Lucrări ştiinţifice **Zootehnie şi Biotehnologii**, vol. **40(1),** (**2007**), Timişoara.

THE INFLUENCE OF K-CASEIN ALLELES ON MILK PRODUCTION AND QUALITY IN A HOLSTEIN-FRISIAN COW POPULATION

INFLUENŢA VARIANTELOR ALELICE ALE GENEI K-CAZEINĂ ASUPRA PRODUCŢIEI DE LAPTE ŞI A CALITĂŢII ACESTUIA ÎNTR-O POPULAŢIE DE VACI HF

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Milk production and its composition are determined by quantitative loci, which under the influence of some environmental factors are producing an allelic variability, meaning a genetic polymorphism of the gene. K-casein is a milk protein whose genetic polymorphism can serve as molecular marker for milk production, composition and industrial processing suitability. The allelic variants for k-casein A and B are the most common and the most important of them. The experiments were conducted on 24 Holstein-Friesian milking cows from a private farm in Giroc. The milk production on a normal lactation is 8444 milk kg/305 days, with a fat percent of 3.9 and a protein percent of 3.3. The cows were divided in three groups AA, AB and BB in function of the genotyped obtained after the allelic variants determination. The DNA isolation was made from hair roots and blood, the cow population studied is not in genetic equilibrium fore k-casein gene, the frequency of allele A is 0.43 and the frequency of B allele is 0.58. The highest genotype frequency was 0.5 for CSN3-AB genotype, the BB genotype had 0.33 frequency, and the lowest frequency was 0.17 for AA genotype. The mean daily milk production from cows with BB genotype for k-casein is significant (p<5%) higher compared to the allelic variant AA. The fat percent is significant higher at the allelic variant AA compared to the other allelic variants (AB and BB) of the k-casein gene. Between the fat percent of the three genotypes variants of K-casein (AA, AB and BB) there are no significant differences.

Key words: *k-casein, genetic polymorphism, milk, milk fat, milk protein*

Introduction

The milk production and its composition are determined by Quantitative Loci, which under the influence of some environmental factors are producing an allelic variability, a genetic polymorphism of the gene in cause. K-casein is a milk protein which genetic polymorphism can serve as molecular marker for milk composition and industry important trades (1, 3). Nowadays there are many methods for analyze the DNA restriction fragments; the analysis is based on PCR method (Polymerase Chain Reaction) (2). These analyzes can be performed on

embryos, very young animals or from cells that have DNA material, and is based on genotyping the alleles of the gene responsible with milk protein (4, 5). The genetic markers play a very important role in pre-selecting or selecting the preimplantational embryos from young bulls ore adult bulls destined to reproduction (progeny test) or cows and heifers.

K-casein (CSN3) has a very important role in protecting the other caseins from precipitation (1). The most important variants of the CSN3 are A and B. The A variant has the Thr (ACC) and Asp (GAT) amino-acids in 136 and 148, respectively, position in the amino-acid chain. At the B variant, Thr (ACC) is replaced by Ile (ATC) and Asp (GAT) is substituted with Ala (GCT). This difference between the two variants can be made by recognizing or not the restriction situs with *Hind* III (273). The modification in the GAT triplet from 148 positions that codifies the Asp amino-acid is destroying the *Hinf* I situs from the B allele CSB3-B (3).

In table 1 are presented the different variants of k-casein.

Table 1

Diffusion and the year of discovery of the k-casein variants in bovines

Variant	Year	Diffusion	Variations	References
A,B	1964	All species	A B Thr(135)- Ile Asp(148) - Ala	Neelin, 1964 Schimdt, 1964 MacKinlay, Wake, 1964 Wochik, 1964
C	1978	Less common	B C Arg(97) - Thr	Di Stasio, Merlin, 1978,1979 Mariani, 1983
B*	1987	Rare	B B* Ile(153) - Thr	Gorodetskij, Kaledin, 1987
E	1989	Not very common	A E Ser(155) . Gly	Erhard&Senf, 1989
F	1992	Rare	A F Asp(148) - Val	Sulimova *et.al.,* 1992
F	1996	Rare	A F Arg(10) - His	Ikonen *et al.,* 1996
G	1996	Rare	A G Asp(148) - Ala	Sulimova *et al*., 1996
G	1996	Rare	A G Arg(97) - Cys	Erhard. 1996
H Az	1974	Not common	A H Thr(135) - Ile	Grosclaude *et al*., 1974
H H	1998			Princenberg&Erhard, 1998 Princenberg *et al,*. 1999
I	1998	Rare	A I Ser(104) - Ala	Princenberg&Erhard, 1998 Princenberg *et al*., 1999
A(I)	1999	Rare	None	Princenberg&Erhard, 1999 Princenberg *et al*., 1999
J	1999	Rare	B J Ser(155) - Arg	Mahe *et al*., 1999

From the data in the table we can notice that the most common variants are: A and B, and the rest of the variants are rare and were found only in some breeds or species.

Material and Methods

The experiments were conducted in a privet farm in Giroc district on a number of 24 milking cows from Holstein-Friesian (H-F) breed with a normal lactation of 8444 milk kg / 305 days, with a fat percent of 3.9 and a protein percent of 3.3. The cows were housed free, on a grid and individual resting arias. The feeding was on discretion on the forage alee. The milking was performed at a milking aria of 5x1 twice a day. Analyze of milk quality was performed as part of the official production control every month. In the calculation was used a standard cow lactation with the quantity, fat and protein calculated on a period of 305 days for every cow.

The cows from the experiment were genotyped for k-casein and its allelic variants A and B. In function of the genotype obtained after the determination of the allelic variants for k-casein the cows were divided into three groups AA, AB and BB. The DNA isolation was performed from the hair roots and blood. The blood was collected from the jugular vein in K-EDTA and preserved at -25°C until analyzes were performed. The sequence of four exons and the introns fragment were amplified using PCR technique. Thirty-two amplification cycles were performed: 94°C – 1 minute; 61°C – 1 minute; 72°C – 1 minute. The amplified product was digested, for three hours at 37°C, with *Hin*III and *Hin*I endonucleases. The electrophoresis separation was performed in 2% agarose gel (Gibco BLR) dissolved in TBE buffer.

Results and Discussions

The frequency of the genotypes and allele for K-casein variants are presented in table 2.

Table 2

The K-casein loci polymorphism

Locus	Genotype	No. animals / %	Genotype frequency	Gene frequency
k-casein (CSN3)	AA	4/17%	0.17	A - 0.42
	AB	12/50%	0.50	
	BB	8/33%	0.33	B - 0.58

From data presented in table 2, it can be seen that the population of cows taken into consideration is not in genetic balance for k-casein gene. The frequency of the A allele is in 0.43 and the frequency of the B allele is 0.58, the frequency of

the CSB-AB genotype (0.50) is the highest, the frequency of the BB genotype is 0.33 and the frequency of the AA genotype is the lowest (0.17).
The milk production, fat percent and protein percent in respect to the k-casein genotype are presented in table 3.

Table 3

Mean milk production on day and on 305 days, fat percent and protein percent for different genotypes of K-casein

Specification	k-casein genotype (CSN3)		
	AA	AB	BB
The genotype frequency	0.17	0.50	0.33
Mean milk production for day and for 305 days	22.5±4.03	28±3.03	29.57±4.4
Fat %	4.04±0.22	3.87±0.11	3.89±0.12
Protein %	3.16±0.65	3.28±0.13	3.4±0.13

From data presented in table we can notice that the mean milk production per day and for 305 days is the highest at BB allelic variant of k-casein gene. Between the mean daily production for k-casein the BB variant and AA we have significant differences at 5% threshold.

The fat percent has the highest value at AA allelic variant of k-casein. Between the AA and AB variants and AA and BB variants we have significant differences at 1% threshold.

The highest protein percent was obtained in the case of BB variant. Between the AA, AB and BB variant we have no significant differences in respect to the mean protein percent per lactation.

Conclusions

The population analyzed is not in genetic balance for K-casein locus (CSN3) taken into consideration, because of the selection process the frequency of the B allele (0.58) is higher compared to the A allele (0.42).

The mean milk production per day at BB allelic variant for k-casein genotype, is significant higher ($p<5\%$) compared to the allelic variant AA.

The fat percent is significant higher ($p<5\%$) at AA allelic variant compared to the other two allelic variants for k-casein AB and BB.

Between the protein percent of the three genotype types of k-casein (AA, AB and BB) there are no significant differences ($p>5\%$).

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Producţia de lapte şi compoziţia acestuia este determinată de către loci cantitativi, care sub influenţa factorilor de mediu externi produc o variabilitate alelică, adică un polimorfism genetic al genei respective. Kapa cazeină este o proteină din lapte a cărei polimorfism genetic poate servi ca şi marker molecular pentru producţia, compoziţia şi proprietăţi pentru industrializare a laptelui. Variantele alelice pentru k-caseină A şi B sunt cele mai răspândite şi cele mai importante. Experienţele s-au desfăşurat într-o fermă privată la Giroc pe un număr de 24 vaci în lactaţie din rase HF cu o producţie medie pe lactaţie normală de 8444 kg lapte / 305 zile, cu un procent de grăsime de 3,9% şi un procent de proteine de 3,3%. În funcţie de genotipul obţinut în urma determinării variantelor alelice pentru k-caseină vacile au fost împărţite în trei grupe AA, AB şi BB. Izolarea ADN s-a efectuat din rădăcina bulbului pilos (păr) şi din sânge. Populaţia de vaci luată în considerare nu este în echilibru genetic pentru gena k-cazeinei. Frecvenţa alelelei A este 0,43 ia frecvenţa alelei B este 0,58. frecvenţa genotipului pentru CSN3-AB este cea mai mare având valoarea de 0,50, frecvenţa pentru genotipul BB este de 0,33 şi frecvenţa pentru genotipul AA este cea mai mică de 0,17. Producţia medie de lapte pe zi la genotipul pentru k-caseină varianta alelică BB este semnificativ mai mare (p>5%) faţă de varianta alelică AA. Procentul de grăsime este semnificativ mai mare la varianta alelică AA faţă de celelalte două variate alelice ale k-cazeinei AB şi BB. Între procentul de proteină a celor trei tipuri de variante genotipice al k-cazeinei (AA, AB şi BB) nu avem diferenţe semnificative.

Cuvinte cheie: *k-cazeină, polimorfism genetic, lapte, grăsime lapte, proteine lapte*