterinary Journal 59*, 453-459.
35. Mayer, F., Erhardt, G., Failing, K., Senfti, B. (1990): Investigation on the relationship between milk yield udder health, milk protein and blood protein polymorphism in cattle. *Animal Breeding 58*, 2593.
36. Milanesi, E., Negrini, R., Schiavini, F., Nicoloso, L. (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.
37. Oner, Y., Elmaci, C. (2006): Milk protein polymorphisms in Holstein cattle. *International Journal of Dairy Technology 59*, 180-182.
38. Popovski, Z.T. (1999): Isolation and characterization of thermostabile DNA polymerase from Bacillus caldolyticus. Skoplje, Master work.
39. Rachagani, S., Gupta, I.D., Gupta, N., Gupta, S.C. (2006): Genotyping of beta-lactoglobulin gene by PCR-RFLP in Sahiwal and Tharparkar cattle breeds. *Bio Med Central Genetics 7*, 31-34.
40. Ren, D.X., Miao, S.Y., Chen, Y.L., Zou, C.X., Liang, X.W., Liu, J.X. (2011): Genotyping of the κ-casein and β-lactoglobulin genes in Chinese Holstein, Jersey and Water buffalo by PCR-RFLP. *Journal of Genetics 90*, 1-5.
41. Sambrook, J., Fritsch, E., Maniatis, T. (1989): Molecular cloning. A laboratory manual. Second edition. Cold Spring Harbor Lab. Press, New York.
42. 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
43. Seegers, H., Fourichon, C., Beaudeau, F., (2003): Production effects related to mastitis and mastitis economics in dairy cattle herds. *Veterinary Research 34*, 475-491.
44. Sevi, A., Taibi, L., Albenzio, M., Annicchiarico, G. (2001): Airspace effects on the yield and quality of ewe milk. *Journal of Dairy Science 84*, 2632-2640.
45. Smithies, O. (1955): Zone electrophoresis in starch gel. *Biochemical Journal 61*, 629.
46. Smithies, O. (1959): An improved procedure for starch gel electrophoresis. *Biochemical Journal 71*, 585.
47. Stojčević-Maletić, J., Vidović, V., Lukač, D., Štrbac, Lj., Stupar, M. (2012): Polymorphism and effect of capa casein on technological quality milk for trapist cheese production. *6th Central European Congress on Food*, Novi Sad, 23-26 May, 1675-1678.
48. Tsiaras, A.M., Bargouli, G.G., Banos, G., Boscos, C.M, (2005): Effect of kappa casein and beta lactoglobulin loci on milk production traits and reproductive performance. *Journal of Dairy Science 88*, 327-334.
49. 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* (2), 91-97.
50. 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 and Animal Husbandry 7*, 396-399.
51. Zhang, F., Huang, J., Li, Q., Ju, Z. (2010): Novel single nucleotide polymorphisms (SNPs) of the bovine STAT4 gene and their associations with production traits in Chinese Holstein cattle. *Genetics and Molecular Research 9*, 4003-4008.