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A note on frequency of A1 and A2 variants of bovine beta-casein locus in Polish Holstein bulls*

S. Kamiński[1], A. Ruść and A. Cieślińska

University of Warmia and Mazury in Olsztyn, Department of Animal Genetics
Oczapowskiego 5, 10-718 Olsztyn, Poland

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ABSTRACT

Besides its nutritional value, bovine beta-casein is a source of bioactive peptides called beta-casomorphins produced during digestion of raw or processed milk. It was shown that the beta-casomorphin 7 originates only from beta-casein variants A1 or B and may be a significant risk factor in human ischemic heart disease, arteriosclerosis, type I diabetes and sudden infant death syndrome. In this study, the frequency of the A1 allele in a sample of Polish Holstein bulls commonly used in artificial insemination (A. I. bulls) was analysed and the potential for production of milk capable of releasing undesirable beta-casomorphin 7 is discussed. Among 143 A. I. bulls, three genotypes were identified (A1/A1, A2/A2, A1/A2) resulting in frequencies of 0.402 and 0.598, for A1 and A2, respectively. Although, the clinical implications of A1 milk on human health is still under discussion it may be necessary to monitor reproductive bulls and decrease the frequency of allele A1.

KEY WORDS: Holstein cattle, beta-casein, polymorphism, casomorphin

INTRODUCTION

The Bovine beta-casein (CSN2) gene belongs to the cluster of 4 casein genes located on chromosome 6. There are 12 genetic variants of CSN2, but only 5 occur in Holstein cattle: A1, A2, A3, B and C, the first two being the most common (Roginsky, 2003). In polypeptide chain position 67, His is substituted by Pro, for A1 and A2, respectively. In the gene coding CSN2, G in position 8101 is substituted by A (GenBank M55158). In addition to its nutritional value, beta-casein is a source of bioactive peptides called beta-casomorphins produced

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[1] Corresponding author: e-mail: stachel@uwm.edu.pl

during the digestion of raw or processed milk or cheese (Jarmolowska et al., 1999; Roginsky, 2003). It was shown that the beta-casomorphin 7 originates only from beta-casein variants A1 or B (Hartwig et al., 1997) and may be a significant risk factor in human ischemic heart disease, arteriosclerosis, type I diabetes and sudden infant death syndrome (Elliot et al., 1999; Thorsdottir et al., 2000; McLachlan, 2001; Sun et al., 2003; Tailford, 2003).

In Poland, research on the frequency of bovine beta-casein variants in bulls was so far not conducted. In this study, an attempt was made to analyse the frequency of the A1 allele in a sample of Polish Holstein bulls used in artificial insemination.

MATERIAL AND METHODS

One hundred forty three A. I. Polish Holstein bulls commonly used in artificial insemination between 2002-2004 in central-northern Poland. Bulls were genotyped in CSN2 locus by PCR-RFLP method using primers designed by Lien et al. (1992). Briefly, one commercial straw of semen was used to isolate genomic DNA by the MasterPurePurification Kit (Epicentre). The primers have the following sequences: CASB 122 L – 5'GAGTCGACTGCAGATTTTCAACATCAGTGAGAGTCA GGCCCTG3' CASB 67 R-5'CCTGCAGAATTCTAGTCTATCCCTTCCCTG GGCCCATCG3'.

To produce 261 bp fragment of CSN2 gene the following PCR mix was composed: 0.4 µl of the primers 122 L and 67 R, each in concentration of 50 pmol/µl, 0.7 U of Tfl Polymerase (Epicentre), 1.25 µl MaserAmp 20 × PCR buffer (Epicentre), 1.5 µl magnesium chloride (15 mM), 2.0 µl enhancer (Epicentre), about 150 ng of genomic DNA and H20 ad 25 µl. Samples were amplified in MJ Research thermocycler under the following conditions: 3 min/ 94°C and 35 cycles of 94°C/25s, 62°C/25s, 72°C/25s.

The yield and specificity of PCR products were evaluated after electrophoresis in 1.5% agarose gel (Promega) with ethidium bromide. The results were observed, analysed and documented by the use of a Fluor-S MultiImager (Bio-Rad). The PCR products were then digested by with Taq I enzyme to generate restriction fragments and electrophoresed in 2.5% agarose gel (ApmliSize, BioRad).

RESULTS AND DISCUSSION

In Figure 1, a typical result of A1/A2 CSN2 genotyping is shown. Among 143 Polish Holstein bulls, three genotypes were identified: A1/A1, A2/A2 and A1/A2, giving a frequency of 0.402 and 0.598, for A1 and A2, respectively

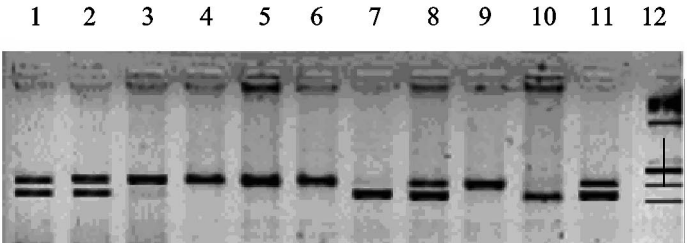

Figure 1. Agarose gel electrophoresis of beta-casein genotypes. Lanes 1, 2, 8, 11 - bulls of A1/A2 genotype. Lanes 3, 5, 9 - bulls of A2/A2 genotype. Lanes 7, 10 - bulls of A1/A1 genotype. Lane 12 - molecular weight marker PhiX 174/Hae III. Restriction fragment 37 bp is not visible on the gel

(Table 1). The frequency of the CSN2 alleles in different breeds and countries is shown in Table 2. This table indicates the general CSN2 allele frequency in

Tabe 1. Frequency of beta-casein A1 and A2 variants in Polish Holstein bulls

CSN2 genotype	Number of bulls	Genotype frequency, %	Frequency of alleles	
A1/A1	16	11.19	A1	A2
A1/A2	83	58.04	0.402	0.598
A2/A2	44	30.77		

Table 2. Occurrence of different beta-casein variants in different breeds and countries

Breed	Country	Frequency of beta-casein alleles		
		B	A1	A2
Guernsey	USA	0.01-0.02	0.01-0.06	0.88-0.97 (0.98)
Jersey	Germany	0.186	0.093	0.721
Jersey	Denmark	0.35	0.07	0.58-0.65
Jersey	New Zealand	-	0.123	0.591
Jersey	USA	0.29-0.37	0.09-0.22	0.49-0.54
Brown Swedish	Germany	0.17	0.108	0.705
Bron Swedish	USA	0.1-0.18	0.14-0.15	0.66-0.72
Simmental	Croatia	0.150	0.190	0.630
Simmental	Germany	-	0.343	0.566
HF	USA	0.01-0.06	0.31-0.66	0.24-0.62
HF	Hungary	0.051	0.456	0.449-0.49
HF	Germany	0.026	0.472	0.496
HF	New Zealand	-	0.465	0.510
Black-and-White	Denmark	0.03-0.08	0.55	0.39
Red-and-White	Sweden	0.008	0.46	0.531
Red-and-White	Germany	0.02	0.573	0.366
Ayrshire	Canada	0-0.003	0.6	0.4
Ayrshire	New Zealand	-	0.432	0.527
Ayrshire	USA	0	0.72	0.28
Ayrshire	Finnland	0.001	0.509	0.490
Red	Denmark	0.04-0.06	0.71	0.23

Europe: in northern Europe, the dominant allele is A1, but in central and southern Europe its frequency steadily decreases. Also, data published by McLachlan (2001) and Laugesen and Elliott (2003) showed A1 and B alleles frequency in many different countries (about 0.43). Our results, for the first time inform about the spread of CSN2 A1 allele and indicate that the frequency of the undesirable allele A1 among Polish Holstein bulls is relatively high (0.41). The population of A.I. bulls is not in genetic equilibrium (A1/A2 genotype is much higher frequent than theoretically expected). This is probably caused by selection pressure in bulls suggesting that this genotype is preferred and probably linked with genes associated with milk performance trait.

Because the method of CSN2 genotyping is based on DNA there is the possibility of fast decreasing the frequency of allele A1 by the monitoring of young and proven bulls and avoiding over-spreading allele A1 through artificial insemination. Including the CSN2 genotype in a breeding program and herd management is a reality in New Zealand. A commercial company A2 Corporation (A2 Corporation, www.a2corporation.com) offers A2 MILK, which is obtained exclusively from cows of the A2/A2 genotype. Although, the clinical implications of A1 milk on human health is still under discussion (Truswell, 2005) and it may be necessary to monitor reproductive bulls and decrease the frequency of allele A1.

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