Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Short Communication

Turk J Vet Anim Sci
(2016) 40: 251-254
© TÜBİTAK
doi:10.3906/vet-1508-7

The effect of DGAT1 polymorphism on milk production traits in dairy cows depending on environmental temperature

Jolanta KOMISAREK*, **Mateusz KOLENDA**
Department of Animal Breeding and Product Quality Assessment, Faculty of Veterinary Medicine and Animal Science, Poznań University of Life Sciences, Poznań, Poland

Received: 04.08.2015 • **Accepted/Published Online:** 02.12.2015 • **Final Version:** 05.02.2016

Abstract: It has long been known that a hot environment negatively influences milk production in cattle. Although molecular mechanisms underlying this phenomenon to a great extent remain to be recognized, they include alterations in lipid metabolism. DGAT1 plays a key role in the synthesis of triacylglycerols and polymorphism of this gene has been proven to have a pronounced influence on milk production. The aim of this study was to establish if the effect of DGAT1:p.K232A on milk-related traits is dependent on environmental thermal conditions. The analysis, performed on 468 cows of the Polish Holstein–Friesian breed, showed that allele DGAT1:K232 significantly increased milk content traits regardless of the temperature. However, its decreasing effect on milk and protein yields as well as the increasing influence on fat yield was not observed in hot weather. Results of this study suggest that the effect of DGAT1:p.K232A on some milk production traits is not constant and may depend on environmental thermal conditions.

Key words: Cattle, DGAT1, environmental temperature, milk production traits

Heat stress is a growing problem in dairy cattle breeding because of global warming and because of the genetic selection for greater production that reduces thermotolerance in cows. The negative influence of elevated environmental temperatures on economically important traits is well recognized in cattle. Thermal stress is responsible for decreased milk yield, milk fat, and protein contents, as well as reduced dry matter intake, health, and fertility (1,2). According to Gorniak et al. (2), even mild heat stress in cows kept in a temperate climate negatively affects milk production. Moreover, the decline in performance they observed could not be explained solely by reduced feed intake, and other factors, including changes in energy metabolism, might have contributed to decreased milk yield (2). At the physiological level, a hot environment was shown to induce several adaptive responses, including alterations of lipid metabolism (3).

Acyl-coenzyme A:diacylglycerol transferase (DGAT1) is a lipogenic enzyme that plays a key role in the synthesis of triacylglycerols (4). An ApA to GpC dinucleotide substitution located in exon 8 of the bovine DGAT1 gene that replaces lysine (K) by alanine (A) in encoded protein (K232A polymorphism) has been proven to have a pronounced influence on milk yield and composition, especially on fat percentage in milk (5–7). Although in many cattle populations DGAT1:p.K232A alleles affected individual milk traits in the same direction, the magnitude of effects was variable between breeds (8), and during lactation (9). Recently, Akbar et al. (10) reported that the expression of this gene in the liver is reduced during the hot season. However, the impact of seasonal ambient air temperature changes on DGAT1 polymorphism effects is not known so far. The aim of this study was, therefore, to establish if the influence of K232A on milk production traits is dependent on environmental thermal conditions.

The analyzed data set comprised 468 dairy cows of the Polish Holstein–Friesian (HF) breed, born between 1999 and 2002, and kept on the same farm under uniform environmental conditions. The animals were daughters of 109 sires, with the sire half-sib family sizes varying between 1 and 53. The average cow's milk yield per 305-day lactation was 7452 kg.

DNA for molecular analyses was extracted from peripheral blood using the standard phenol method. The biological material was collected in accordance with the ethical standards of the Local Ethics Committee for Animal Research. Genotypes of DGAT1:p.K232A were determined using the PCR-RFLP method, as described by Szyda and Komisarek (11), with the use of the following primers (5'-3'): F:TGCCGCTTGCTCGTAGCTTTGGCC* and

* Correspondence: komisjol@up.poznan.pl

R:ACCTGGAGCTGGGTGAGGAACAGC. In the F primer, the restriction site for enzyme BglI (GCCNNNNNGGC) was introduced through purposeful mismatching of one base (marked with an asterisk). Selected PCR-RFLP conditions for the DGAT1:p.K232A polymorphism are presented in Table 1.

Phenotypes were obtained from the routine national milk recording system and comprised monthly test-day records for milk, fat, and protein yields (kg) and protein and fat contents (%). Only phenotypes of the first 3 lactations were considered. A total of 6173 test day records were collected between July 2004 and September 2007. Data on the average daily air temperatures were obtained from the Polish Institute of Meteorology and Water Management - National Research Institute. The temperatures were measured on days of milk collection, in the weather station located near the dairy farm maintaining the cows analyzed in this study.

Pearson's correlation coefficients were used to determine if a significant relationship exists between the average daily environmental temperature and cows' milk production traits. The effect of DGAT1:p.K232A polymorphism on milk yield and composition was analyzed by ANOVA, followed by Tukey's post-hoc test, for the following air temperature ranges: 1) <5 °C, 2) ≥5 < 20 °C, 3) ≥20 < 28 °C, and 4) ≥28 °C. For these classes, the numbers of available test day records were 1850, 2708, 907, and 708, respectively. The statistical model included effects of sire, DGAT1 genotype, lactation number, and year-season of calving. Additionally, the allele substitution effects (α/2) were estimated by regressing the number of copies of DGAT1 allele K against the tested trait values. All statistical analyses were performed with the procedures of the SAS 9.3 package (12).

Among 468 cows examined, 72 KK, 230 KA, and 166 AA genotypes were identified. Allele frequencies (0.4 and 0.6 for DGAT1:K232 and DGAT1:232A alleles, respectively) observed in this study did not differ notably from those previously reported for other HF cattle populations (7,13). The genotypes were distributed according to the Hardy–Weinberg equilibrium.

The average daily environmental temperatures measured at days of milk collection ranged from –4.6 °C to 31.9 °C. There was a significant negative correlation between air temperature and milk fat yield as well as milk composition traits, with the strongest correlation with fat percentage. Milk and protein yields did not correlate with environmental thermal conditions (Table 2). In the association analysis carried out without air temperature taken into account, the DGAT1:K232 allele significantly decreased milk and protein yields and increased fat yield and fat and protein contents (data not shown). These results are consistent with the findings of many previous studies performed in several dairy cattle populations (e.g., 6,8,13). To the best of the authors' knowledge, the influence of weather conditions on DGAT1 effects on milk yield and composition has not been investigated to date, although Duchemin et al. (14) found significant K232A genotype by season interactions for some fatty acid contents in milk. Results of the association analysis considering temperature effect are shown in Table 3. Summarizing, the increasing impact of the K232 allele on milk content traits, especially milk fat percentage, was observed regardless of the environmental thermal conditions. The other traits, however, were significantly affected only at the lower air temperatures (milk yield < 28 °C; fat and protein yields < 20 °C).

Results of this study provide additional evidence that the effect of DGAT1:p.K232A on some production traits is not constant in cattle. In our previous study, the effect of this polymorphism on fat and protein contents in milk showed changes over time, being low in the first stage of lactation, and increased as lactation progressed (9). The significant impact of lactation stage on K232A

Table 1. Selected PCR-RFLP conditions for the DGAT1:p.K232A polymorphism.

Annealing temp. (°C)	PCR product size (bp)	Restriction enzyme	Digestion temp. (°C)	Digestion product size (bp)
58.5	378	BglI	37.0	K (AA): 96 and 282 A (GC): 28, 96, and 254

Table 2. Pearson's correlation coefficients (and P values) between average daily air temperature and milk-related traits.

	Milk (kg)	Fat (%)	Protein (%)	Fat (kg)	Protein (kg)
Pearson's correlation coefficient (r)	–0.0007	–0.1555	–0.0492	–0.0948	–0.0167
P value	0.9563	<0.0001	0.0001	<0.0001	0.1894

Table 3. Estimated AA and AK DGAT1 genotype effects expressed in contrast to the KK genotype, and regression coefficients for the number of copies of the DGAT1:K232 allele representing half of the allele substitution effects (and P values) in different environmental temperatures.

Environmental temperature	Trait	Genotype effect[1]			DGAT1:K232 allele substitution effect	
		AA	AK	Overall P	α/2	P
<5 ºC	Milk (kg)	4.02**	3.38**	<0.0001	−1.73	<0.0001
	Fat (%)	−0.72**	−0.40**	<0.0001	0.35	<0.0001
	Protein (%)	−0.19**	−0.13**	<0.0001	0.09	<0.0001
	Fat (kg)	−0.09**	−0.06*	0.0023	0.04	0.0132
	Protein (kg)	0.10**	0.09*	0.0019	−0.04	0.0005
≥5 < 20 ºC	Milk (kg)	3.07**	2.04**	<0.0001	−1.48	<0.0001
	Fat (%)	−0.62**	−0.32**	<0.0001	0.31	<0.0001
	Protein (%)	−0.23**	−0.16**	<0.0001	0.10	<0.0001
	Fat (kg)	−0.06*	−0.02	0.0118	0.03	0.0224
	Protein (kg)	0.05**	0.03*	0.0102	−0.02	0.0059
≥20 < 28 ºC	Milk (kg)	2.67**	1.34	0.0352	−1.30	0.0142
	Fat (%)	−0.68**	−0.39**	<0.0001	0.33	<0.0001
	Protein (%)	−0.16**	−0.10**	<0.0001	0.08	<0.0001
	Fat (kg)	−0.07*	−0.05	0.0922	0.02	0.0537
	Protein (kg)	0.06*	0.02	0.0952	−0.01	0.0614
≥28 ºC	Milk (kg)	1.07	−0.18	0.2698	−0.66	0.1954
	Fat (%)	−0.62**	−0.27**	<0.0001	0.31	<0.0001
	Protein (%)	−0.10*	−0.03	0.0230	0.05	0.0041
	Fat (kg)	−0.06*	−0.04	0.0626	0.02	0.0533
	Protein (kg)	0.01	−0.02	0.5063	−0.01	0.5131

[1]Significance: *P ≤ 0.05, **P ≤ 0.01.

effects was recently confirmed by Bovenhuis et al. (15). Other reports suggest that the effect of DGAT1 may also depend on breed (8,13,16) and parity (15,16). Now it seems that the impact of K232A on milk yield traits in hot seasons may be decreased. This is in agreement with the recent findings reported by Akbar et al. (10) that hepatic expression of DGAT1 in peripartal dairy cows is greater in spring than in summer. As the K232A alleles encode forms of the enzyme with different maximal velocity (17), a declined expression may result in a lesser polymorphism effect. On the other hand, DGAT1 effects on milk content traits, especially fat percentage, were not affected by environmental thermal conditions. Temperatures observed in this study, however, were rather moderate, possibly not high enough to reduce the gene effect on those traits. Nevertheless, DGAT1 does not seem to be one of the genes responsible for the negative correlation between temperature and milk composition.

Results of this study suggest that the effect of DGAT1:p.K232A on some milk production traits in cattle is not constant and may depend on environmental thermal conditions. These findings give a better understanding of how genes and the environment interact to produce variations in the phenotype. As DGAT1 is one of the most promising candidate genes controlling many economically important traits, these results may be also important for implementation of this locus to dairy cattle breeding programs. To confirm the conclusion of this study, further investigations with higher temperatures and more accurate temperature measurements are needed.

References

1. De Rensis F, Scaramuzzi RJ. Heat stress and seasonal effects on reproduction in the dairy cow - a review. Theriogenology 2003; 60: 1139-1151.
2. Gorniak T, Meyer U, Südekum KH, Dänicke S. Impact of mild heat stress **Final Version:** 05.02.2016 on dry matter intake, milk yield and milk composition in mid-lactation Holstein dairy cows in a temperate climate. Arch Anim Nutr 2014; 68: 358-369.
3. Tao S, Connor EE, Bubolz JW, Thompson IM, Do Amaral BC, Hayen MJ, Dahl GE. Short communication: Effect of heat stress during the dry period on gene expression in mammary tissue and peripheral blood mononuclear cells. J Dairy Sci 2013; 96: 378-383.
4. Farese RV Jr, Cases S, Smith SJ. Triglyceride synthesis: insights from the cloning of diacylglycerol acyltransferase. Curr Opin Lipidol 2000; 11: 229-234.
5. Grisart B, Coppieters Q, Farnir F, Karim L, Ford C, Berzi P, Cambisano N, Mni M, Reid S, Simon P et al. 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 Res 2002; 12: 222-231.
6. Sanders K, Bennewitz J, Reinsch N, Thaller G, Prinzenberg EM, Kühn C, Kalm E. Characterization of the DGAT1 mutations and the CSN1S1 promoter in the German Angeln dairy cattle population. J Dairy Sci 2006; 89: 3164-3174.
7. Kaupe B, Brandt H, Prinzenberg EM, Erhardt G. Joint analysis of the influence of CYP11B1 and DGAT1 genetic variation on milk production, somatic cell score, conformation, reproduction, and productive lifespan in German Holstein cattle. J Anim Sci 2007; 85: 11-21.
8. Suchocki T, Komisarek J, Szyda J. Testing candidate gene effects on milk production traits in dairy cattle under various parameterizations and inheritance modes. J Dairy Sci 2010; 93: 2703-2717.
9. Szyda J, Komisarek J, Antkowiak I. Modelling effects of candidate genes on complex traits as variable in time. Anim Genet 2014; 45: 322-328.
10. Akbar H, Bernabucci U, Basiricò L, Morera P, Loor JJ. Temporal gene expression profiling of liver from peripartal dairy cows during spring and summer. J Dairy Sci 2013; 96 (E-Suppl. 1): 119.
11. Szyda J, Komisarek J. Statistical modelling of candidate gene effects on milk production traits in dairy cattle. J Dairy Sci 2007; 90: 2971-2979.
12. SAS Institute Inc. SAS/STATR 9.3 User's Guide. Cary, NC, USA: SAS Institute Inc., 2011.
13. Gautier M, Capitan A, Fritz A, Eggen A, Boichard D, Druet T. Characterization of the DGAT1 K232A and variable number of tandem repeat polymorphisms in French dairy cattle. J Dairy Sci 2007; 90: 2980-2988.
14. Duchemin S, Bovenhuis H, Stoop WM, Bouwman AC, van Arendonk JA, Visker MH. Genetic correlation between composition of bovine milk fat in winter and summer, and DGAT1 and SCD1 by season interactions. J Dairy Sci 2013; 96: 592-604.
15. Bovenhuis H, Visker MH, van Valenberg HJ, Buitenhuis AJ, van Arendonk JA. Effects of the DGAT1 polymorphism on test-day milk production traits throughout lactation. J Dairy Sci 2015; 98: 6572-6582.
16. Thaller G, Krämer W, Winter A, Kaupe B, Erhardt G, Fries R. Effects of DGAT1 variants on milk production traits in German cattle breeds. J Anim Sci 2003; 81: 1911-1918.
17. Grisart B, Farnir F, Karim L, Cambisano N, Kim JJ, Kvasz A, Mni M, Simon P, Frère JM, Coppieters W et al. Genetic and functional confirmation of the causality of the DGAT1 K232A quantitative trait nucleotide in affecting milk yield and composition. Pc Natl Acad Sci USA 2004; 101: 2398-2403.