Animal Science Papers and Reports vol. 22 (2004) no. 3, 307-313
Institute of Genetics and Animal Breeding, Jastrzębiec, Poland

Effects of DGAT1 variants on milk production traits in Jersey cattle

Jolanta Komisarek*, Krystyna Waśkowicz, Arkadiusz Michalak, Zbigniew Dorynek

Department of Cattle Breeding,
The August Cieszkowski Agricultural University of Poznań,
Wojska Polskiego 71 A, 60-625 Poznań, Poland

(Received March 23, 2004; accepted August 18, 2004)

The aim of the study was to analyse the K232A polymorphism in the acyl CoA:diacylglycerol acyltransferase gene (*DGAT1*) and to estimate the effects of lysine (*K*) and alanine (*A*) encoding variants on milk yield and composition in cattle. A total of 100 Jersey cows was tested for *DGAT1* genotypes using the PCR-RFLP technique. The *K* and *A* allele frequencies were 0.83 and 0.17, respectively. Effect of the K232A polymorphism on most considered traits was observed. The lysine encoding variant was associated with the high milk fat yield as well as fat and protein contents, whereas the alanine allele was related to the increased milk yield. The most significant result was obtained for the fat content of milk. None of the dominance effects proved to be significantly different from zero, that indicates the additive effect of the gene.

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

Fat is one of the major components of milk and is a key source of energy to the suckling young mammals. Milk fat is primarily composed of triglycerides (triacylglycerols or TAG), a typical storage form of lipids, which account for over 95% of the total milk fat [Jensen 2002 – a review].

One of the key enzymes in triglyceride metabolism is acyl CoA:diacylglycerol acyltransferase (DGAT), an integral microsomal membrane enzyme that catalyses

*e-mail: komisjol@jay.au.poznan.pl

the termal step in both two major pathways of TAG synthesis [reviews: Lehner and Kuksis 1996, Farese *et al*. 2000]. The reaction involves the joining of fatty acyl CoA to diacylglycerol, which can be supplied either by hydrolysis of phosphatidic acid in the glycerol phosphate pathway, or by acylation of monoacylglycerol in the monoacylglycerol pathway. Until now, two acyl CoA:diacylglycerol acyltransferases – DGAT1 and DGAT2 – have been identified [Cases *et al*. 1998, 2001].

The knock-out of mouse *DGAT1* gene indicates its crucial role for lactation. Females deficient in this enzyme are characterized by a complete lack of milk production, most probably because of impaired triglycerides synthesis in the mammary gland [Smith *et al*. 2000].

In cattle, *DGAT1* is considered to be a very strong positional candidate gene for fat per cent of milk. It is located on the centromeric end of the bovine chromosome 14, within a region that contains quantitative trait *locus* (QTL) influencing milk yield and composition [Coppieters *et al*. 1998, Heyen *et al*. 1999, Riquet *et al*. 1999, Farnir *et al*. 2002]. It was recently shown that this QTL variation is most likely caused by a nonconservative ApA→GpC dinucleotide substitution in exon 8 of gene *DGAT1*, changing lysine to alanine at position 232 (K232A mutation) of the encoding protein [Grisart *et al*. 2001, Winter *et al*. 2002].

The aim of the present study was to analyse the relationship between K232A polymorphism in *DGAT1* gene and milk production traits in Jersey cattle.

Material and methods

The study included 100 Jersey cows, born between 1996 and 2000, maintained on the Siedlec farm belonging to Horse Stud, Iwno, Poland.

Genotypes of gene *DGAT1* were analysed using the PCR-RFLP technique. Genome DNA was isolated from whole blood with a method described by Kanai *et al.* [1994].

Primers for PCR amplification (MWG-Biotech, Germany) were designed using the Primer3 software (http://www.genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi) on the basis of gene sequence available in the GenBank (accession number AY065621), as follows:

DGAT1-F: 5'-CACCATCCTCTTCCTCAAGC-3' and
DGAT1-R: 5'-ATGCGGGAGTAGTCCATGTC-3'

The PCR reaction volume of 25 µl contained 30-50 ng of genomic DNA, 1 unit of Taq DNA polymerase (FERMENTAS), 1× PCR buffer with $(NH_4)_2SO_4$ (FERMENTAS), 1 µM of each primer, 2 mM $MgCl_2$, 5% DMSO and 200 µM of each dNTP (FERMENTAS). Amplifications were carried out using a T Gradient thermocycler (BIOMETRA) as follows: 94°C for 5 min; 30 cycles consisting of 30 s at 94°C, 30 s at 58,5°C and 40 s at 72°C; 72°C for 5 min.

The amplified fragments (5 µl of PCR products) were digested with 10 units of *Cfr*I restriction endonuclease (FERMENTAS) at 37°C for at least 2.5 h, and next sub-

jected to electrophoretic separation in 1.5% agarose gel (BASICA LE GQT, Prona) in 1× TBE buffer.

The effect of *DGAT1* genotypes on milk traits was tested using the GLM procedure of the SAS package [SAS 1989]. The statistical model included effects of *DGAT1* genotype, sire, herd-year-season of calving and lactation number. The records of milk, fat and protein yields as well as fat and protein contents were obtained from the official milk recording system. The analysed *locus* was expressed either as genotype classes or as regressions on the amount of the alternative allele and dominance.

Results and discussion

The PCR amplification yielded a 777 bp long *DGAT1* gene fragment. One *Cfr*I restriction site was found in the K (lysine) allele, at position 653. In the A (alanine) allele, an additional restriction site at position 202 was present (Fig. 1).

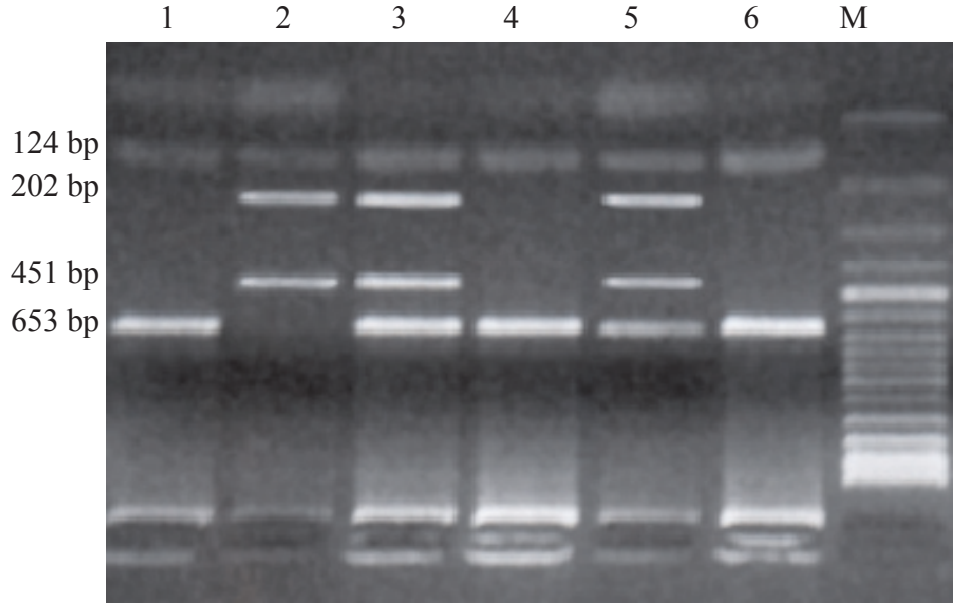

Fig. 1. DGAT1 genotyping with PCR-RFLP method. Lanes 1, 4, 6 – *KK* genotype; lane 2 – *AA* genotype; lanes 3, 5 – *KA* genotype; M – DNA marker: GeneRuler™ DNA Ladder Mix (FERMENTAS).

Among the 100 animals examined, 72 *KK*, 22 *KA* and 6 *AA* genotypes were identified. This gives frequencies of 0.83 and 0.17 for *K* and *A* alleles, respectively. A comparable values of 0.88 for lysine and 0.12 for alanine were reported by Spelman *et al*. [2002] for New Zealand Jersey bulls. In the Holstein-Friesian (HF) breed, allele frequencies of *DGAT1* differ considerably between populations. The *K* variant frequencies range

from 0.35 to 0.7 [Grisart *et al*. 2001, Spelman *et al*. 2002, Winter *et al*. 2002, Thaller *et al*. 2003]. The lowest values were obtained for HF animals of the US and Dutch origin [Grisart *et al*. 2001, Spelman *et al*. 2002], selected mainly for milk yield. The New Zealand Holsteins, selected with a special emphasis on the fat, and, until recently, also on the protein contents of milk, are characterized by a *K* allele frequency of 0.7, similar to that of the Jersey breed [Grisart *et al*. 2001, Spelman *et al*. 2002]. Winter *et al*. [2002], within the German HF population, observed the distinct differences in allele frequencies between individuals with different breeding values for milk fat content. The lysine encoding variant was more frequent in the group of animals with high breeding values. The same tendency was shown for Fleckvieh and Braunvieh German breeds [Winter *et al*. 2002]. Thus, the *K* allele seems to be related to the elevated milk fat per cent in cattle. Association studies of variation in *DGAT1* gene and milk production traits support the conclusion arising from the allele frequencies data.

Table 1. Effect of K232A mutation in gene *DGAT1* on milk traits in Jersey cows – least squares means and their standard errors for *DGAT1* genotypes, A→K allele substitution effects, and *KA* dominance effects

Genotype	No. of animals	Milk (kg)		Fat				Protein			
				kg		%		kg		%	
		mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
KK	72	4237^{aA}	663	249^{a}	44	5.88^{aA}	0.47	168^{a}	28	3.97^{aA}	0.22
KA	22	4615^{a}	894	243^{a}	43	5.19^{aC}	0.34	177^{a}	32	3.78^{aB}	0.16
AA	6	4693^{a}	577	224^{aB}	32	4.77^{aC}	0.27	171	24	3.65^{aB}	0.25
A→K allele substitution effect		-228*	92	12.81*	5.44	0.56***	0.06	-1.41	3.69	0.16***	0.03
KA dominance effect		151	143	5.87	8.72	-0.14	0.09	7.58	5.71	-0.03	0.04

^{aA} Within columns means bearing the same superscripts differ significantly at: small letters – P≤0.05; capitals – P≤0.01.
*Significant at P≤0.05.
***Significant at P≤0.001.

The effects of K232A mutation on milk yield and composition in Jersey cows are presented in Table 1. Although the most significant results were obtained for fat, *DGAT1* polymorphism affected the other traits considered, as well. In most cases, the heterozygous genotype values were found between alternate homozygotes. Additionally, none of the dominance effects proved to be significantly different from zero, that indicates the exclusively additive effect of the gene. The lysine encoding variant was associated with increased fat and protein contents. In contrast, its impact on milk yield appeared to be negative. As a consequence, the influence of *DGAT1* variants on protein yield was not distinct. However, in spite of the antagonistic effects on milk yield and content traits, the *K* allele significantly increased the yield of fat.

The results presented here are in the good agreement with those previously reported for several dairy cattle breeds, including Jersey [Spelman *et al*. 2002], Ayrshire [Spelman *et al*. 2002], Fleckvieh [Thaller *et al*. 2003] and Holstein-Friesian [Grisart *et al*. 2001, Spelman *et al*. 2002, Thaller *et al*. 2003]. Although the magnitude of the K232A mutation effects on milk traits differed between populations, the direction was always the same. The lysine variant was consistently associated with high fat and protein contents and fat yield, whereas the alanine variant increased milk and protein yields. The same trend was generally observed in the present study. Only the impact of *K* allele on protein yield decrease was not evident (Tab. 1). Although the *KK* homozygotes were characterized by a lowest trait value, the allele substitution effect was not significant. The reason for this could be the very limited number of *AA* homozygotes (6 animals) as well as the smaller influence of *DGAT1* variants on protein yield compared with the other milk traits that was reported also by Grisart *et al*. [2001] and Thaller *et al*. [2003], or both.

There are strong evidences that K232A substitution in *DGAT1* is responsible for the variation of QTL controlling milk yield and composition on bovine chromosome 14 [Grisart *et al*. 2001, Winter *et al.* 2002]. Until recently, however, it could not be completely excluded, that the association observed is due to another causal mutation located in the same, or in another gene being in the linkage disequilibrium with the lysine/alanine *DGAT1* variants. The latest report of Grisart *et al.* [2004] confirmed the causality of the K232A mutation. They demonstrated that the $K \rightarrow A$ substitution affected the acyl CoA:diacylglycerol acyltransferase enzymatic activity. The amount of triglycerides synthesized by the enzyme variant including lysine was approximately 1.5 times greater than that synthesized by the enzyme variant with alanine, what is in agreement with phenotype effects of both alleles. The positively charged, hydrophilic lysine residue at position 232 of the DGAT1 protein seems to be, therefore, more efficient in binding of acyl CoA than a neutral, hydrophobic alanine residue.

Recently, it has been shown that K232A *DGAT1* polymorphism is not responsible for all the genetic variation at the centromeric end of chromosome 14 for milk-related traits [Bennewitz *et al*. 2004]. Thus, at this region, the additional source of variation exists, either within the acyl CoA:diacylglycerol acyltransferase or in another *locus*. The gene content of the chromosomal region flanking *DGAT1* was determined by Winter *et al.* [2004]. Their results can be the basis for future studies testing the *DGAT1* neighboring *loci* as a candidate genes for milk traits in cattle.

REFERENCES

1. BENNEWITZ J., REINSCH N., PAUL S., LOOFT C., KAUPE B., WEIMANN C., ERHARDT G., THALLER G., KÜHN C., SCHWERIN M., THOMSEN H., REINHARDT F., REENTS R., KALM E., 2004 – The DGAT1 K232A mutation is not solely responsible for the milk production quantitative trait locus on the bovine chromosome 14. ***Journal of Dairy Science*** 87, 431-442.
2. CASES S., SMITH S.J., ZHENG Y.-W., MYERS H.M., LEAR S.R., SANDE E., NOVAK S., COLLINS C., WELCH C.B., LUSIS A.J., ERICKSON S.K., FARESE JR R.V., 1998 – Identification of a gene encoding an acyl CoA:diacylglycerol acyltransferase, a key enzyme in triacylglycerol synthesis. ***Proceedings of the National Academy of Sciences, USA*** 95, 13018-13023.

3. CASES S., STONE S.J., ZHOU P., YEN E., TOW B., LARDIZABAL K.D., VOELKER T., FARESE JR R.V., 2001 – Cloning of DGAT2, a second mammalian diacylglycerol acyltransferase, and related family members. ***Journal of Biological Chemistry*** 276, 38870-38876.
4. COPPIETERS W., RIQUET J., ARRANZ J.-J., BERZI P., CAMBISANO N., GRISART B., KARIM L., MARCQ F., MOREAU L., NEZER C., SIMON P., VANMANSHOVEN P., WAGENAAR D., GEORGES M., 1998 – A QTL with major effect on milk yield and composition maps to bovine chromosome 14. ***Mammalian Genome*** 9, 540-544.
5. FARESE JR R.V., CASES S., SMITH S.J., 2000 – Triglyceride synthesis: insights from the cloning of diacylglycerol acyltransferase. ***Current Opinion in Lipidology*** 11, 229-234.
6. FARNIR F., GRISART B., COPPIETERS W., RIQUET J., BERZI P., CAMBISANO N., KARIM L., MNI M., MOISIO S., SIMON P., WAGENAAR D., VILKKI J., GEORGES M., 2002 – Simultaneous mining of linkage and linkage disequilibrium to fine map quantitative trait loci in outbred half-sib pedigrees: revisting the location of a quantitative trait locus with major effect on milk production on bovine chromosome 14. ***Genetics*** 161, 275-287.
7. GRISART B., COPPIETERS W., FARNIR F., KARIM L., FORD C., BERZI P., CAMBISANO N., MNI M., REID S., SIMON P., SPELMAN R., GEORGES M., SNELL R., 2001 – Positional candidate cloning of a QTL in dairy cattle: Identification of a missense mutation in the bovine DGAT1 gene with major effect on milk yield and composition. ***Genome Research*** 12, 222-231.
8. GRISART B., FARNIR F., KARIM L., CAMBISANO N., KIM J.-J., KVASZ A., MNI M., SIMON P., FRERE J.-M., COPPIETERS W., GEORGES M., 2004 – Genetic and functional confirmation of the causality of the DGAT1 K232A quantitative trait nucleotide in affecting milk yield and composition. ***Proceedings of the National Academy of Sciences, USA*** 101, 2398-2403.
9. HEYEN D.W., WELLER J.I., RON M., BAND M., BEEVER J.E., FELDMESSER E., DA Y., WIGGANS G.R., VANRADEN P.M., LEWIN H.A., 1999 – A genome scan for QTL influencing milk production in dairy cattle. ***Physiological Genomics*** 1, 165-175.
10. JENSEN R.G., 2002 – The composition of bovine milk lipids: January 1995 to December 2000. ***Journal of Dairy Science*** 85, 295-350.
11. KANAI N., FUIJI T., SAIKI K., TOKOYAMA T., 1994 – Rapid and simple method for preparation of genomic DNA from easily obtainable clotted blood. ***Journal of Clinical Pathology*** 47, 1043-1044.
12. LEHNER R., KUKSIS A., 1996 – Biosynthesis of triacylglycerols. ***Progress in Lipid Research*** 35, 169-201.
13. RIQUET J., COPPIETERS W., CAMBISANO N., ARRANZ J.-J., BERZI P., DAVIS S., GRISART B., FARNIR F., KARIM L., MNI M., SIMON P., TAYLOR J.F., VANMANSHOVEN P., WAGENAAR D., WOMACK J.E., GEORGES M., 1999 – Identity-by-decent fine-mapping of QTL in outbred populations: Application to milk production in dairy cattle. ***Proceedings of the National Academy of Sciences, USA*** 96, 9252-9257.
14. SAS, 1989. SAS/STAT User's Guide, Version 6, 4th ed. SAS Inst., Inc., Cary, NC.
15. SMITH S.J., CASES S., JENSEN D.R., CHEN H.C., SANDE E., TOW B., SANAN D.A., RABER J., ECKEL R.H., FARESE JR R.V., 2000 – Obesity resistance and multiple mechanisms of triglyceride synthesis in mice lacking Dgat. ***Nature Genetics*** 25, 87-90.
16. SPELMAN R.J., FORD C.A., MCELHINNEY P., GREGORY G.C., SNELL R.G., 2002 – Characterization of the DGAT1 gene in the New Zealand dairy population. ***Journal of Dairy Science*** 85, 3514-3517.
17. THALLER G., KRÄMER W., WINTER A., KAUPE B., ERHARDT G., FRIES R., 2003 – Effects of DGAT1 variants on milk production traits in German cattle breeds. ***Journal of Animal Science*** 81, 1911-1918.
18. WINTER A., ALZINGER A., FRIES R., 2004 – Assessment of the gene content of the chromosomal regions flanking bovine DGAT1. ***Genomics*** 83, 172-180.

19. WINTER A., KRÄMER W., WERNER F.A.O., KOLLERS S., KATA S., DURSTEWITZ G., BUITKAMP J., WOMACK J.E., THALLER G., FRIES R., 2002 – Association of a lysine-232/alanine polymorphism in a bovine gene encoding acyl-CoA:diacylglycerol acyltransferase (DGAT1) with variation at a quantitative trait locus for milk fat content. ***Proceedings of the National Academy of Sciences, USA*** 99, 9300-9305.

Jolanta Komisarek, Krystyna Waśkowicz,
Arkadiusz Michalak, Zbigniew Dorynek

Wpływ polimorfizmu w genie *DGAT1* na cechy mleczności bydła rasy jersey

Streszczenie

Celem pracy była analiza polimorfizmu K232A w genie acylotransferazy diacyloglicerolowej (*DGAT1*) oraz oszacowanie wpływu alleli kodujących lizynę (*K*) i alaninę (*A*) na wydajność i skład mleka bydła. Badaniem objęto 100 krów rasy jersey. Genotypy *DGAT1* identyfikowano metodą PCR-RFLP. Frekwencje alleli *K* i *A* wynosiły odpowiednio 0.83 i 0.17. Zaobserwowano wpływ polimorfizmu K232A na większość analizowanych cech. Allel kodujący lizynę związany był z wyższym procentem tłuszczu i białka w mleku oraz wyższą wydajnością tłuszczu, natomiast allel kodujący alaninę zwiększał wydajność mleka. Najbardziej istotny wynik uzyskano dla zawartości tłuszczu w mleku. Dla żadnej z analizowanych cech efekt dominacyjny nie był istotnie różny od zera, co wskazuje na addytywne działanie genu.