STUDY OF THE MILK PROTEIN GENETIC CHARACTERIZATION IN LATVIAN DAIRY CATTLE BREEDS POPULATIONS

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ABSTRACT
The aim of this study in Laboratory of Molecular Genetic Research of Faculty of Agriculture in LLU was to perform an initial characterization of milk quality at the protein level of β-casein (*CSN2*) alleles A1 (*CSN2*A1) and A2 (*CSN2*A2) and also at the protein level of κ-casein (*CSN3*) in two different Latvian cattle breeds (Latvian Brown and Latvian Blue). A wide variation in the *CSN3* allele B frequency among Latvian Blue (LZ) and Latvian Brown (LB) breeds was found suggesting that the molecular selection for animals carrying the allele B could impact breeding programs for dairy production in Latvia. The genotyping of *CSN2* alleles A1 and A2 is of the practical importance since the *CSN2*A1 associated with the liberation of β-casomorphin-7 and other bioactive peptides with the opioid nature and cause of human non-communicable diseases. The relatively high incidence of the *CSN2* allele A2 is characterisation for the Latvian cows breed populations and this special allele distribution could be used to develop selection strategies to breed specialised lines of Latvian local breeds. The results obtained are as follows: the LB breed (n=96) allele's frequency of *CSN2*A1 is 0.703, the LZ breed (n=95) allele's frequency of *CSN2*A1 is 0.811.

KEY WORDS: cows, κ-casein, β-casein, gene polymorphism, Gene Assisted Selection (GAS).

INTRODUCTION

Caseins are milk proteins secreted by mammary gland cells. They constitute about 78-82% of bovine milk proteins and subdivided into four main groups: αS1-casein, αS2-casein, β-casein (*CSN2*), and κ-casein (*CSN3*) (Eigel et al., 1984). These proteins and their genetic variants have been extensively studied and reported as an important factor associated with lactation performance, milk composition and cheese yield efficiency (Aleandri et al., 1990). Bovine casein is encoded by a 200 kb DNA fragment located at chromosome (Chr) 6 q31-33 (Ferretti et. al., 1990) arranged in the order of αS1, αS2, β and κ. κ-CN fragment spans the 13 kb DNA sequence divided into five exons and intervening sequences and constitutes about 25% of the casein fraction (Martin et al., 2002). The αS1-, β-, and αS2-casein is at least 70 kb away from them (Ferretti et al., 1990).

Kappa-casein (*CSN3)* has been extensively studied for its role in stabilizing the casein micelles and its influence on the manufacturing properties of milk. For several breeds, the genetic variability in the *CSN3* locus has been reported each with a different allelic frequency based on genetic diversity among breeds. Various allelic variants have been described for *CSN3* gene in different cattle breeds, which include alleles A, B, C, E, F, G, H, I and AI (See review by Soria et al., 2003). Among these, variants of *CSN3* alleles A and B are most commonly found and variant of *CSN3* allele B is predominantly concerned with processing properties of milk and has better lactodynographic properties (Lin et al., 1992). In variant B, due to a single base mutation in the *CSN3* locus, isoleucine substitute threonine and aspartic acid is substituted by alanine (Pinder et al., 1991). Selection for the B allele of *CSN3* gene is integrated into cattle breeding programs in many countries and it also have should be done in Latvia too.

Controversial and still unresolved is the role of β-casein *(CSN2)* genetic variants A1 and A2 on dietetic value of milk and it impact up on human health. Studies of *CSN2* genetic polymorphism, which began in 1961 with works of R. Aschaffenburg (1) have a wealth of material on the *CSN2* gene, more than 10 genetic variants and their occurrence in different breeds of cows and their possible correlation with productivity traits. Therefore unanswered questions remain on the role of *CSN2* allele A1 and partly also of *CSN2* allele A2 in liberation of β-casomorphin-7 and other bioactive peptides in the human digestive tract, and their further fate. Many studies point to opioid nature of β-casomorphin-7 and other peptides and the possible effects up on the central nervous system, a certain relationship with the child sudden death syndrome, atherosclerosis, cardiovascular disease and insulin-dependent diabetes mellitus (DM-1) (McLachlan, 2001, Elliot et al., 1999, Teschemacher, 2003). The trustful association between using of *CSN2*A1 milk and DM-1 had shown by epidemiological situation in 16 countries of the World, and had calculated correlation of that factor which is very high (r=0.75) (Laugesen un Elliott, 2003). The first, which championed the need to reduce the frequency of A1 variant in herds, use only sires with the A2A2 genotype, were dairy cattle breeding organizations in New Zealand (A2 Corporation, 2009). This company sell the special branded milk of *CSN2*A2 Premium Brand A2 to New Zealand, the USA, Australia and Asia. We reserve the doubt that it is appropriate to identify someone A1 allele incidence in terms of Latvian dairy cattle populations. Milk protein gene research is needed to obtain the information GAS program development in Latvia.

MATERIALS AND METODS

Animals were chosen at random from different dairy cattle heards in Latvia. The material involved 191 cows. The blood was taken from the jugular vein and collected in K3-EDTA coated sterile vaccutainers and stored at -20°C until used for DNA extraction. DNA for the research of k-casein (CSN3) was extracted using the Fermentas Genomic DNA Purification Kit # KO512. CSN3 alleles A and B were identified using the PCR-RFLP (Polymerase Chain Reaction and Restriction Fragment Length Polymorphism) method in accordance with methodology provided by Medrano and Aguilar-Cordova (10) and Sulimova (15). DNA was extracted using the QIAGENE DNeasy Blood@ Tissue kit (USA). The *CSN2* alleles A1 and A2 were identified using the PCR-RFLP method in accordance with methodology provided by Medrano and Sharrow (11) and McLachlan (13) with some our–modifications. DNA primers described by McLachlan (2006) were used to PCR amplification.

RESULTS AND DISCUSSION

Milk protein genetic polymorphism was studied in Latvia raised breeds – Latvian Brown (LB) and Latvian Blue (LZ). LB breed at the number of animals is the leading in Latvia (150,000) but the LZ breed is one hundred times smaller but it is maintained as a genetic resource population. The study found the extent in which the favoured alleles of β- and κ-casein are represented in both breeds.

Beta-casein. The most common is *CSN2* allele A2. Unfortunately the adverse *CSN2A1* frequency of *CSN2A* in Latvian Brown breed population is a high level (Table1); in the preliminary estimation we found frequency of *CSN2* allele A1 0.697, but in the extended material the result of estimation was a little bit greater - 0.703. but for Latvian Blue breed population the adverse allele's A1 frequency have a much higher level: in the preliminary estimation we found frequency of *CSN2* allele A1 is 0.779 (n=61), but in the extended material the result of estimation - 0.811 (n=81).

Table 1

Frequency of β-casein (*CSN2*) genotypes and alleles estimated in Latvian Brown (LB) dairy cow breed population

Genotypes and alleles	Preliminary results (Smiltina,2010)				Extended material			
	n	Frequency			n	Frequency		
		De facto	HW	+/-		De facto	HW	+/-
A1A1	13	0.394	0.486	-0.092	41	0,427	0,494	-0,067
A1A2	20	0.606	0.422	+0.184	53	0,552	0,418	0,135
A2A2	0	0.000	0.092	-0.092	2	0,021	0,088	-0,067
Total	33	1.000	1.000	0	96	1,000	1,000	-
A1		0.697	-	-	-	0,703	-	-
A2		0.303	-	-	-	0,297	-	-

HW – Hardy-Weinberg equilibrium

Breeding opportunities for increasing the desired allele A2 frequency of A2A2 and A1A2 determine the genotype of animals available. Over the two varieties of material (n = 191) found only 4 animals (2%) with the desired genotype A2A2. Also, heterozygous animals appeared relatively small quantities. Over the two varieties were a total of 85 (44.5%) female animals with the genotype A1A2. The animals of the *CSN2* undesired genotype A1A1 directly off of the reproduction is not inconceivable, but if they will have mated with the A2A2 genotype *CSN2* breeding, then in the F1 generation will be able to obtain heterozygous individuals. The *CSN2*A2 frequency increases it is necessary to detect A2A2 bulls and the maximum use of breeding work. For increasing of the frequency of CSN2A2 need to discover A2A2 bulls and the maximum use of breeding work.

Kappa – casein. In our research of *CSN3* the most common allele B have shown (Table 2) that frequency of allele B is 0.316 for LB breed and 0.317 – for LZ breed.

Table 2

The corresponding genotypes and allele frequency's analysis of *CSN3* alleles A and B in Latvian cow breeds

Genotypes and genes	Latvian Brown			Latvian Blue
	Our results Cows, n=30	(Paura, 2009) Bulls, n=19	(Paura,2009) Cows, n=30	Our results Cows, n=71
AA	0.467	0.632	0.733	0.465
AB	0.433	0.368	0.200	0.437
BB	0.100	0	0	0.099
A	0.684	0.816	0.833	0.683
B	0.316	0.184	0.167	0.317

Planning of the 2 mutually independent gene sampling (*CSN2*A2 and *CSN3*B), selection will also determine the effectiveness of the desired gene coincidence readable animals. Our data sample of 108 cases, data have simultaneously on the two locus (3.tab.). These data failed to find not one case where the animals would be homozygous for the preferred alleles while both locus. In seven cases of the 108 possible (6.49%)

was found in one of the preferred homozygous alleles and heterozygous - in the second one. Since the two varieties of the desired alleles' frequencies (CSN2A2 and CSN3B) are in the minority, then in the genotype composition (3.tab.) only 25% of animals have in the desired allele's homo- or heterozygous state, which have also characterized by a random potential of selected for breeding cows.

Table 3

The incidence of the desirable genotypes of *CSN2* and *CSN3* in the total sample of LB and LZ dairy cows breeds (N=108)

N=108	The number of desired genotypes				Total	x
	22BB	22AB	12BB	12AB		
n	0	1	6	20	27	x
%	0	0,93	5,56	18,52	25,00	100,00

Explanations: 22BB – *CSN2* A2A2, *CSN3* BB;
22AB –*CSN2* A2A2, *CSN3* AB;
12BB – *CSN2* A1A2, *CSN3* BB;
12AB – CSN2 A1A2, *CSN3* AB.

Favorite opportunities may be able to find the desired alleles homozygous bulls and they are widely used in further breeding work.

CONCLUSIONS

1. The frequency of an undesirable bovine milk β-casein allele A1 (*CSN2A1*) has very high in both populations of the Latvian dairy breeds: Latvian brown breed – 0.703 (n=96), Latvian Blue breed – 0.811 (n=95).
2. The frequency of an undesirable bovine milk β-casein allele A1 (*CSN2A1*) can reduce by the breeding activities and can create the herds and breeds populations of the *CSN2* genotype A2A2 (homozygous for desirable alleles). Critically important is to detect A2A2 breeding bulls and use artificial insemination.

If we want also to increase milk κ-casein B allele frequency at the same time, then the task becomes more complicated, because in the tested two varieties of the breeding material (n=108) we did not find noone animal homozygous for both the preferred alleles' locus (A2A2BB). Work should begin with the heterozygous animals.

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