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Association of polymorphism κ-casein gene with longevity and lifetime production of Holstein-Friesian cows in Vojvodina

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Abstract

The aim of this study was to identify frequencies of alleles and genotypes, to evaluate their frequency in population of Holstein-Friesian cows, and to investigate association of κ-casein genotypes with longevity and lifetime production of cows. Blood samples were collected from 192 Holstein cows which have finished their production. Cows were reared at one farm in Vojvodina in Serbia. In the current study the following ratios of κ-casein genotypes were found: 0.50 were AA, 0.40 were AB and 0.10 were BB. Frequencies of alleles A and B were 0.70 and 0.30, respectively. Based on these results, it can be concluded that cows with heterozygous genotype AB had the longest life (2809 days) with the highest number of productive days (2062), while the cows which were homozygous recessive of genotype BB had the shortest life expectancy (2627 days) and the lowest number productive days (1878). The same trend was observed with regard to the production results of cows of these genotypes, where statistically significant differences have been observed ($P<0.05$) in the most traits between cows of heterozygous and homozygous genotypes, while between cows of dominant and recessive homozygous genotype statistically significant differences were not observed ($P>0.05$). The effect of dominant genes in observed traits was greater than the additive effect of genes. From the ratio of the additive and dominant gene effects, it can be seen that the average degree of dominance in observed traits was positive.

Key words: polymorphism, κ-casein, productive life of cow

Introduction

One of the primary interests among breeders of dairy cows is a cheaper and more economical production, which is reflected in the amount of milk produced and obtained calves per cow. This can only be achieved if cows remain long enough in production, without compromising health or reproductive performance. Greater number of lactations, and good reproductive health, high productivity and long life period reduce annual costs of herd turn-overs, thereby getting a higher number of cows in late lactation, which has significantly higher production compared to cows in early lactation. The longevity of a cow is normally defined as the length of its productive life in the herd (Jovanovac et al., 2013; Effa et al., 2013). Longevity, as measured by the length of productive life, demonstrates the ability of the individual to remain as long as possible in the production, and the ability for no any reason to be

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extracted from heard or even due to low production. From an economic and the selection point of view can be concluded that the ability of cows for high production and reproduction through many years desirable traits in dairy cattle breeding (Chirinosa et al., 2007; Effa et al., 2013).

Selection at the level of genes has become a powerful tool for genetic improvement of animal selection and production (Rachagani and Gupta, 2008; Dogru and Ozdemir, 2009).The most commonly studied genetic forms of milk protein is casein, which in its various forms makes up 80 % of all milk protein, of which κ-casein of 12 % of the total casein, which is the most common genetic form of the test. The κ-casein variants A and B differ at amino acid 136 and 148 (Lin et al., 1992). In position 136, The (ACC) is replaced by Ile (ATC), and in position 148, Asp (GTA) is replaced by Ala (GCT). 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 (Hallen et al., 2008; Alipanah et al., 2007; Kastonina et al., 2004; Celik, 2003), and in physiological process such as cytotoxic and antibacterial effects that enhance immunity (Hamza et. al., 2010; Matin and Otani, 2002). Genotype κ-casein in addition to being associated with lactation traits, he has a great influence on physiologically processes involved in improving immunity and therefore the longevity of cows (Matin and Otani, 2002). It is thus very useful to study the genetic variations of candidate genes and their association with milk production and milk traits (Caroli et al., 2009; Khatib et al., 2007).

Soria et al. (2003) reported 11 different types of alleles κ-casein (A, B, C, D, E, F, G, H, A1), while in their research Caroli et al. (2009) reported 14 polymorphic forms of κ-casein (A, A1, B, B2, C, D, E, F1, F2, G1, G2, H, I, J). In addition, other genetic forms were also detected in several breeds, but often low frequency (Sulimova et al., 2007). However, the two most common alleles that is present in dairy herds are A and B allele (Dokso et al., 2014; Ju et al., 2011; Anggraeni et al., 2010). By literature, the B allele has previously found to be associated with thermal resistance, shorter coagulation time, better curdling and micelles of different sizes, which are preferable in cheese making. K-casein B allele was reported to have a favourable and significant effect on both milk and milk protein yield (Patel et al., 2007).

The aim of the research was to identify the frequency of alleles and genotypes of κ-casein, and to examine their association with the total lifetime production of cows in Vojvodina.

Materials and methods

The study included 192 Holstein-Friesian cows from one farm in Vojvodina, which ended its production, so that we can determine the total lifetime production of each cow. The main criteria for cows excluding from production was the decrease in daily milk yield beneath the 10 kg per day which were not fertile. After collection of blood the samples were kept at 4 °C until isolation of DNA. Isolation of DNA was performed using standard procedures (Sambrook et. al., 1989) which includes a lysis protocol with Proteinase K in the presence of detergent, phenol-chloroform extraction end ethanol precipitation. After that, 300 ng of DNA were 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 mM KCl); 20 pM of each primer; 2.5 mM dNTP; 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 for 1 min, hybridization at 65 °C, 1 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. 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 for digestion

The effects of additivity and dominance were calculated using the methodologies described by Falconer and Mackay (1996). The additive effect was calculated as half of the difference between the homozygote genotypes (AA - aa)/2, while the dominant effect was calculated as the deviation of the heterozygote genotype from the homozygote genotype (Aa- 0.5 (AA +aa)).

The chi-square test (χ^2) was used to check whether the populations were in Hardy-Weinberg equilibrium. For investigated traits adjusted mean LSM (Least Square Means) and standard errors of the means (SE_{Lsm}) were calculated with in statistical softwere Statistica 12.

The linear model was as follows:

$$Y_{ijkl} = \mu + YS_i + L_{ij} + G_{ijk} + e_{ijkl}$$

where Y_{ijkl} = mean phenotypic value of observed trait; μ = average mean value of observed traits; YS_i = fixed effect of years and season; L_j = fixed effect of lactation number; G_k = fixed genotype effects and e = random error.

Results and discussion

The genotypic frequencies and gene frequencies of κ-casein phenotypes found in the studied population of cows are presented in Table 1. Among 192 cows, 97 were of the κ-casein AA genotype, 77 were of genotype AB, and 18 were of BB genotype. The frequencies of genotypes AA, AB and BB were 0.50, 0.40 and 0.10, 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. Since the obtained value of $\chi2$ test 0.203 and p 0.903, it can be concluded that the frequencies of genotypes in observed population does not differ significantly from those frequencies that are expected. The frequency of alleles A and B, which was derived from the frequency of genotypes, was 0.70 for allele A and 0.30 for allele B. This ratio expresses preliminary information about the presence of different genotypes of κ-caseins in the black and white Holstein cows in the analyzed population.

Based on the obtained frequency of dominate alleles, which was higher than the frequency of the recessive alleles, it can be concluded that the expression of characteristics prevalent dominant over recessive genes.

In Table 2 is presented the least-squares means (LSM), errors of the means (SE_{Lsm}) and analysis of investigated traits. From the table we can see that the longest life expectancy had cows of heterozygous AB genotype, whereas cows of dominant homozygous genotype AA and recessive homozygous genotype had a shorter life span for 136 and 182 days, compared to cows of heterozygous genotype. The largest number of lactations and therefore most of the lactation days number in the life had also cows of AB genotype ($P<0.05$), whereas cows BB and AA genotypes had shorter lactation number for 157 days, or 138 days compared to the heterozygous cows genotype. Cows with genotype AB achieved maximum lifetime milk production ($P<0.05$) (to 519 kg with respect to the cow and BB and 345 kg in comparison to the AA genotype cow), milk fat ($P<0.05$) (for 134 kg of the cows in relation to the BB and 97 kg of the cows in relation to the AA genotype) and proteins ($P<0.05$) (for kg of 169 in relation to the cow BB, and 100 kg in relation to the cow of AA genotype), whereas the percentage of the milk fat and the protein was not significantly different between genotypes ($P>0.05$). Productive life of cows ranged from 1878 days in cows genotype BB, then 1912 days in the cow genotype AA, while cows of heterozygous genotype AB achieved a productive life of 2062 days.

Vidović et al. (2013) reported that cows with κ-casein AA and AB genotypes produced respectively

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

Frequencies	Casein genotypes			Allelic frequency	
	AA	AB	BB	A	B
Observed	97	77	18	0.70	0.30
Genotype	0.50	0.40	0.10		
Expected	94.08	78.72	19.2		
Genotype	0.49	0.41	0.09		
χ^2 = 0.203	df = 2	*p value* = 0.903			

322.72 and 464.73 kg more milk per year compared to cows of casein BB genotype (P<0.05). Hristov et al. (2012) found that the cows of heterozygous κ-casein genotype AB had about 12 % higher milk yield production and milk fat production in compare to the cow of homozygous BB genotype, and approximately for 7 % in compare to the cows of AA genotype. In the research of Denisenko (2004), milk of cows with BB genotype had the highest percentage of fat and protein in relation to the other two genotypes (AA, AB), with significant differences in milk yield, fat and protein percentage. Dokso et al. (2014) in their research show that the most unfavorable genotype is κ-casein, given the amount of milk produced during the first three standard lactation of Simmental cows found it to BB, while cows with AA genotype produced the highest amount of milk.

On the basis of phenotypic values of observed traits depending on the genetic variants of κ-casein, it may be noted that the additive and dominant genes play an important role (Table 2). In addition to additive (individual allelic effects), dominance

Table 2. Lifetime production of Holstein-Friesian cows, depending on the κ-casein genotype

Traits	Genotype of κ-casein	LSM	SE_{Lsm}	Effects	
				Additive [a]	Dominant [d]
The lifetime of a cow, days	AA	2673.16[a]	86.17	22.83	158.83
	AB	2809.16[b]	95.96		
	BB	2627.50[a]	95.88		
Number of cows lactations during the life	AA	4.33[a]	0.19	-0.02	0.21
	AB	4.56[b]	0.21		
	BB	4.38[a]	0.43		
Number of cows lactations days during the life	AA	1518.48[a]	68.66	9.16	146.86
	AB	1656.18[b]	76.46		
	BB	1500.16[a]	156.08		
Lifetime milk productivity, kg	AA	38639.08[a]	1827.31	861.98	4329.03
	AB	42106.12[b]	2034.81		
	BB	36915.11[a]	4153.53		
Lifetime milk fat productivity, kg	AA	1267.24[a]	59.03	22.67	119.96
	AB	1364.54[b]	65.74		
	BB	1221.89[a]	134.19		
Lifetime milk fat productivity, %	AA	3.28	0.01	-0.01	-0.05
	AB	3.24	0.01		
	BB	3.31	0.03		
Lifetime milk protein productivity, kg	AA	1213.26[a]	123.09	34.45	134.90
	AB	1313.71[b]	139.57		
	BB	1144.36[a]	261.12		
Lifetime milk protein productivity, %	AA	3.14	0.04	0.02	0.00
	AB	3.12	0.04		
	BB	3.10	0.09		
Productivity life of cow, days	AA	1912.29[a]	85.96	16.98	167.43
	AB	2062.74[b]	95.72		
	BB	1878.33[a]	195.39		

P<0.05 - different small letters; P>0.05 - same small letters effects (specific effects for allele combinations) as well as interactions between κ-casein alleles and genes of the populations (epistatic effects) are possible. However, the effect of dominant genes on the observed traits was greater than the additive effect of genes. From the ratio of the additive and dominant gene effects, it can be seen that the average degree of dominance in observed traits were positive, indicating partial dominance in the inheritance of traits. Positive additive gene effect was detected in the majority of traits, except for the total number of lactations and lifetime production of milk fat which recorded a slightly negative effect of additive genes.

Conclusions

The proportions of the three κ-casein genotypes found in the cow population studied were similar to other studies. The ratios of κ-casein genotypes were found: 0.50 were AA, 0.40 were AB and 0.10 were BB. Frequencies of alleles A and B were 0.70 and 0.30, respectively. Based on the gained results ($P<0.05$) it can be concluded that the cows heterozygous for genotype AB lived the longest with the best productive results during the exploitation period in regard to cows of dominant AA and recessive BB homozygous genotype. Considering genetic aspects and productivity, it would be desirable to increase the share of cows with genotype AB. Attention should be also paid to environmental factors in milk production as well. A genetic screening program for breeding dairy cattle should be set up in Vojvodina to increase possibilities for profit.

Povezanost polimorfizma gena κ-kazeina s dugovječnošću i životnom proizvodnjom krava holštajn-frizijske pasmine u Vojvodini

Sažetak

Cilj istraživanja bio je identificirati frekvencije alela i genotipova u populaciji krava holštajn-frizijske pasmine goveda, te istražiti njihovu povezanost s dugovječnošću i životnom proizvodnjom. Uzorci krvi uzeti su od 192 krave koje su završile svoju životnu proizvodnju. Krave su uzgajane na farmi u Vojvodini (Srbija). Identificirane su sljedeće frekvencije genotipova κ-kazeina: 0,50 za AA, 0,40 za AB i 0,10 za BB. Frekvencije alela A i B bile su 0,70, odnosno 0,30. Na temelju dobivenih rezultata može se utvrditi da su krave heterozigotnog genotipa AB bile najdugovječnije (2809 dana) s najvećim brojem produktivnih dana (2062) i ostvarile najbolju životnu proizvodnju, za razliku od krava homozigotnog recesivnog genotipa BB, koje su imale najkraći životni vijek (2627 dana) i najmanji broj produktivnih dana (1878). Ista tendencija zapaža se kada su u pitanju i proizvodni rezultati krava ovih genotipova, gdje su utvrđene statistički značajne razlike ($P<0{,}05$) u većini promatranih osobina između krava heterozigotnog i homozigotnog genotipa, dok između krava dominantnog i recesivnog homozigotnog genotipa nije utvrđena statistički značajna razlika ($P>0{,}05$). Aditivni i dominantni učinak gena kod većine promatranih osobina bio je pozitivan. Upotrebom polimorfnih oblika gena kao genetskih molekularnih markera u mliječnom govedarstvu značajno bismo poboljšali proizvodne osobina mliječnih goveda, uvođenjem poželjnih genotipova krava u populaciju.

Ključne riječi: polimorfizam, κ-kazein, produktivan život krava

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