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An effect of the *DGAT1* gene polymorphism on breeding value of Polish Holstein-Friesian sires

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The effect of the GC→AA polymorphism in the *DGAT1* gene causing amino acid substitution (lysine – *K* to alanine – *A*) was estimated on the breeding value (BV) of 89 Polish Holstein-Friesian sires. A fragment of 411 bp of the *DGAT1* gene was analysed by RFLP (*Cfr1*) or MSSCP (screening for other substitutions). Only GC→AA in exon 8 dinucleotide substitution was identified causing K232A amino acid substitution. The frequencies of *K* and *A* alleles were 0.54 and 0.46, respectively. The *KK* genotype (compared to *AA* and *KA*) was found associated with higher BV for fat and protein content of milk and lower BV for milk and protein yields. Distribution of the polymorphism within the Holstein-Friesian population, as well as its effect on bull BV are discussed.

KEY WORDS: breeding value / cattle / *DGAT1* / Holstein-Friesian / milk production traits

A quantitative trait *loci* (QTLs) for fat content of milk were mapped to the centromeric region of the bovine chromosome 14 (BTA14). In that region the acyl-CoA:diacylglycerol acyltransferase 1 (*DGAT1*) gene as a strong candidate gene for milk production traits was independently indicated by Winter *et al.* [2002] and Grisart *et al*. [2002]. Moreover, Kaupe *et al.* [2007] showed that the *K* allele of the *DGAT1* gene could have a possible negative effect on the maternal non-return rate and in consequence on the length of productive life. The DGAT1 protein is an enzyme catalyzing the final step of triglyceride syntesis. Numerous mutations in the bovine *DGAT1* gene were found, but only one, localized in exon 8 at positions 10433 and

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10434, resulting in lysine for alanine substitution (K232A) has been shown to be associated with milk production traits. In particular, the lysine encoding allele (allele *K*) is associated with increased fat content of milk compared to the alanine amino-acid residue (allele *A*) at position 232 [Winter *et al*. 2002, Grisart *et al*. 2002]. The polymorphism in question was analysed with the bovine microarray containing 16 SNPs by Kaminski *et al*. [2006] who showed that the combined genotypes of the *DGAT1* and leptin genes are associated with fat yield, and fat and protein contents of milk. Szyda and Komisarek [2007] analysed an effect of nine SNPs in *BTN1A1*, *LEP*, *LEPR* and *DGAT1* genes on milk production traits. They showed that K232A polymorphism in the *DGAT1* gene had a much larger additive effect on milk, fat and protein yields than the other polymorphisms considered. Moreover, it was shown that the *K* allele is related to an increase of saturated and decrease of unsaturated fatty acids share in milk what may have a negative effect on human health [Schennink *et al.* 2007]. In addition, it was shown that the *K* allele has a positive effect on intramuscular fat content of beef in Charolaise and Holstein cattle [Thaller *et al.* 2003a]. However, Casas *et al*. [2005] reported no significant association to exist between the K232A polymorphism and carcass traits in zebu cattle.

Milk production traits are determined by genetic and numerous nongenetic factors which are not easy to estimate when analysed with limited data. It can be done more efficiently during routine estimation of breeding value. Thus, the effects of polymorphisms could be estimated based on analysis of breeding values which may contain also effects of major genes.

The aim of the current report was to present the results of evaluation the effect of the K232A polymorphism on breeding value for milk production traits of Polish Holstein-Friesian bulls.

Material and methods

The polymorphism of the *DGAT1* gene was studied in 89 Polish Holstein-Friesian (HF) sires from the local AI centre (Tulce near Poznań), born in 1990-1997, and having the estimated breeding value (BV). Genomic DNA was extracted from peripheral blood using the phenol-chloroform method according to the standard protocol, and from semen samples with the use of a commercial kit Genomic Mini (A&A Biotechnology, Gdansk, Poland). A fragment of 411 bp of *DGAT1* gene was amplified by PCR using a T-gradient thermocycler (BIOMETRA, Goettingen) with the use of primers according to Winter *et al*. [2002]. The PCR reaction conditions were as follows: initial denaturation at 94ºC for 5 min, 35 cycles of denaturation at 94ºC for 40 s, annealing of primers at the temperature at 61.5ºC for 40 s, elongation at 72ºC for 1 min, and final elongation at 72ºC for 10 min. The PCR products were separated by electrophoresis in 1.5% agarose gel with a TBE buffer, stained with EtBr, and visualized and photographed in UV light.

Bulls were genotyped with two techniques. Restriction fragment length polymorphism (RFLP) was identified according to Winter *at al.* [2002]. The PCR product, digested over 12 hours by the *Cfr*I restriction enzyme, was separated on 3% agarose gel. In addition, the multi-temperature single strand conformation polymorphism (MSSCP) was applied. Briefly, 4 µl PCR product were added to 16 µl loading dye (96% formamide, 0.05% bromophenol blue, 0.05% xylem cyanol). The samples were denaturated at 95°C for 5 min, cooled on ice for 5 min and loaded onto a 9% polyacrylamide gel. The conditions of vertical electrophoresis were as follows (temperature-voltage-time): 30ºC-175V-2h, 15ºC-280V-1.5h, and 5ºC-400V-2h. The gels were stained with the use of 0.2% silver nitrate solution and developed with the solution of sodium carbonate.

The estimated BVs for production traits were derived from the recent official estimations of 89 Polish HF sires [Żarnecki *et al*. 2007]. The effect of genotype on breeding value for milk yield, fat yield, protein yield, and fat and protein content was estimated with analysing least squares means using one-way ANOVA.

Results and discussion

The MSSCP technique appeared to be a reliable alternative for the RFLP test. Moreover, the technique made it possible to detect other polymorphisms, apart from the K232A, in the studied fragment. The only three patterns observed in MSSCP technique were confirmed by RFLP and were in accordance with three genotypes: *KK*, *KA* and *AA*. No other MSSCP patterns were observed, thus the authors assumed that in the analysed fragment only the dinucleotide (GC→AA) substitution, causing the K232A amino acid change, was present (Fig.1). The genotype frequency was 0.18 for *AA*, 0.55 for *KA* and 0.27 for *KK*. The frequencies of the *K* and *A* alleles were 0.54 and 0.46, respectively. This distribution is in accordance with data reported by Kaupe *et al.* [2007] for the German Holstein breed (0.549 and 0.451, respectively). A wide range of distribution of the K232A polymorphism in 38 *Bos indicus* and *Bos taurus* breeds was presented by Kaupe *et al.* [2004]. They did not detect the *K* allele in Belgian Blue (beef), Gelbvieh, Hereford, Pinzgauer and Slavonian Syrmian breeds, whereas in the Nellore and White Fulani breeds the frequency reached 0.99 and 0.92, respectively. The K232A polymorphism in the Holstein-Friesian breed was presented in several reports and the frequency of the *K* allele ranged from 0.27 to 0.65 (Tab. 1). Citek *et al.* [2007] analysed the K232A polymorphism in two groups of Holstein-Friesian sires: born in 1993 and between 1998 and 2001. They observed a tendency of increasing the frequency of the *A* allele in bulls born in 1998-2001, probably due to selection for protein yield.

Recent studies have been focused on search for another source of genetic variation in the centromeric region of BTA14, influencing milk fat content. Kühn *et al.* [2004] suggested that besides the K232A polymorphism some phenotypic variation in fat content of milk was associated with the variable number of tandem repeats (VNTR)

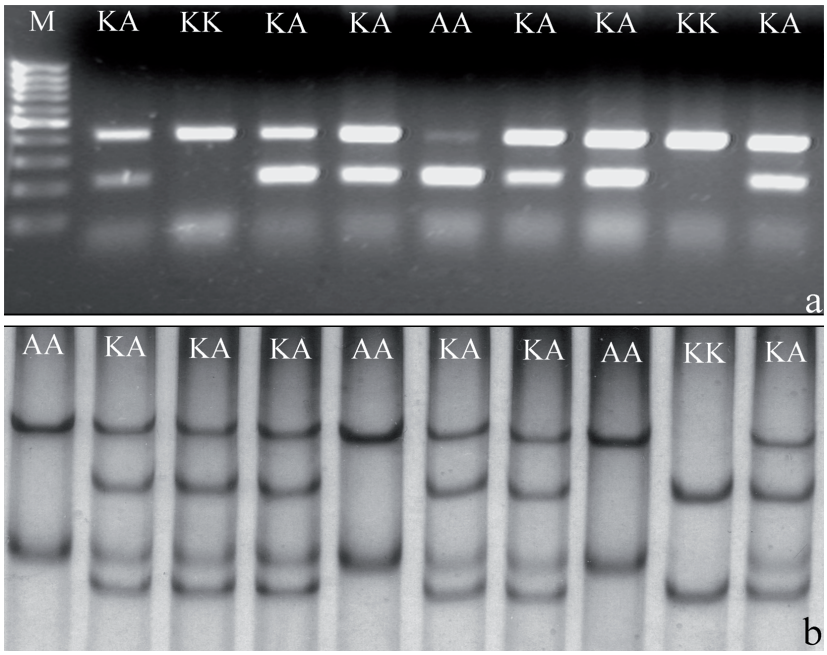

Figure 1. Electrophoretic distribution of the *DGAT1* gene fragments; a – after PCR-RFLP analysis (M – length marker Gene Ruler™ 100bp DNA Ladder Plus (FERMENTAS); b – MSSCP pattern of the analysed gene fragment.

Table 1. Distribution of the *DGAT1* polymorphism in Holstein-Friesian cattle

Variety	N	Sex	Frequency		Reference
			K	*A*	
German Holstein-Friesian	1291	m	0.549	0.451	Kaupe *et al*. [2007]
	200*	m	0.37	0.63	
	66**	m	0.40	0,60	Citek *et.al*. [2007]
	79	nm	0.42	0.58	Kaupe *et al*. [2004]
	28	m+f	0.446	0.554	Thaller *et al*. [2003a]
	858	m	0.548	0.452	Thaller *et al*. [2003b]
	40	m	0.35	0.65	Winter *et al*. [2002]
Dutch Holstein-Friesian (1818 males) and New Zealand Holstein-Friesian (529 females)	2347	m+f	0.63	0.37	Grisart *et al*. [2002]
Dutch Holstein-Friesian	1762	f	0,40	0,60	Schennink *et al*. [2007]
New Zealand Holstein-Friesian	1527	m	0.6	0.4	Spelman *et al*. [2002]
Brasilian Holstein	50	m	0.27	0.73	Lacorte *et al*. [2006]
Polish Holstein-Friesian	244	m	0.65	0.35	Pareek *et al*. [2005]
	213	f	0.48	0.52	
	177	f	0.40	0.60	Strzalkowska *et al*. [2005]
	89	m	0.54	0.46	this study

m – males; f – females; nm – not mentioned; *born in 1998-2001; **born in 1993.

in the 5'-flanking region of the *DGAT1* gene. The polymorphic motif $(CCCGCC)_n$ is a potential binding site for the Sp1 transcription factor, and thus could affect the expression of the *DGAT1* gene. Moreover, Fürbass *et al*. [2006] suggested that an allele containing seven repeats may cause fat content increase in *AA* animals, comparable to the *KK* genotype effect. Recently, Gautier *et al.* [2007] analysed the effects of *DGAT1* K232A and VNTR polymorphisms in three breeds of French dairy cattle. They showed that different alleles of the VNTR were significantly associated with fat content (%) variation in the breeds studied. Therefore, Gautier *et al*. suggested the existence of another causative polymorphism (besides VNTR and K232A) in the centromeric region of *BTA14* responsible for variation in milk production traits.

The present report shows a relationship between the BV for selected milk production traits and *DGAT1* genotype in sires (Tab. 2). The *KK* genotype was associated with highest BV for fat content of milk, what is in accordance with the results of Winter *et al.* [2002], Grisart *et al*. [2002] and Thaller *et al*. [2003b]. On the other hand, however, *KK* animals presented the lowest BV for milk yield and the highest for protein content. The highest protein content seemed to be related with the lowest milk yield, causing the lowest BV for protein yield. A similar observation concerning lower milk and protein yields was presented by Spelman *et al.* [2002], who analysed milk production traits in Holstein-Friesian, Jersey and Ayrshire cattle in New Zealand. Moreover, Spelman *et al.* [2002] found that the *KK* genotype was associated with higher milk fat yield. Such a tendency was also presented in the current report, the intergenotype differences being, however, not significant. Citek *et al*. [2007] also observed that the *K* allele was related to the higher BV in terms of fat yield and content, whereas the *A* allele for milk and protein yield.

Table 2. Least squares means (LSM) and their standard errors (SE) for breeding values of sires with different *DGAT1* genotypes. Values denote deviations from the official genetic base defined as the mean breeding values of cows born in the year 2000

Geno-type	Number of animals		Milk yield (kg)	Fat yield (kg)	Protein yield (kg)	Fat content (%)	Protein content (%)
AA	16	LSM	166.18^{A}	-3.47	1.51^{A}	-0.19^{Ac}	-0.07^{A}
		SE	102.24	3.59	3.06	0.05	0.03
KA	49	LSM	19.90^{B}	-0.15	-0.98^{B}	-0.03^{Bc}	-0.03^{B}
		SE	58.42	2.05	1.75	0.03	0.02
KK	24	LSM	-350.21^{AB}	2.01	-9.19^{AB}	0.31^{AB}	0.04^{AB}
		SE	83.48	2.93	2.51	0.04	0.02

$^{aA....}$Within columns means bearing the same superscripts differ significantly at: small letters – $P \leq 0.05$; capitals – $P \leq 0.01$.

This study showed higher BVs of sires for fat content and lower for milk and protein yields as associated with *DGAT1 KK* genotype what confirmed the earlier reports on the combined effect of the K allele. Thus, taking into consideration the breeding goal to increase protein yield, the elimination of the K allele may be postulated. However, it may negatively affect the fat yield of milk.

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Wpływ polimorfizmu genu *DGAT1* na wartość hodowlaną buhajów rasy polskiej holsztyńsko-fryzyjskiej

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

Celem pracy była analiza polimorfizmu K232A (substytucja lizyny alaniną) w białku DGAT1 oraz ocena wpływu tego polimorfizmu na wartość hodowlaną 89 buhajów rasy polskiej holsztyńsko-fryzyjskiej. Techniką PCR amplifikowano fragment 411 nukleotydów genu *DGAT1*, który następnie analizowano dwiema technikami: RFLP (z zastosowaniem enzymu restrykcyjnego *Cfr*I) oraz MSSCP (dla poszukiwania innych substytucji). Zidentyfikowano tylko dinukleotydową substytucję GC→AA, wywołującą zmianę sekwencji aminokwasów (K232A). Frekwencja allelu *K* wyniosła 0,54, a allelu *A* – 0,46. Wykazano, że buhaje o genotypie *KK* mają wyższą wartość hodowlaną dla cech zawartości tłuszczu i białka, a niższą dla wydajności mleka i białka mleka niż buhaje *AA* i *KA*. Omówiono stopień rozprzestrzenienia badanego polimorfizmu w rasie holsztyńsko-fryzyjskiej oraz jego wpływ na wartość hodowlaną buhajów.