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Heritability and correlations of milk traits in the view of kappa-casein genotypes in Vojvodina Holstein-friesian dairy cattle

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Summary

In the current study, among 420 Holstein cows, the following ratios of κ-casein genotypes were found: 0.25 were AA, 0.52 were AB and 0.23 were BB. Frequencies of alleles A and B were 0.51 and 0.49, respectively. Cows with κ-casein AA and AB genotypes produced 322.72 and 464.73 kg more milk per year compared to cows of casein BB genotype (P<0.05), respectively. There were no significant differences in the amount of milk produced by cows of AA and AB casein genotypes. There were no statistically significant differences in overall yields (kilograms per year) of milk fat and milk protein between κ-casein genotypes of cows. In contrast, milk from genotype BB cows contained a significantly (P<0.05) higher percentage of milk fat compared to the milks of casein genotypes AA and AB. In spite of the different κ-casein genotypes having a significant influence on milk yield and fat content, heritability estimations showed that all three genotypes were of medium heritability, and heritability did not differ significantly between them. Variability values (of examined traits) were stable and large enough to ensure selection gains in future generations.

Key words: κ-casein genotypes, milk traits, heritability

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 a great economic importance for selection and can increase productivity in livestock (Vidović and Lukač, 2010).

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 is affected 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 a place in modelling genetic progress and selection efficiency to improve milk traits in dairy cattle.

Since selection efficiency is based on the dam side, but the outcomes are seen in the offspring later chosen to be sires, knowledge of κ-casein genotype variation can play an important role.

For this reason, qualitative characters, such as polymorphisms in blood groups, enzymes, blood

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serum proteins or milk protein types are among those being investigated for the possibilities which they provide for improving the accuracy of sires and cows genetic merit estimation and practicing selection at an earlier age (Lin et al., 1992). Studies have been conducted to determine the frequencies of genetic variants of milk proteins in different cattle breeds (Vohra et al., 2006).

Kappa-casein is of special interest for a milk protein polymorphism due to its known relation with milk quality and composition (Scheepers et al., 2010). Kappa-caseins are milk proteins secreted by mammary gland cells (Azevedo et al., 2008). Kappa-casein constitutes approximately 12 % of the total casein (Azevedo et al., 2008). Normally, cow's milk contains 3 to 4 % protein, of which 80 % is κ-casein and 20 % is whey protein. These whey proteins and the κ-caseins are a source of minerals and amino acids for the calves, and they also play a crucial role in the coagulation and curdling of milk (Patel et al., 2007).

Milk protein polymorphisms attract considerable interest because of their potential use as an aid to genetic selection and to genetic characterization of bovine breeds (Golijow et al., 1996; Kemenes et al., 1999; Caroli et al. 2004; De Marchi et al., 2008). The κ-casein variants A and B differ at amino acid 136 and 148 (Lin et al., 1992; Alexander at. al., 1988). In position 136, The (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 (Golijow et al., 1996; Del Lama and Zago, 1996; Kemenes et al., 1999). 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; Kastonina et al., 2004; Denisenko and Kalashnikova, 2004; Konovalova 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). 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.

With techniques based on DNA analysis, which include polymerase chain reaction and restriction fragment length polymorphisms (PCR-RFLP) methods (Medrano and Aguilar-Cordova, 1990; Denicourt et al., 1990), it is possible to determine the κ-casein genotype of all individuals in a given population under selection, regardless of sex, age or physiological status. As a result, it is now possible to include information on milk protein genotypes into selection programs, which should result in more accurate predictions of breeding values of animals to be selected, and thus improve response to selection. Genetic variability in the κ-casein locus has been reported for several breeds, with allelic frequencies incorporated into studies on genetic diversity among breeds (Golijow et al., 1996; Del Lama and Zago, 1996; Kemenes et al., 1999).

Black and white Holstein cattle breed is an excellent population for monitoring of κ-casein influence on the quantity and composition of milk, because it is a breed that was created by many years of artificial selection on milk traits. The aim of this study was to identify alleles (A and B) and genotypes (AA, AB and BB) of kappa casein, evaluate their frequency in the population of cows, and study the effect of the kappa casein genotypes on milk quantity and composition. Moreover, the purpose of the study was to estimate the genetic parameters of milk traits for different κ-casein genotypes which could be detected in young calves, and used to evaluate their potential use for selection.

Material and methods

The study included 420 Holstein cow, daughters of 18 sires (6 for each genotype group AA, AB and BB). 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 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. Direct counting was used to estimate phenotype and allele frequencies of κ-casein genetic variants. The chi-square test (χ^2) was used to check whether the populations were in Hardy-Weinberg equilibrium. Mixed Model Equation (MME) was used to analyze fixed effects including: year-season, lactation and κ-casein genotype. Sire was random effect. Heritability was estimated from half sibs groups for each κ-casein genotype effect and the total genotype effect used all data. The following 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 κ-casein phenotypes are presented in Table 1. 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 Hanusova 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, as did Golijow et al. (1999) in Argentina (A=0.65, B=0.34). According to 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. Kappa-casein B allele was reported to have a favourable and significant effect on both milk and milk protein yield (Patel et al., 2007).

Table 2 shows the effect of the κ-casein gene on the milk production traits in the cows studied. Cows with κ-casein AA and AB genotypes produced respectively 322.72 and 464.73 kg more milk per year compared to cows of casein BB genotype (P <0.05). There were no significant differences in the amount of milk produced by cows of AA and AB casein genotypes. In a study of Bovenhuis et al. (1992), κ-casein genotype had a significant effect on milk production (P<0.001), with Pantaneiro cows of the BB genotype that produced 173 kg less milk than AA cows. In another study, Alipanah et al. (2005) showed that the milk from cows with the BB genotype contained the highest percentage of fat and protein among the genotypes studied.

According to Denisenko (2004) and Patel et al. (2007), cheese production can be increased by 5% if milk derives from cows of the κ-casein BB genotype, when compared to the milk from AA animals. In the Holstein and Jersey breeds it has been shown that the B allele is associated with higher protein content in milk (Denisenko, 2004), and it has been suggested that appropriate weights could be given to genotypic information and polygenic breeding value in order to improve selection response (Van Arendonk and Bovenhuis, 1996).

The B alleles may allow improvement in the quality of milk for manufacturing processes, primarily because milk from cows possessing the B allele at the κ-casein locus was superior for cheese making, due to faster coagulation and firmer curd (Lunden et al., 1997).

Table 1. 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 2. Quantity and quality of milk yield in lactation with different κ-casein genotypes

Analyzed parameters of cow milk		AA	AB	BB
		$\bar{x} \pm S$	$\bar{x} \pm S$	$\bar{x} \pm S$
Milk yield (kg)		8582.72±1570.43[a]	8724.73±1403.17[a]	8260±1254.63[b]
Fat	(kg)	278.02±46.49	279.68±42.31	269.95±41.63
	(%)	3.25±0.18[a]	3.21±0.18[a]	3.27±0.17[b]
Protein	(kg)	283±50.88	274.50±21.46	264±62.22
	(%)	3.19±0.27	3.14±0.13	3.13±0.98

P<0,01 - different small letters; P<0,05 - same small letters

Table 3. Influence of different genotypes of cows (AA, AB, BB) on the fat, protein content and yield of milk

Source of variation		DF	SS	MS	F	P
Milk yield (kg)		2	2018.67	1009.33	2.02	0.133
Fat	(kg)	2	3662	1831	0.93	0.395
	(%)	2	0.228	0.114	3.54	0.029*
Protein	(kg)	2	7.89.3	394.6	0.21	0.813
	(%)	2	0.013	0.006	0.12	0.886

*P<0,05 - significant; DF - degree of freedom; SS - sum of squares; MS - middle of the squares; F - value; P - probability

Previous studies have shown that milk from A allelic variants of κ-casein required a longer time for renneting (Lunden et al, 1997; Kubarsepp et al., 2005) and gave lower cheese yield with lower protein content (Braunschweig et al., 2000; Miceikiene et al., 2005; Molina et al., 2006). The cheese yield from cows with genotype BB was 10 % higher when compared to AA cows (Azevedo et al., 2008).

In presented study there were no statistically significant differences in yields of milk fat and milk protein (kilograms) between κ-casein genotypes of cows, but in contrast, there were statistically significant differences in the percentage of milk fat produced by cows of the AA, AB and BB genotypes. Milk from genotype BB cows contained a slightly higher percentage of milk fat compared to the milks of casein genotypes AA and AB (P<0.05) (Table 2). In study of Botaro et al. (2009), cows of AB and BB genotypes showed higher milk fat content when compared to the AA genotype. Because of the effects of κ-casein genetic variants on cheese yield, selection of animals with the favourable κ-casein B allele is desirable.

Kappa-casein genotype had a significant impact on the milk fat content, but had no influence on the other observed properties of milk (fat and protein yield, content of protein; Table 3). In a

Table 4. Heritability of milk traits at different κ-casein genotypes

Cow milk traits		AA	AB	BB	All Genotype
		$h^2 \pm S_h{}^2$	$h^2 \pm S_h{}^2$	$h^2 \pm S_h{}^2$	$h^2 \pm S_h{}^2$
Milk yield (kg)		0.245±0.20	0.246±0.19	0.238±0.24	0.261±0.12
Fat	(kg)	0.236±0.18	0.239±0.21	0.229±0.23	0.223±0.11
	(%)	0.239±0.19	0.243±0.21	0.234±0.23	0.209±0.10
Protein	kg)	0.227±0.22	0.223±0.20	0.221±0.24	0.214±0.11
	(%)	0.224±0.23	0.231±0.20	0.212±0.24	0.201±0.09

study of Alipanah et al. (2005), the effect of polymorphism of κ-casein on fat and protein content was determined. Bovenhuis et al. (1992) found that the κ-casein genotypes had a great effect on protein content, such that κ-casein BB cows produced milk with 0.08 % higher protein content compared to AA cows. The same authors found that the effect of κ-casein genotype on fat content was not determined, while for fat yield, the B allele was associated with a significantly lower production of fat when compared to the A allele.

Even though different κ-casein genotypes had a significant influence on milk yield and fat content, heritability estimates showed the genotypes were only of medium heritability, and the heritability did not differ significantly between the genotypes. The additive genetic variation for the different genotypes of the examined traits was similar for each genotype, and large enough to provide new genetic progress and gains in the future. However, the standard error for the different genotypes shows the necessity of using larger animal populations in future studies.

Conclusions

The proportions of the three κ-casein genotypes found in the cow population studied were similar to other studies. The cows studies carried allele A and allele B in almost equal numbers (51 % carried allele A; 49 % carried allele B). Cows with κ-casein AA and AB genotypes produced respectively 322.72 and 464.73 kg more milk per year compared to BB genotype casein cows. These differences were statistically significant ($P<0.05$). Milk from BB cows contained the most milk fat. It would be desirable to increase the proportion of BB cows, in order to produce more milk fat and hence, more dairy products. If market requires, it would be better to breed more AA and AB cows, as they produce more milk overall, and more protein. In some case farmers should be aware of the milk type they require for their individual market (for cheese, raw milk, cream, butter, whey etc). Even for the genotypes differences regarding κ-casein, the heritability estimations for examined traits were similar. Attention should be paid also to environmental factors in milk production as well. 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.

Nasljednost i korelacije osobina mlijeka u odnosu na genotipove kapa kazeina u holštajn-frizijskih mliječnih goveda u Vojvodini

Sažetak

U istraživanje je bilo uključeno 420 krava holštajn pasmine, kod kojih su utvrđeni sljedeći odnosi κ-kazeinskih genotipova: 0,25 - AA, 0,52 - AB i 0,23 je bilo BB genotipa. Frekvencija alela A bila je 0,51 i alela B 0,49. Krave κ-kazeinskog genotipa AA i AB proizvele su 322,72, odnosno 464,73 kg više mlijeka godišnje u odnosu na krave BB kazeinskog genotipa ($P<0,05$). Nisu utvrđene značajne razlike u količini mlijeka između krava AA i AB κ-kazeinskog genotipa. Također, nisu utvrđene niti statistički značajne razlike u ukupnoj količini (kilograma godišnje) mliječne masti i bjelančevina između κ-kazeinskih genotipova krava. Nasuprot tome, utvrđene su statistički značajne razlike u postotku mliječne masti u mlijeku krava BB genotipa, u odnosu na krave AA i AB kazeinskog genotipa. Unatoč različitim kapa kazeinskim genotipovima, koji imaju značajan utjecaj na prinos mlijeka i postotak mliječne masti, procijenjena heritabilnost osobina pokazuje da su kod sva tri genotipa srednjeg

stupnja nasljednosti, gdje se nasljednosti između njih nisu razlikovale. Varijabilnosti promatranih osobina bile su stabilne i dovoljno velike da osiguraju selekcijski napredak u budućim generacijama.

Ključne riječi: κ-kazeinski genotipovi , mliječne osobine, nasljednost

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