

GH and CSN3 gene polymorphisms and their impact on milk traits in cattle

**D. Lechniak, T. Strabel, D. Przybyła, G. Machnik
and M. Świtoński¹**

*August Cieszkowski Agricultural University of Poznań,
Department of Genetics and Animal Breeding
Wołyńska 33, 60-637 Poznań, Poland*

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ABSTRACT

Associations of milk production traits with growth hormone (L/V) and κ -casein (A/B) gene polymorphisms were investigated in this study. Official breeding values of genotyped 99 AI bulls for milk, protein, fat yields as well as fat and protein contents were used to study genotype effects on the production traits. Effects of four single-locus genotypes (LL, LV, AA and AB) and four two-loci genotypes (AALL, AALV, ABLL and ABLV) were estimated. The AB genotype showed an advantage over the AA homozygote in terms of fat and protein contents. No significant relationships between the GH polymorphism and the breeding values were found. The analysis of the effect of two-loci genotype reflected the relationships observed for single-locus analysis and ABLL and ABLV genotypes were related to higher breeding values for fat (ABLV) and protein (ABLV and ABLL) contents. The contradictory effects of the studied genetic variants are discussed.

KEY WORDS: GH, CSN3, polymorphism, milk traits, cattle

INTRODUCTION

K-casein (CSN3) as well as growth hormone (GH) genes are considered to be potential genetic markers for the milk production trait in cattle. Among several mutations in the bovine growth hormone gene (bGH), the most investigated one is

¹ Corresponding author

the point mutation causing the leucine/valine substitution in the 127 position of the aminoacid chain (Seavey et al., 1971). With regard to the CSN3 locus, the A and B alleles appear with the highest frequency among cattle breeds (for review see Kamiński, 1996). An extensive amount of work has been done on associations between the A/B or L/V polymorphisms and productive traits in cattle; however, published results seem to be contradictory.

The aim of the present study was to evaluate the effect of the CSN3 (A and B alleles) and GH (L and V alleles) loci on milk production traits, based on the breeding values of genotyped AI bulls.

MATERIAL AND METHODS

Altogether, 99 AI proven bulls of dairy breeds were examined in this study (25 Holstein-Friesian -HF, and 74 crossbred – Polish Black-and-White with a predominant share of the HF genes).

Primers for PCR amplification of the CSN3 gene were designed, based on the gene sequence published by Alexander et al. (1988):

Forward primer -

5'- AAGAAATAATACCATTCTGCATAATTTATTTTTTTTACAG - 3'

reverse primer 5'- GGCTGTTATTCATTTTGCCTTATTTTTTACCTG -3'

The PCR products were digested with Hinf I and Hind III (Promega) restriction enzymes.

The data on the frequency of the GH variants was already published by Lechniak et al. (1999).

Official breeding values (EBV) for milk, fat, protein yields and fat and protein contents estimated on the national scale were used to analyse the association between genotypes and production traits (Żarnecki et al., 2001). The effect of genotype on consecutive traits was tested using the least square analysis of variance (SAS) based on the following model:

$$y_{ij} = \mu + a_i + e_{ij}$$

where y_{ij} is the bull EBV of a production trait, μ is the general mean, a_i is the fixed effect of genotype i , e_{ij} – residual effect associated with ij -th observation.

Single-locus and two-loci analyses were carried out separately. In the single-locus model the effect of the GH and CSN3 genotypes on milk traits was analysed in separate runs.

RESULTS AND DISCUSSION

Among genotyped bulls the following allele frequencies were found: A – 0.81, B – 0.19 and L – 0.88, V – 0.12. The frequencies were similar among pure HF and crossbred bulls, however the incidence of the V allele in the pure HF individuals was especially low (0.04). The observed frequencies confirmed the already described tendency (Lucy et al., 1993; Lien et al., 1999). Due to the low number of bulls with BB (four individuals) and VV (two individuals) genotypes, the analysis was carried out only for selected single-locus (AA, AB, LL, LV) and two-loci (AALL, AALV, ABLL and ABLV) genotypes.

The effect of the single-locus genotype is presented in Table 1. The AB bulls were characterised by a higher breeding value for fat ($P < 0.05$) and protein contents ($P < 0.01$). No significant relationships were found at the GH locus. The analysis of the effect of two-loci genotype revealed that bulls carrying the heterozygous status of CSN3 locus presented higher breeding values for fat (ABLV) and protein (ABLL and ABLV) contents when compared with bulls carrying AALL genotype (Table 2). It can be assumed that the effect of the AB genotype was not influenced by the GH locus (LL or LV). Thus, a lack of interaction between these two loci is postulated.

The average milk production recorded for the progeny of bulls used in this study was rather low ($\bar{x} = 4\ 725$ kg). It allows to speculate that the expression of genetic variance was limited.

Previously published reports on the effect of the GH and CSN3 polymorphisms on lactation traits are rather contradictory. There are reports on the lack of any associations with regards to A/B polymorphism at CSN3 locus (Velmala et al., 1995). On the other hand, considering the milk yield, higher milk production was noticed for the heterozygous HF and crossbred cows (Ng-Kwai-Hang et al., 1991;

TABLE 1
Least square means for breeding values of analysed AI bulls according to their genotypes

Trait	Bull's genotype at locus			
	CSN3		GH	
	AA	AB	LL	LV
No of bulls	62	28	73	19
Milk yield, kg	351.35	214.18	329.93	193.58
Fat yield, kg	15.34	15.54	15.12	14.95
Protein yield, kg	9.02	7.38	8.86	5.78
Fat, %	0.01 ^a	0.13 ^a	0.02	0.15
Protein, %	-0.04 ^A	0.02 ^A	-0.003	-0.004

^{A,a} – columns with the same superscript differ significantly (^A $P < 0.01$; ^a $P < 0.05$)

TABLE 2

Least square means for breeding values of AI bulls according to their combine genotypes at both analysed loci

Trait	Bull's genotype at locus			
	AALL	ABLV	ABLL	ABLV
No of bulls	50	12	20	7
Milk yield, kg	373.30	259.92	263.95	79.86
Fat yield, kg	15.26	15.67	16.65	13.73
Protein yield, kg	9.60	6.62	8.71	4.36
Fat, %	-0.01 ^A	0.10	0.10	0.22 ^A
Protein, %	-0.05 ^{A,B}	-0.04	0.01 ^A	0.05 ^B

^A – columns with the same superscript differ significantly ($P < 0.05$)

Walawski et al., 1994; Freyer et al., 1999). However, in the study of Bovenhuis et al. (1992) the AA genotype showed an advantage for HF and crossbred cattle. At the same time a lower casein and a higher whey protein content was found for daughters of the AA Swiss Brown bulls (Braunschweig et al., 2000). Moreover, the A allele was associated with a higher protein yield and content (Lien et al., 1995; Kamiński, 2000), whereas usually the B allele is attributed to the higher fat, protein and casein contents (Ng-Kwai-Hang et al., 1991; Bovenhuis et al., 1992; Mao et al., 1992; Juszczak et al., 2001; Panicke et al., 2001).

The results published on the associations between the L/V polymorphism and milk production traits are also not consistent and vary depending on the analysed cattle breeds. The L allele was attributed to the higher milk production for the HF cows, while the V variant was reported to be more effective for the Jersey breed (Lucy et al., 1993; Lee et al., 1996). However Sabour et al. (1997) and Panicke et al. (2001) did not observe relationship between the L/V polymorphism and milk production. With respect to milk protein and fat contents no relationship was found between GH genotypes and those traits (Lucy et al., 1993). On the other hand the latest study by Grochowska et al. (2001), performed on Polish HF cattle, revealed significant differences in milk and protein yields in accordance with the L/V polymorphism with the LV genotype being superior to others. Moreover, the highest fat yield was noticed for the LL homozygotes. Our results do not support these observations.

Extensive studies on the identification of quantitative trait loci (QTL) in the bovine genome brought numerous reports indicating a tentative localisation of chromosomal regions carrying genes influencing milk production. QTLs influencing milk, fat and protein yields, localised close to the casein genes on the sixth chromosome have been reported (Kuhn et al., 1999; Velmala et al., 1999). These findings are supported by a report by Freyer et al. (1999) on the linkage between CSN3 locus

and QTLs for fat and protein contents. However, up to date there has been no report on any QTL identified on the bovine chromosome 19 carrying the GH locus (for review see Kurył, 2000).

Contradictory results on the effect of the GH and CSN3 polymorphisms indicate that the breed/population effect on the studied traits is predominant. Since populations differ from each other with regard to gene pools and environmental conditions, thus variable effects of the analysed polymorphisms were estimated by different authors. It was probably due to the action of other unknown genes or/and interactions between them. For instance, Brockmann et al. (2000) showed that the obese phenotype in mice is caused by many gene interactions (IGFBP, IGF, leptin genes). Thus it is proposed that unknown interactions between both studied genes (GH and CSN3) and the other ones may influence lactation traits differently in different populations. Furthermore, A/B and L/V polymorphic forms do not exert major effects on the milk production traits.

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STRESZCZENIE

Wpływ polimorfizmu genów hormonu wzrostu (GH) i kappa kazeiny (CSN3) na produkcję mleka u bydła

Przeprowadzono analizę wpływu polimorfizmu genu hormonu wzrostu (L/V) oraz kappa-kazeiny (A/B) na produkcję mleka u bydła. W badaniach wykorzystano oszacowane wartości hodowlane 99 buhajów objętych analizą dla następujących cech: wydajność mleka, tłuszczu i białka oraz zawartość tłuszczu i białka w mleku. Ze względu na małą liczebność osobników w niektórych klasach genotypowych, w analizie wykorzystano 4 genotypy dla jednego locus (LL, LV, AA i AB) oraz 4 genotypy uwzględniające oba loci (AALL, AALV, ABLL i ABLV). Wartość hodowlana buhajów o genotypie AB była istotnie wyższa pod względem procentu tłuszczu i białka w mleku. Nie stwierdzono istotnych różnic między genotypami w locus GH. Analiza związku genotypów uwzględniających oba loci odzwierciedliła tendencję obserwowaną dla genotypów z uwzględnieniem jednego locus. Istotnie wyższy procent tłuszczu w mleku przekazywały potomstwu buhaje o genotypie ABLV, podczas gdy samce o genotypach ABLV i ABLL wyższy procent białka. W pracy przedyskutowano niejednoznaczne wyniki dotychczasowych badań nad wpływem omawianego polimorfizmu na produkcję mleka.