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A relationship between *DGAT1* K232A polymorphism and selected reproductive traits in Polish Holstein-Friesian cattle

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The aim of the study was to analyse the relationship between *DGAT1* K232A polymorphism and bulls' breeding value for five reproductive traits – age at first insemination, calving-to-conception interval, calving-to-first service interval, non-return rate in heifers, and non-return rate in cows. The investigation was conducted on 264 Polish Black-and-White (Polish Holstein-Friesian) bulls from the active dairy population. *DGAT1* genotypes were identified using the PCR-RFLP technique. The *K* and *A* allele frequencies were 0.38 and 0.62, respectively. Homozygotes *AA* were characterized by highest breeding values of non-return rates, both in heifers and in cows. On the contrary, for the age at first insemination and for calving-to-conception and calving-to-first service intervals, the most favourable seemed to be the *KK* genotype. However, the significant effect of *AA* ($P \leq 0.01$) was obtained only for non-return rate in cows.

KEY WORDS: breeding value /cattle / *DGAT1* / gene polymorphism / non-return rate / reproductive traits

Declined reproduction efficiency related to the high milk production became a worldwide problem in dairy cattle breeding. High-yielding cows in early lactation require more energy than is available from their diet. This leads to the negative energy balance (NEB) which, in turn, negatively influences ovarian activity and largely determines the length of calving-to-first ovulation interval and the probability of subsequent conception [Butler 2003, Reist *et al.* 2003]. Several strategies can

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*Corresponding author e-mail: komisjol@jay.au.poznan.pl be applied to alleviate the NEB in early postpartum period and thus to decrease the risk of reproductive failures. The traditional approach includes increasing dietary energy concentration through the partial substitution of forages with concentrates or fat supplements. This is, however, associated with possible negative effects such as acidosis or reduced dry matter intake. An alternative approach is to decrease the calorific demand of milk production. As fat is the most energetically expensive to synthesize component of milk, reduction of its yield and content of milk may influence the severity and duration of NEB in high-producing dairy cows. Fat is the most variable among the major milk components and its synthesis is affected by many environmental and genetic factors.

Acyl-CoA:diacylglycerol acyltransferase (DGAT1) is an enzyme playing the key role in synthesis of major milk lipids – triacylglycerols [Farese *et al*. 2000]. In cattle, two polymorphisms identified in the *DGAT1* gene – K232A and promoter VNTR – are considered to affect milk yield and composition markedly [Bennewitz *et al*. 2004, Kühn *et al*. 2004, Gautier *et al*. 2007]. K232A is a nonconservative ApA to GpC dinucleotide substitution located in exon 8, that replaces lysine by alanine at position 232 of the encoding protein [Grisart *et al*. 2002, Winter *et al*. 2002]. Many investigations confirmed the pronounced effect of this mutation, especially on milk fat production [Grisart *et al*. 2002, Winter *et al*. 2002, Thaller *et al*. 2003, Sanders *et al*. 2006, Gautier *et al*. 2007].

The present study aimed at analysing the relationship between *DGAT1* K232A polymorphism and breeding value of bulls of selected reproductive traits in cattle.

Material and methods

The study included 264 Polish Black-and White (Polish Holstein-Friesian) bulls from the active dairy population, born from 1996 to 2001.

DNA for molecular analyses was extracted from peripheral blood or semen using the standard phenol method. Genotypes were determined using the PCR-RFLP technique. Primers for the PCR were established from *DGAT1* gene sequence available in the GenBank database (accession number AY065621) with the use of Primer3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3.cgi):

F: 5'- TGCCGCTTGCTCGTAGCTTTGGCC* -3, and
R: 5'- ACCTGGAGCTGGGTGAGGAACAGC -3'.

In the forward primer, the restriction site for enzyme *Bgl*I was introduced through purposeful unpairing of one base (marked with an asterisk). PCR amplification was performed in a TGradient thermocycler (BIOMETRA, Germany). The reaction mixture contained in 10 μl a total of 20-50 ng genomic DNA, 0.5 units of *Taq* polymerase (FERMENTAS, Lithuania), 1×PCR buffer with $(NH_4)_2SO_4$, 2 mM $MgCl_2$, 7% dimethyl sulfoxide, 1 μM of each primer (Institute of Biochemistry and Biophysics, Polish Academy of Sciences) and 200 μM of each dNTP (FERMENTAS, Lithuania). Reaction conditions included initial denaturation at 94°C (5 min) followed by 30 cycles consisting of denaturation (94°C, 30 s), annealing (58.5°C, 30 s), synthesis (72°C, 35 s), and final synthesis (72°C, 5 min). Amplified fragments were digested overnight at 37°C using 5 units of the *Bgl*I restriction endonuclease (FERMENTAS, Lithuania). Digestion products were subjected to electrophoresis in 3% ethidium bromide-stained agarose gel (BASICA GQT, Prona, Spain).

Effects of *DGAT1* genotypes on bulls' breeding values (EBV) were tested by one-way ANOVA [SAS 1999] for five following reproductive traits:

– age at first insemination – AFI;
– calving-to-conception interval after first calving – CCI;
– calving-to-first service interval after first calving – CFI;
– non-return rate to 56 days after first insemination in heifers – NRRH;
– non-return rate to 56 days after first insemination in cows – NRRC.

Used were the breeding values estimated (EBV) on the national scale in 2007 (http://wycena.izoo.krakow.pl/). The higher breeding values for AFI, CCI and CFI may be interpreted as indicative of decreased age at first insemination of daughters, and shorter intervals from first calving-to-conception and from first calving-to-first service in daughters, respectively. The higher breeding values for NRRH and NRRC indicate the increased per cent of daughters which have become pregnant after first insemination.

Results and discussion

The PCR reactions resulted in 378 bp-long products. After digestion with the *Bgl*I restriction enzyme the alanine encoding allele (*A*) was cleaved into three fragments – of 254 bp, 96 bp and 28 bp – while lysine encoding allele (*K*) was visible in gel as two bands – of 282 bp and 96 bp. Among the 264 bulls examined, 40 *KK*, 120 *KA* and 104 *AA* genotypes were identified. This gives frequencies of 0.38 and 0.62 for *K* and *A* alleles, respectively. Earlier, the lysine encoding variant frequency in Holstein-Friesian cattle was reported to range from 0.37 [Gautier *et al*. 2007], through 0.55 [Thaller *et al*. 2003, Kaupe *et al*. 2007] and 0.61 [Sanders *et al*. 2006] to 0.70 [Grisart *et al*. 2002].

In this study the observed genotypes distribution deviated from the Hardy-Weinberg equilibrium (χ^2=6.51, P≤0.05), with excess of *KA* heterozygotes mainly at the expense of homozygotes *AA*. The excess of heterozygotes compared with their expected distribution may indicate the presence of overdominant selection. On the other hand, however, it may also result from a small number of animals in the analysed sample.

Several studies confirmed that *DGAT1* K232A polymorphism is responsible for variation in milk-related traits in cattle. The lysine-encoding variant is generally related to markedly elevated fat content and a less distinctively elevated protein content, while its impact on both milk and protein yield is negative. In spite its relation with the lower milk yield, *K* allele is positively associated with fat yield [Grisart *et* *al*. 2002, Winter *et al*. 2002, Thaller *et al*. 2003, Gautier *et al*. 2007, Sanders *et al*. 2006]. A pronounced influence of *DGAT1* on milk-related traits, especially on milk fat yield, has been reported in many cattle populations, including the Jersey [Spelman *et al*. 2002], Fleckvieh [Thaller *et al*. 2003], Normande [Gautier *et al*. 2007] and Angeln [Sanders *et al*. 2006] breeds, as well as German [Thaller *et al*. 2003], Dutch [Grisart *et al*. 2002], French [Gautier *et al*. 2007], Polish [Szyda and Komisarek 2007], and New Zealand [Grisart *at al*. 2002] Holstein-Friesians. Additionally, Grisart *et al*. [2004] provided the functional confirmation of the K232A mutation effect on DGAT1 enzymatic activity. They demonstrated *in vitro* a significantly higher efficiency in triglyceride synthesis of the enzyme variant including lysine compared to the variant with alanine.

In this report, an effect of *DGAT1* gene polymorphism was analysed on five fertility traits of cattle. The results are presented in Table 1. Bulls homozygous for the *A* allele, that is regarded as variant decreasing fat and energy content of milk, were characterized by a highest breeding values for non-return rates, both in heifers and in cows. On the contrary, for the AFI, CCI and CFI, the most favourable seemed to be the *KK* genotype. However, the significant effect was identified only for NRRC. Although the effect of *DGAT1* on fertility traits was not intensively examined so far, observations of Kaupe *at al*. [2007] also indicate the possible relationship between an alanine encoding variant and increased non-return rate in cattle.

Table 1. Means and their standard deviations (SD) for estimated breeding values (EBV) of selected reproductive traits in Polish Black-and-White (Polish Holstein-Friesian) bulls

Reproductive trait		*DGAT1* genotype			P
		KK (n=40)	*KA* (n=120)	*AA* (n=104)	
AFI	mean	105.00	104.25	103.52	0.5509
	SD	8.95	9.41	11.66	
CCI	mean	96.65	95.47	95.95	0.7466
	SD	7.63	8.76	8.83	
CFI	mean	97.19	95.26	96.76	0.4471
	SD	7.75	11.97	8.84	
NRRH	mean	98.96	100.62	102.03	0.1196
	SD	10.34	11.57	11.15	
NRRC	mean	94.11^{A}	95.49^{a}	98.72^{Aa}	**0.0097**
	SD	8.64	10.17	10.21	

n – number of bulls.

AFI – age at first insemination; CCI – calving-to-conception interval; CFI – calving-to-first service interval; NRRH – non-return rate to day 56 after first insemination in heifers; NRRC – non-return rate to day 56 after first insemination in cows.

^{aA}Within rows means bearing the same superscript differ significantly at: small letters – P≤0.05; capitals – P≤0.01.

The mechanism underlying the positive influence of the *A* allele on non-return rate could be similar to the effect of administration of a dietary conjugated linoleic acid (CLA) to cows. *Trans*-10 *cis*-12 and several other CLA isomers inhibit the mammary lipid synthesis [Liu *et al*. 2006, Perfield *et al*. 2007]. Supplementing the diet with CLA promotes the milk fat depression and may improve the overall energy balance in the early lactation [Kay *et al*. 2007, Odens *et al*. 2007]. Although the positive effect of CLA on reproductive traits in cattle has not been confirmed, Bernal-Santos *et al*. [2003] and Castañeda-Gutiérrez *et al*. [2005] observed a tendency of shortening the calving-to-first ovulation interval and increasing the pregnancy rate in CLA-administered cows.

The *DGAT1* K232A mutation could affect fertility in a similar way. It is well established that NEB in the postpartum period, probably acting through the combined signaling of various metabolites and hormones, including glucose, IGF-I, insulin and nonesterified fatty acids, reduces the pulsative pituitary gonadotropin release necessary for the development of ovarian follicles and oocytes [Gong *et al*. 2002, Leroy *et al*. 2005, 2006]. Thus, as a consequence of lower energy expenditure in a K232A-*AA* cows producing less fat in milk, the NEB severity could be reduced and ovulation rate increased. Effect of *DGAT1* polymorphism on reproductive traits in dairy cattle should be, however, confirmed in the further investigations.

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Zależność między polimorfizmem *DGAT1* K232A a wybranymi cechami reprodukcyjnymi bydła rasy polskiej holsztyńsko-fryzyjskiej

Streszczenie

Celem pracy była analiza zależności między polimorfizmem *DGAT1* K232A a wartością hodowlaną buhajów pod względem pięciu cech reprodukcyjnych – wieku pierwszego unasienienia, okresu międzyciążowego, okresu od ocielenia do pierwszego unasienienia, wskaźnika niepowtarzalności unasieniania jałówek oraz wskaźnika niepowtarzalności unasieniania krów. Badaniem objęto 264 buhaje rasy polskiej holsztyńsko-fryzyjskiej z aktywnej populacji bydła mlecznego. Genotypy *DGAT1* identyfikowano metodą PCR-RFLP. Frekwencje alleli *K* i *A* wynosiły odpowiednio 0,38 i 0,62. Zwierzęta homozygotyczne pod względem allelu *A* charakteryzowały się najwyższą wartością hodowlaną wskaźnika niepowtarzalności unasieniania, zarówno jałówek, jak i krów. Natomiast pod względem wieku pierwszego unasienienia i okresów międzyciążowych oraz okresów od ocielenia do pierwszego unasienienia najkorzystniejszy okazał się genotyp *KK*. Statystycznie istotny ($P \leq 0,01$) wpływ genotypu *AA* udowodniono jednak tylko dla wskaźnika niepowtarzalności unasieniania krów.