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Effect of the *DGAT1* gene polymorphism on milk production traits in Black-and-White (Friesian) cows*

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The effect was studied of the lysine/alanine (K232A) diallelic polymorphism in acylCoA-diacylglycerol-acyltransferase 1 (DGAT1) on milk production traits in a sample of Polish-Black-and-White (Friesian) cows. The considered polymorphism results from AA→GC nucleotide substitution in *DGAT1* gene exon 8.

Genotyped were 177 cows out of which 86 were included in the association study. The *DGAT1* genotype showed no significant effect on the daily milk yield. Moreover, no differences were found between genotypes in daily yield of fat-corrected-milk (FCM) and mean daily value-corrected milk (VCM). The cows of *DGAT1* genotype *AA/GC* yielded milk with significantly higher fat and total protein content than those of *GC/GC* genotype. Moreover, the *AA/GC* cows produced milk with higher total solids and solids-non-fat content. The total milk protein to milk fat ratio in *AA/GC* cows was 0.79, while 0.83 in *GC/GC* cows. No significant differences were found between genotypes in the weight of milk components produced daily. The study showed some associations occurring between genetic variants at the coding region of the bovine *DGAT1* gene and milk production traits of Polish Black-and-White (Friesian) cattle; in particular the *AA* allele (coding for lysine) appeared favourable for fat and protein content of milk.

KEY WORDS: cattle /gene *DGAT1* / gene polymorphism / milk production traits

A quantitative trait *locus* (QTL) for fat content (%) of milk fat has been mapped consistently to the centromeric region of the bovine chromosome 14 (BTA14). Two independent studies have identified the nonconservative lysine/alanine mutation

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(K232A) in the acylCoA-diacylglycerol-acyltransferase 1 (DGAT1) as likely to be the reason for the variation observed. Winter *et al*. [2002] showed that *DGAT1* maps close to the region of a quantitative trait locus (QTL) on bovine chromosome 14 for variation in fat content of milk. Sequencing the *DGAT1* from pooled DNA revealed significant frequency shifts at several variable positions between groups of animals with high and low breeding values for fat content of milk in different breeds. Among the variants was a substitution of lysine by alanine (K232A), with the lysine-encoding allele being associated with higher milk fat content. Haplotype analysis indicated the lysine variant to be ancestral. Independently, Grisart *et al.* [2002] mapped a quantitative trait *locus* (QTL) with a major effect on milk composition – particularly fat content – to the centromeric end of bovine chromosome 14. They demonstrated that a very strong candidate is acylCoA-diacylglycerol-acyltransferase 1 gene (*DGAT1*) and a K232A substitution in the DGAT1 protein which has a major effect on fat content of milk and other milk characteristics.

The effect of *DGAT1* polymorphism on dairy traits was further confirmed by numerous authors [Spelman *et al*. 2002, Fisher and Spelman 2004, Kaupe *et al.* 2004, Bennewitz *et al*. 2004]. Significant results were identified for milk fat, milk protein, and milk yield for Jerseys and Holstein-Friesians, and only for milk yield for Ayrshires. The average allele substitution effects were 2 to 3 kg of protein and 120 to 130 kg of milk. For milk fat yield, the average allele substitution effect was 6 kg for Holstein-Friesians and 3 kg for Jerseys. Weller *et al*. [2003] genotyped for *DGAT1 locus* in the Israeli Holstein population and found that the *DGAT1* polymorphism was associated with milk yield, fat yield, and fat and protein content. Thaller *et al.* [2003] used granddaughter designs for Fleckvieh and German Holsteins to estimate allele frequencies and gene substitution effects for milk, fat, and protein yield, as well as fat and protein content. Effects of *DGAT1* variants on the content traits were pronounced; on average for both breeds the estimates of the gene substitution effect for the lysine-encoding variant were 0.31 per cent points for fat content and 0.06 per cent points for protein content. Conversely, negative effects of the lysine variant of -242 to -180 kg for Fleckvieh and -260 to -320 kg for Holsteins were revealed for milk yield from first to third lactation.

The association between the K232A polymorphism and milk production traits was studied in Polish Jersey cattle by Komisarek *et al.* [2004]. Significant effect was observed on most traits considered – the lysine encoding variant was associated with elevated milk fat yield as well as fat and protein contents, whereas the alanine allele was related to the increased milk yield. Most significant result was obtained for the fat content of milk.

The *DGAT1* encodes for acylCoA-diacylglycerol-acyltransferase 1, a microsomal enzyme that catalyses the final step of triglyceride synthesis [Cases *et al.* 2001]. Studies carried out on mice indicated that animals lacking both copies of *DGAT1* (gene knockout) were completely devoid of milk secretion because of deficient triglyceride synthesis in the mammary gland [Smith *et al.* 2000]. Grisart *et al.* [2004], by using a baculovirus system, have expressed both *DGAT1* alleles in Sf9 cells and showed that

the K allele, causing an increase in fat content of milk *in vivo*, was characterized by a higher V_{max} in producing triglycerides than the allele *A*. Kühn *et al*. [2004] showed that in German Holstein population alleles of the *DGAT1* promoter derived from the variable number of tandem repeat (VNTR) polymorphism were associated with milk fat content in animals homozygous for the allele 232A at *DGAT1*. Due to the presence of a potential transcription factor binding site in the 18-mer element of the VNTR, the variation in the number of repeats might be causal for the variability in the transcription level of the *DGAT1* gene.

In most studies concerning the associations between genotype and production traits the results are highly dependent on a breed, an animal population, and even on a herd. Therefore, we decided to study the effect of the diallelic polymorphism in the *DGAT1* exon 8 on milk production traits in a sample of Polish Black-and-White (Friesian) population.

Material and methods

Animals

Genotyped were 177 Polish Black-and-White (Friesian) dairy cows from the Institute of Genetics and Animal Breeding Experimental Farm, Jastrzębiec, sharing more than 80% of Holstein-Friesian blood and sired by 48 bulls. Eighty-six cows were included in the study of the effect of *DGAT1* polymorphism on milk traits.

All cows were kept in loose barn and fed *ad libitum* with Total Mixed Ration based on corn silage, wilted grass silage and concentrates, supplemented with minerals and vitamins. Cows were milked twice a day. Milk yield from both milkings was recorded individually and milk samples were collected once a month. Over three consecutive years the herd mean milk yield rose from 7142 kg (year 1) to 8244 kg (year 3). Simultaneously, fat and protein contents decreased from 4.34 to 3.51% and from 3.75% to 3.37%, respectively.

Milk production data were collected throughout three consecutive lactations for every cow. In order to facilitate statistical evaluation, a production data record was included into analyses provided the following two conditions were fulfilled: (1) number of cows of a given genotype exceeded 20 and (2) a cow had at least one paternal half-sister with full data records. As consequence, in the final dataset used for analyses at least 20 cows and at least two sires represented every genotype. As a result, only two genotypes – *AA/GC* and *GC/GC* – could be considered for final calculations. The eventual dataset had 2095 test-day records of 86 cows (56 with *AA/GC* and 30 with *GC/GC* genotype), sired by 20 sires.

Blood withdrawal procedure was approved by the Local Ethics Commission (permission No. 67/2001).

DNA isolation from whole blood

An authorized veterinarian collected blood for isolation of DNA from the jugular

vein. Blood was collected on K_2-EDTA and stored at -25°C for a few weeks or at -75°C up to several months. The isolation of DNA from whole blood was done with a rapid method described by Kanai *et al.* [1994].

***DGAT1* genotyping**

The GC/AA polymorphism in exon 8 of the *DGAT1* gene was identified using RFLP-*Cfr*I as described by Winter *et al.* [2002]. The following primers were used to amplify a 411-bp DNA fragment encompassing parts of intron 7 and exon 8 of the *DGAT1* gene:

F – 5'-TCAGGATCCAGAGGTACCCAG-3' and

R – 5'-GGGGTCCAAGGTT-GATACAG-3'.

The polymerase chain reactions were performed using a PCR-mix with both primers (each at a final concentration of 2 pmol/µl), 1 U Taq polymerase (SIGMA), 1 µl Taq polymerase buffer, dNTPs of 2.0 mM, approx. 100 ng of genomic DNA, and H_2O up to 10 µl. The following PCR protocol was used: 1 min at 94°C, 1 min at 61°C, and 1 min at 72°C – 34 cycles. The yield and specificity of the PCR reactions were evaluated by electrophoresis of the products in 2% agarose gels (GIBCO) with ethidium bromide.

The PCR products were digested in 10-µl aliquots with 10 U of *Cfr*I restriction nuclease (BioLabs, New England, USA) for 3 h at 37°C. Restriction fragments were subjected to electrophoresis in 2% agarose/ethidium bromide gels (GIBCO, BRL, England) in 1 × TBE buffer (0.09 M Tris-boric acid, 0.002 M EDTA). The gels were examined under UV light and documented in a FX Phosphoimager apparatus (Bio-Rad).

Analysis of milk composition

The fat, protein and lactose contents of milk were determined in fresh samples using Milko Scan 104A/B. Per cent of total solids in each sample was expressed as a sum of per cent of fat, total protein, lactose and minerals and the solids-non-fat as total solids minus fat.

Statistical

Data were analysed with the repeatability mixed model used in authors' earlier study [Maj *et al*. 2004]. Effects of test-day, lactation number (parity), and stage of lactation were found to be significant in preliminary analyses. As interactions between lactation number (parity) and lactation stage were proved significant, in final analyses lactation curves were fitted within parity.

The model used for all traits and all considered polymorphisms was:

$$y_{ijklmn} = Gen_i + Parity_j + (\sum b_r LP_r)_j + tday_k + sire_l + cow_m + (cow \times parity)_{mj} + e_{ijklmn}$$

where y is the individual measure of considered trait in a given test-day.

The fixed effects in the model were: *Gen* – the considered polymorphism (with

either 2 or 3 levels) and 3 *Parity* subclasses. The *LPs* are Legendre polynomials of standardized days-in-milk (days in lactation), which were fitted as fixed covariates within each *Parity* subclass, in order to represent changes of considered traits due to stage of lactation. Fixed regressions were fitted up to the 5th power of Legendre polynomials (r=1, ….5). Legendre polynomials are commonly used for test-day models and were shown to be better than others, such as logarithmic polynomials [e.g. Kettunen *et al.* 2000]. The effect of date of test-day (*tday*), with 50 levels, was considered as random. Animal effects were *cow*, cow-by-parity (*cow* × *parity*) as specific effect of *n*-th cow in her *j*-th lactation, and *sire* of a cow. The MIXED Procedure in SAS [1999] was used for computations.

Size of the dataset was too small to consider genetic variance in analyses, thus genetic relationships between animals were ignored. All random effects were assumed to be uncorrelated and follow a normal distribution.

Results and discussion

Detection of allelic variation in the bovine *DGAT1* gene was carried out using RFLP-*Cfr*I (Fig. 1). The *DGAT1* polymorphism results from AA→GC substitution at nucleotide positions 10433-10434 within exon 8, causing a substitution of lysine by alanine at position 232 of DGAT1 protein. Both alleles were equally amplified and addition of 5% DMSO to the PRC reaction had no effect on the yield of PCR products (as it was suggested by Winter *et al.* [2002]). Two alleles (*AA* and *GC*) and three genotypes were identified. The *AA/GC* genotypes of the DAGT1 gene were estimated

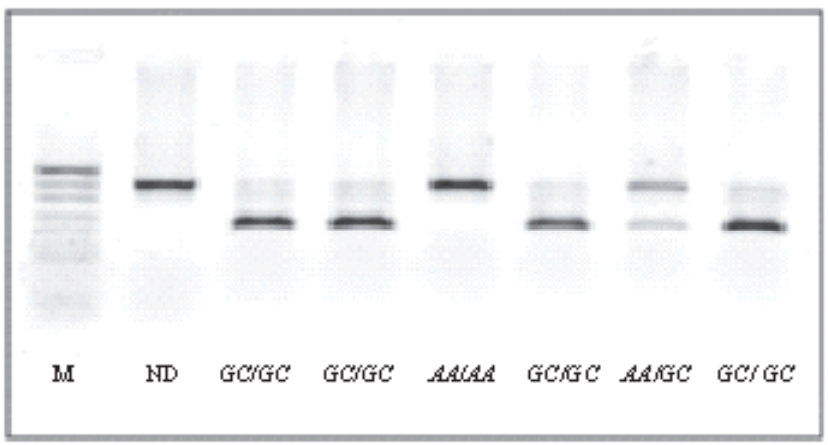

Fig.1. Agarose gel electrophoresis to detect RFLP-*Cfr*I in the exon 8 of the bovine acylCoA-diacylglycerol-acyltransferase 1 (*DGAT1*) gene. M – 11-1444 bp DNA marker (*Hae*III and *Taq*I digest of pUC19, InGen, Poland); ND – non-digested 411-bp PCR product. *AA/AA*, *GC/GC*, *AA/GC – DGAT1* genotypes.

in the group of 177 animals of dairy Black-and-White (Friesian) cattle. The frequency of *AA/AA*, *AA/GC*, and *GC/GC* genotypes was 0.11, 0.58, and 0.31, respectively. The *AA* and *GC* alleles were represented with a frequency of 0.40 and 0.60, respectively.

Effects of *DGAT1* genotype on daily milk yield and milk composition are presented in Table 1. *DGAT1* genotype did not affect the daily milk yield significantly. There was only a tendency to higher milk yield in cows of *GC/GC* genotype (with alanine at position 232 of the protein). Moreover, no differences were found in daily fat corrected milk (FCM) yield and average daily value-corrected milk (VCM) yield. Although cows carrying *GC/GC* gene variant yielded about 1.2 kg more milk per day, the difference in VCM yield was less by 1 kg when compared with *DGAT1 AA/GC* genotype cows. This could be a result of higher fat and total protein content of milks from *AA/GC* cows.

The *AA/GC* cows produced milk containing significantly more fat and total protein than those of *GC/GC* genotype (4.46% *vs* 4.16% and 3.55 vs 3.45%, respectvely). The fat content of milk of *AA/GC* cows was higher by 0.5 per cent points (pp) than in *GC/GC* cows. Higher fat and total protein content of milk of *AA/GC* cows resulted in the higher gross energy concentration. Moreover, the differences in fat and total protein content of milk significantly affected its total solids and solids-non-fat content. The cows carrying *DGAT1 AA/GC* genotype produced milk with total solids by 0.3 pp higher than those carrying *DGAT1 GC/GC* variant. The solids-non-fat content of milk in *AA/GC* cows was significantly higher than in milk of *GC/GC* cows – by 0.17 pp. The total milk protein to milk fat ratio was 0.79 and 0.83 in cows carrying *AA/GC* or *GC/GC* genotype, respectively. The higher ratio in the former was possibly related to less differences between protein and fat content than those found in the *AA/GC* cows.

No significant inter-genotype differences were

Table 1. Least squares means (LSM) and their standard errors (SE) for daily milk yield, milk energy, and per cent of major milk constituents as referred to *DGAT1* genotype

DGAT1 genotype	n[a]	Milk yield (kg)		Fat-corrected milk yield (kg)		Gross energy (MJ/l)		Total solids (%)		Solids-non-fat (%)		Fat (%)		Total protein (%)		Lactose (%)	
		LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE
AA/GC	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
GC/GC	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

[a] Number of milk samples analysed

[a,b] Within columns means bearing different superscripts differ significantly at P<0.01

found in milk components yielded per day (Tab. 2). However, a significantly higher total protein and fat content of milk in *AA/GC* genotype cows obviously resulted in higher production of milk components per day (non-significant) in spite of less daily milk yield (Tab. 1 and 2).

The gene encoding for acylCoA-diacyl-glycerol-acyltransferase 1 (DGAT1) was recently identified [Grisart *et al*. 2002, Winter *et al*. 2002] as the one underlying the quantitative trait *locus* (QTL) for milk production traits in the centromeric region of the bovine chromosome 14. The two alleles, the lysine variant (increasing fat and protein content) and the alanine variant (increasing milk and milk protein yield), were postulated at *DGAT1*. This notion was at least partially confirmed in the present study. Heterozygotic Black-and-White cows carrying one *AA* allele (coding for lysine at position 232) produced milk with significantly higher content (%) of fat and protein. Although differences in the daily milk yield were not found significant, cows of homozygous *GC* genotype (with alanine) produced daily by 1.2 kg more milk than *AA/GC* heterozygotes. The data for *AA/AA* homozygotes are not available, due to a small representation of this genotype in the population studied.

The results presented here show associations between genetic variants at the coding region of the bovine *DGAT1* gene and milk production traits in Polish Black-and-White (Friesian) cattle. In particular the *AA* allele (coding for lysine) appeared favourable for fat and protein content of milk. However, to draw final conclusions these studies should be extended to a larger population of cattle, preferably with the use of a reference family.

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Table 1. Least squares means (LSM) and their standard errors (SE) for daily yield of major milk constituents as referred to DGAT1 genotype

DGAT1 genotype	n*	FCM (kg)**		Solids-non-fat (g)		Total solids (g)		Fat (g)		Protein/fat		Protein (g)		Lactose (g)	
		LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE
AA/GC	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
GC/GC	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

*Number of milk samples analysed

**FCM – [illegible]

No significant differences were identified between genotypes

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Wpływ polimorfizmu genu *DGAT1* na cechy produkcji mlecznej krów rasy czarno-białej (fryzyjskiej)

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

Zbadano wpływ polimorfizmu lizyna/alanina (K232A) genu acylotransferazy diacylglycerol-acyloCoA-1 (*DGAT1*) na cechy produkcji mlecznej polskiego bydła czarno-białego (fryzyjskiego). Badany polimorfizm wynika z podstawienia nukleotydów AA→GC w eksonie 8 genu. Genotyp w *locus DGAT1* zidentyfikowano u 177 krów, spośród których 86 wykorzystano do badania związku polimorfizmu z wydajnością i składem mleka. Genotyp *DGAT1* nie wpływał istotnie na dzienną wydajność mleka. Nie stwierdzono także jego wpływu na wydajność mleka skorygowanego na zawartość tłuszczu (FCM) oraz na VCM (*value corrected milk*). Mleko krów o genotypie *AA/GC* cechowała istotnie wyższa zawartość tłuszczu i białka całkowitego niż stwierdzona w mleku krów o genotypie *GC/GC*. Ponadto, mleko krów o genotypie *AA/GC* zawierało więcej suchej masy i suchej masy beztłuszczowej. Stosunek białka całkowitego do tłuszczu był korzystniejszy w mleku krów o genotypie *GC/GC* (0,83) niż o genotypie *AA/GC* (0,79). Nie wykazano istotnego wpływu genotypu *DGAT1* na dobową ilość produkowanych składników mleka. Uzyskane wyniki wykazały wpływ genotypu *DGAT1* na niektóre cechy produkcji mlecznej krów. Allel *AA DGAT1* (kodujący lizynę) okazał się szczególnie korzystny z punktu widzenia zawartości (%) tłuszczu i białka w mleku.