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Relationship between growth hormone, κ-casein and β-lactoglobulin genotypes and selected biochemical blood indicators in young Friesian cattle

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Two-hundred-sixty-five young cattle of both sexes, progeny of thirty-one Polish Friesian AI bulls were genotyped for growth hormone (*GH*), κ-casein (*CSN3*), and β-lactoglobulin (*LGB*) gene variants. Associations were evaluated between genetic variants in the three *loci* and concentration of triiodothyronine (T_3), thyroxine (T_4), insulin (Ins), glucose (Glu), urea (Ur), creatinine (Cr), cholesterol (Chol), as well as activity of alkaline phosphatase (AP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST). Only the level of Ins and Ur were not found related to the *loci* considered. The level of T_3 was affected by the interaction between the *GH* and *LGB loci,* while that of T_4 by the epistasis of *GH*, *CSN3* and *LGB*. Cr and Chol levels depended on both *CSN3* and *LGB* as well as their interaction. *GH* × *CSN3 loci* were found involved in the AP, all the three *loci* in ALT, and *GH* × *LGB* in AST activity moulding.

KEY WORDS: β-lactoglobulin / blood / cattle / growth hormone / κ-casein / polymorphism

Marker-assisted selection can be a powerful tool in selection programmes of farm animals. In particular, due to its wide growth-promoting and anabolic effect, growth hormone (GH) appears to be an ideal marker for milk and meat production potentials in farm animals. Simultaneously, κ-casein (CSN3) and β-lactoglobulin (LGB) are proteins expressed in milk and due to their polymorphism may be informative molecular markers for yield and composition of milk. Moreover, a significant correlation has been described between growth rate and *CSN3* and *LGB* genotypes in Hereford and Friesian cattle [Moody *et al.* 1994, Oprządek *et al.* 1999]. Blood biochemical indica-

tors may be interpreted as metabolic markers measured during normal growth of an animal. It is accepted that measuring concentrations of blood hormones and metabolites is likely to be useful in animal production. The use of biochemical indicators may be particularly successful in improvement of dairy cattle, where sex-limited production of milk precludes testing of bulls and young heifers on the basis of phenotypic performance. The use of such markers would speed up the genetic progress and reduce the costs of identifying individuals which are genetically superior. Benefits, though to a lesser extent, could also be expected from selection for growth traits, which are not sex-limited and can be measured at an early stage of an animal's life. However, some important beef production traits (dressing percentage, carcass composition *etc*.) cannot be measured on live animals. It is necessary, therefore, to rely upon an indirect selection for correlated traits [Bittante *et al.* 1987]. The effect of using biochemical markers as selection criteria depends on the genetic correlation between the marker(s) and production trait(s) – Christensen [1987]. Moreover, the effect depends on a design of the breeding programme in which the marker is used.

The present study aimed at searching for relationship between genotype at three *loci* and animal's metabolic traits described by levels of three selected hormones, four metabolites and three enzymes.

Material and methods

Young Polish Friesian cattle (142 bulls and 123 heifers), the progeny of 31 sires, were genotyped for growth hormone (*GH*), κ-casein (*CSN3*) and β-lactoglobulin (*BLG*) gene variants. Genotypes of *GH*, *CSN3* and *LGB* were determined using the PCR-RFLP technique. Crude DNA was prepared from blood samples according to Kawasaki [1990]. *GH* genotypes were identified with *Alu*I as described by Lucy *et al.* [1993]. Primer sequences and PCR conditions used for *CSN3* were those described by Kamiński and Figiel [1993]. Amplified DNA was digested with *Hind*III restriction nuclease and analysed electrophoretically in 2% agarose gel. The PCR/RFLP of *LGB* was performed according to Medrano and Aquilar-Cordova [1990] using *Hae*III restriction nuclease.

The blood serum level of triiodothyronine (T_3), thyroxine (T_4), insulin (Ins), glucose (Glu), urea (Ur), creatinine (Cr) and cholesterol (Chol), and activity of alkaline phosphatase (AP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were recorded four times during the 42-day test period (starting at the beginning of seventh month of life) in which feed intake and growth rate were individually controlled. First sampling was done three days before the start of month 7 and second sampling three days later, after 36 h fasting. The procedure of sampling was repeated 6 weeks later. The blood was obtained by jugular vein puncture into Monovette tubes, at 6:00 a.m. Samples were centrifuged, and blood serum separated and stored at -20°C until analysed. Concentrations of hormones were measured with radioimmunoassay kits (OPiDI Świerk, Poland) while levels of metabolites and activity of enzymes using commercial kits (BIOCHEMTEST, Gliwice, Poland). The relationship between

genotypes of *GH*, *CSN3* and *LGB* and the blood biochemical indicators were studied assuming two *loci* epistasis. The genotype substitution of LL to VV, AA to BB and AA to BB effects for *GH*, *CSN3* and *LGB*, respectively, as well as the dominance effects off LV, AB and AB were estimated as dependent on the accompanying genotypes at the other two *loci.*

The model of analysis included fixed effects of sex, year-season of sampling, number of sampling, age at sampling nested within sampling number.

$$y_{ijklmno} = \mu + SEX_i + YS_j + SN_k + \beta(Age_{ijklmno} - Age) + \beta_k(Age_{ijklmno} - Age_k) + GH_l + CSN3_m + LGB_n + GH \times CSN3_{lm} + GH \times LGB_{ln} + CSN3 \times LGB_{mn} + e_{ijklmno}$$

where:

$y_{ijklmno}$ – studied trait;

μ – overall mean;

SEX_i – effect of the *i*-th sex (i = 1, 2);

YS_j – effect of the *j*-th year-season (j = 1... 7);

SN_k – effect of the *k*-th blood sampling (k = 1, 2, 3, 4);

$\beta(Age_{ijklmno} - Age)$ – overall regression on age at sampling;

$\beta_k(Age_{ijklmno} - Age_k)$ – regression on age at sampling nested within sampling number;

GH_l – effect of the *l*-th *GH* genotype (LL, LV, VV);

$CSN3_m$ – effect of the *m*-th *CSN3* genotype (AA, AB, BB);

LGB_n – effect of the *n*-th *LGB* genotype (AA, AB, BB);

$e_{ijklmno}$ – random error.

The sires were assumed indifferent with regard to their "random" genotype. To avoid confusing the effect of a sire with that of genotype the sires were not included in the model.

Results and discussion

Means for biochemical components of blood serum are shown in Table 1. Generally, hormones concentration, metabolites level and activity of enzymes were within the range reported by other authors [Schams *et al.* 1991, Graml *et al.* 1995].

Numbers of animals of *GH, CSN3*, and *LGB* genotypes are given in Table 2. The frequencies of different gene variants appear similar to those reported earlier for Polish Friesian (Black-and-White) cattle by Zwierzchowski *et al.* [1995] and Klauzińska *et al.* [2000].

Direct effects of *GH*, *CSN3* and *LGB* genotypes and additive, dominance and epistatic effects across the traits and *loci* studied, as well as their significance are given in

Table 1. Least squares means (LSM) and their standard errors (SE) for biochemical components of blood serum

Component	LSM	SE
Triiodothyronine (T_3, ng/ml)	1.23	0.04
Thyroxine (T_4, ng/ml)	74.16	2.37
Insulin (Ins, μIU/ml)	7.21	0.50
Glucose (Glu, mmol/l)	4.20	0.08
Urea (Ur, mmol/l)	3.64	0.07
Creatinine (Cr, mg/100 ml)	1.74	0.02
Cholesterol (Chol, mg/100 ml)	83.47	2.63
Alkaline phosphatase (AP, U/l)	48.12	2.63
Alanine aminotransferase (ALT, IU/l)	18.10	0.52
Aspartate aminotransferase (AST, IU/l)	17.98	0.55

Table 2. Number of animals classified according to growth hormone, κ-casein, and β-lactoglobulin genotypes

Locus	Genotype	Number of animals
Growth hormone (*GH*)	LL	116
	LV	91
	VV	58
κ-casein (*CSN3*)	AA	151
	AB	100
	BB	14
β-lactoglobulin (*LGB*)	AA	111
	AB	97
	BB	57

Table 3. Out of the three hormones studied only the Ins level was not found related to the *loci* considered. T3 level was significantly affected by the interaction between *GH* and *LGB loci,* while T4 by *CSN3* with *GH* and *LGB* epistasis. Glucose level depended on *LGB*, due to the dominance effect at that *locus*. Urea level was not related to the accompanying genotypes, while Cr and Chol levels depended on both *CSN3* and *LGB* as well as their interaction. *GH* × *CSN3 loci* were found involved in the AP, all three *loci* in ALT, and *GH* × *LGB* in AST activity moulding.

So far, no such studies on the association between gene polymorphism and biochemical indicators have been reported. Genetic and physiological background of the associations between the three *loci* analysed and biochemical blood indicators observed in this study is not clear. All the three *loci* – *GH*, *LGB* and *CSN3* – are located in the cattle genome on three different chromosomes (19, 11 and 6, respectively). Gene coding

Table 3. Statistical significance of diallelic genotype, additive, dominance effects and epistatic effects across blood indicators and focal models

Com-pound	Diallelic genotype[1] effects across focus (genotype of higher value)	Diallelic additive[2] effects across focus (homozygote of higher value)	Diallelic dominance[3] effects across focus (direction of effect)	Epistasis[4] (accompanying genotype with which the effect is highest)
TC	GH CSN3 LGB			aGH × LGB*(BB) dLGB × GH*(LL)
TG	GH CSN3 LGB			dGH × CSN3*(A-A) aCSN3 × GH*(LL) aCSN3 × LGB**(A-A) aLGB × CSN3** (A-A)
Ins			no association found	
Glu	GH CSN3 LGB*(AB)		*(+)	
Ur			no association found	
Cr	GH CSN3 LGB			dCSN3 × LGB*(AB) aLGB × CSN3*(AB) dLGB × CSN3*(AB)
Chol	GH CSN3**(BB) LGB*(A-A)	**(BB) *(A-A)	*(-)	dGH × LGB*(BB,A-A), aCSN3 × LGB*(A-A) dCSN3 × LGB*(AB),aLGB × CSN3**(A-A,AB) dLGB × GH*(BB), dLGB × CSN3*(AB)
AP	GH CSN3 LGB			aGH × CSN3**(A-A), dGH × CSN3**(AB) aCSN3 × GH*(LL) dCSN3 × GH**(L-V)
ALT	GH CSN3**(BB) LGB		*(-)	dGH × LGB*(AB,BB) aCSN3 × GH*(LL) aLGB × GH** (L-V)
AST	GH CSN3 LGB			aGH × LGB** (BB), dGH × LGB**(AB) aLGB × GH** (L-V) dLGB × GH*(L-V)

[1] Differences in FA mean levels between genotypes are given for loci.
[2] Difference between the homozygous genotypes
[3] Deviation from the means of the homozygotes
[4] Differences between additive and dominance effects at one locus as dependent on the genotype at another locus
a = additive effects across locus
d = dominance effects across locus
* = significance at P TO 05
** = significance at P TO 01
Symbols for blood indicators are explained in Table 1

for AST was assigned to chromosome 2, and that coding for AP – to chromosome 26 (ArkDB bovine genome database, Roslin Institute www.thearkdb.org) Chromosomal locations of ALT and Ins genes in the cattle genome are still not established. Blood concentrations of compounds included in this study are obviously controlled by many genes, located in different chromosomes. Thus, relations shown in this report cannot be solely explained by genetic linkage.

Many results have been published concerning the effect of L/V *GH* gene polymorphism on dairy and meat traits in cattle. In most studies L allele was considered to be associated with higher milk production [Lucy *et al.* 1993]. It was also shown, that L/V genotype of *GH* was associated with GH blood concentration of Friesian bulls [Grochowska *et al.* 1999]. A significant effect of *GH* L/V genotype on carcass gain of Simmental bulls has been shown by Schlee *et al.* [1994] and Chrenek *et al.* [1998], who demonstrated that the meat classification score was significantly higher for LL than for LV and VV genotypes. In Friesian (Black-and-White) bulls body weight and feed intake were also strongly related to the *GH* genotype, the LV heterozygotes were the heaviest and consumed most [Zwierzchowski *et al.* 1998]. The LL and LV genotypes proved to be associated with higher meat deposition than VV homozygotes [Oprządek *et al.* 1999].

In addition to studies directed on *GH* level and polymorphism, concentrations of a variety of metabolites in circulation (e.g, free fatty acids, glucose, urea) as well as insulin, thyroid hormones, and glucagon have been evaluated as reflectors of genetic merit of animals. Many of those studies focused on responses relative to feeding, fasting, or other metabolic challenges. For example, progeny of bulls of a high milk yield merit had, after fasting, lower concentrations of urea but higher concentrations of free fatty acids than that of bulls of a low milk yield merit [Tilakaratne *et al.* 1980]. Those and other results were reviewed by Wooliams and Lovendahl [1991] and Akers [2000]. It was emphasised, however, that differences in metabolite concentrations due to breeding value of animals are often small, not significant, and frequently not repeatable. Studies on Friesian calves in New Zealand [Min *et al.* 1993] failed to confirm differences in blood urea between fasted animals of low *vs.* high genetic merit, but did confirm the differences in glucose and insulin secretion reported earlier by Xing *et al.* [1988]. It should be noted, however, that determinations of blood plasma or serum components often give conflicting or disappointing results. The discrepancies may possibly result from the effect of environmental factors and choice of animals.

Robinson *et al.* [1992] summarized the reports on hormones and/or metabolites as indicators of dairy merit and found them useful in discriminating types of cattle with different dairy potential. Xing *et al.* [1988] reported significant dairy merit differences existing among young calves for blood glucose, insulin and growth hormone levels, that all were higher in high- than in low-index animals. Similar differences for glucose and insulin were reported by Min *et al.* [1993] who, however, found no differences for urea, creatinine and free fatty acids between calves of high- and low-breeding index. Xing *et al.* [1991] reported a line of high dairy index yearling heifers that, after feeding,

showed lower plasma urea and higher glucose level than heifers from a low-index line; basal levels of insulin, free fatty acids, creatinine and growth hormone did not differ between the two lines.

Also the activity of ALT and AST are anticipated as possible indicators of animal productivity. Both are involved in production of energy from gluconeogenetic amino acids – alanine and aspartic acid [Mithieux 2001]. Plasma insulin is negatively correlated with milk production [Hart *et al.* 1979], and administration of exogenous hormone reduces milk yield [Cowie *et al.* 1980]. It has been demonstrated by Xing [1989] and Michel *et al.* [1991] that the insulin response to exogenous glucose or arginine is greater in high- than in low-producing dairy cattle.

High correlations between serum indicators and production traits call for using the former as markers for predicting genetic merit of the latter. Peterson *et al.* [1982] reported the correlation between blood serum AP activity and milk, fat and protein yield to reach 0.94, 0.91 and 0.89, respectively. High correlations with the three production traits were also found for creatinine, urea nitrogen and uric acid. The authors anticipate that selection for some of these indirect traits, particularly for AP should be, at least in theory, as effective as direct selection based on dairy performance data.

The results presented here suggest that certain biochemical blood indicators can be used to study the direction and intensity of metabolic processes in association with genetic changes of production traits.

All the three polymorphic *loci* studied – *GH, CSN3* and *LGB* – are of economic importance in dairy cattle. They appear to be marker *loci* for some active protein and metabolite levels which themselves may be indicative of animal's production potential. Moreover, in predicting productivity of animals, a fixed combined marker genotype should be considered rather than just a single *locus*.

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Związek między poziomem wybranych wskaźników biochemicznych krwi a genotypami GH, κ-kazeiny i β-laktoglobuliny bydła czarno-białego

S t r e s z c z e n i e

Zbadano polimorfizm genu hormonu wzrostu, κ-kazeiny i β-laktoglobuliny u 265 sztuk młodego bydła cb. W surowicy krwi oznaczono poziom triiodotyroniny (T_3), tyroksyny (T_4), insuliny (Ins), glukozy (Glu), mocznika (Ur), kreatyniny (Cr) i cholesterolu (Chol), a także aktywność fosfatazy zasadowej (AP), aminotransferazy alaninowej (ALT) i aminotransferazy asparaginianowej (AST). Oszacowano powiązania między wymienionymi trzema *loci* a podanymi wskaźnikami biochemicznymi krwi. Nie stwierdzono zależności między koncentracją insuliny i mocznika a badanymi genotypami. Interakcja między *GH* a *LGB* wpływała na koncentrację T_3, natomiast na koncentrację T_4 wpływała epistaza między *GH*, *CSN3* i *LGB*. Poziom Cr i Chol wiązał się z *CSN3* i *LGB* oraz z ich interakcją. Wykazano związek między *loci GH* × *CSN3* a aktywnością AP oraz między *loci GH* × *LGB* a aktywnością AST. Aktywność ALT wiązała się ze wszystkimi trzema badanymi *loci*.