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THE EFFECTS OF POLYMORPHISM IN THE *DGAT1* GENE ON ENERGY BALANCE AND MILK PRODUCTION TRAITS IN PRIMIPAROUS HOLSTEIN COWS DURING THE FIRST SIX MONTHS OF LACTATION

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Abstract

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The aim of this study was to analyze the effects and significance of DGAT1 K232A polymorphism on milk production traits – milk yield, content of protein, fat and fat-to-protein ratio in milk during the first six months of lactation, with respect to the course of the body condition. The influence of year, calving season and month of lactation on these traits was also assessed. The investigation was conducted on 246 primiparous Holstein cows, calved from 2009 to 2011. DGAT1 genotypes were identified using the polymerase chain reaction – the restriction fragment length polymorphism technique (PCR-RFLP). The A and K allele frequencies were 0.73 and 0.27. Homozygotes KK were characterized by significantly ($P<0.01$- 0.05) highest milk fat content during the whole observed period on average as well as in almost all individual months after parturition. Homozygotes AA reached significantly highest average milk yield in almost all observed months and in the whole observed period ($P<0.01$-0.05), which is related to statistically significantly ($P<0.01$- 0.05) lowest percentage content of milk components - protein, fat and fat-to-protein ratio in cows of this allelic variant. The low fat-to-protein ratio indicates a positive effect of the AA genotype on body condition score changes reflecting energy balance level. The most intensive body condition score changes occurred in homozygotes KK during the first month of lactation but this was not significantly different. Average daily milk production annually significantly increased ($P<0.01$) as well as intensity of body condition score changes ($P<0.01$). The most significant body condition changes were detected during the summer months.

Key words: genetic polymorphisms, DGAT1, PCR-RFLP, dairy - cows, indicators of milk production traits, body condition score, negative energy balance

Abbreviations: BCS = body condition score, NEB = negative energy balance, PCR-RFLP = polymerase chain reaction – the restriction fragment length polymorphism, DGAT1 = Acyl-CoA: diacylglycerol acyltransferase, QTL = quantitative trait loci, DNA = deoxyribonucleic acid, £PLI = Profitable Lifetime Index

Introduction

During the last decades, the major goal of dairy cattle husbandry was to improve milk production traits. Better nutrition and management, as well as intensive genetic selection led to radically increased milk yield per cow (Dematawewa and Berger, 1998). However, increase in the genetic value for milk yield did not parallel increase in genetic merit for feed intake and resulted in an ongoing increase in negative energy balance (NEB) during early lactation (de Vries and Veerkamp, 2000). The result is dairy cows that promptly mobilize their body reserves at the expense of their own health and fertility (Buckley et al., 2003).

Fat to protein ratio is the most important energy status indicator. The optimum ratio lies in the range between 1.2 and 1.4, with the lower value connected to acidosis and the higher value usually indicates NEB (Buttchereit et al., 2010; Čejna and Chládek, 2005). During NEB, adipose tissue mo-

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bilization is higher due to energy undersupply, followed by an increase in milk fat content and a decreased milk protein content caused by a decrease in microbial protein synthesis in the rumen (de Vries and Veerkamp, 2000).

It is now well-known that NEB is accompanied by negative consequences, such as increase in incidence of metabolic disease and reduced reproductive performance (Rajala-Schultz and Frazer, 2003; Westwood et al., 2002; Pryce et al., 1999; Van Arendonk et al., 1989). The decline in reproductive efficiency accompanying the high milk performance has become a worldwide problem affecting the dairy industry (Washburn et al., 2002; Lucy, 2001). Over the last 10 to 15 years, global dairy cattle breeding programmes have seen a substantial move from production orientated goal traits towards fitness traits (Miglior, 2004). As an example, in the United Kingdom, current breeding goals include increasing milk, fat and protein yields plus longevity. These traits are combined as a Profitable Lifetime Index, or £PLI, designed to maximize the economic return from a cow during her expected productive life (Pryce and Veerkamp, 2001). A similar necessity was determined worldwide (Přibyl et al., 2004; Šafus et al., 2005). Longevity is one of the main research topics in different cattle production systems and breeds (Raguz et al., 2011) and also depends on the cow‘s ability to cope with metabolic load to avoid high negative energy balance after calving.

Acyl-CoA: diacylglycerol acyltransferase (DGAT1) is an enzyme playing the key role in the synthesis of major milk lipids – triacyglycerols (Anton et al., 2012; Farese et al., 2000). Milk fat contains approximately 98% triglycerides so *DGAT1* has an important effect on content of fat in milk (Koopaei et al., 2012). The *DGAT1* gene is located at the centromeric end of chromosome 14 (BTA 14) inside the quantitative trait loci (QTL) with major effect on milk production (Gautier et al., 2007; Kühn et al., 2004; Grisart et al., 2002). An ApA to GpC dinucleotide substitution located in exon 8 of *DGAT1* that replaces positively charged lysine by neutral alanine at position 10 433 a 10 434 and at the 232th residue of the encoding protein (K232A polymorphism). It has been proved to have a pronounced effect on milk production traits in different breeds of cattle, especially on production of fat of the KK genotype (Anton et al., 2012; Oikonomou et al., 2009; Gautier et al., 2007; Kaupe et al., 2007; Sanders et al., 2006; Strzałkowska et al., 2005; Winter et al., 2002). Also, found were possible pleiotropic effects of K232A polymorphism on selected reproduction traits in cattle. Oikonomou et al. (2009) showed that replacement of the lysine by the alanine variant led to a reduction in conception rate in the first 305 days of lactation and an increase in reproductive problems.

It seems justified to presume that genes affecting milk yield and composition may also alter the calorific demand for milk production and influence the severity and duration of NEB in early lactation (Komisarek et al., 2011). From the results of previous studies, it can be concluded that the *DGAT1* gene can be used as a gene marker for assisted selection in milk composition traits (Molle et al., 2012).

The objective of this study was to investigate the effect of *DGAT* gene polymorphism on milk production traits and energy balance during the first six months of lactation, with respect to the course of body condition.

Materials and Methods

A total of 246 Holstein cows from the Dairy Farm of the Czech University of Life Sciences Prague were included in this study. The primiparous cows were calved between 2009 and 2011. Included were calving data and milk recording data -milk yield, content of protein, fat and fat-to-protein ratio in milk, during the first 6 months of lactation. At the same time, body condition score was evaluated by a methodology valid for Holstein cattle with an accuracy of 0.25 points. Body condition score was evaluated at calving and then every month with a difference of ± 1 day. Blood was collected from each cow. An anticoagulant (EDTA) was added to the blood samples and then stored at -20°C. Genomic DNA was extracted from the blood using the standard proteinase K method (Kawasaki, 1990). The relationship between *DGAT1* K232A polymorphisms and selected production traits (Kaupe et al., 2007; Banos et al., 2008) is well known. We use this gene in our research because of the pronounced effect of this mutation on milk fat production (Winter et al., 2002) which can affect the body condition score. Genotypes were determined using the PCR-RFLP technique. Primers for the PCR were established based on gene sequences available in the GenBank database (accession numbers: DGAT1 – AY065621). Komisarek and Michalak (2008) used the primers.

F: 5′- TGCCGCTTGCTCGTAGCTTTGGCC -3, and

R: 5′- ACCTGGAGCTGGGTGAGGAACAGC -3′.

PCR amplification was performed in a thermocycler (Bio-Rad, USA). Amplified fragments were 378 bp length and digested overnight at 37°C, usualy 12 – 16 hours,with *Bgl*I restriction endonuclease (Biogen, Czech Republic) (Komisarek, Michalak, 2008) in a thermostat (Memmert, Germany). Digestion products were subjected to electrophoresis in 2.5% ethidium bromide-stained agarose gel (BioRad, USA).

The effect of DGAT1 genotypes on selected milk production traits were tested by multivariate analysis of variance (MANOVA) using the GLM procedure of the SAS 9.2 software (SAS Institute Inc. 2002-2005). For the calculations were chosen the following models:

$$Y_{ijkl} = ц + A_i + S_j + G_k + \beta\ (Yield_{ijkl} - Yield_{0000)} + e_{ijkl,}$$

where: Y_{ijkl} - measured value of dependent variable;
ц - overall mean of dependent variable;
A_i - fixed effect of calving year i (3 levels);
S_j - fixed effect of calving season j (4 levels);
G_k - fixed effect of genotypes (3 levels);
b(Yield $_{ijkl}$ – Yield $_{0000)}$ - regression on the average daily milk yield;
e_{ijk} - random residual effect.

$y_{ijkl} = ц + A_i + S_j + G_k + \beta (BCS_{ijkl} – BCS_{0000)} + e_{ijkl,}$
where: ц - overall mean of dependent variable;
A_i - fixed effect of calving year i (3 levels);
S_j - fixed effect of calving season j (4 levels);
G_k - fixed effect of genotypes (3 levels);
b(BCS_{ijkl} – $BCS_{0000)}$ - regression on changes of BCS from calving till first months of calving;
e_{ijk} - random residual effect.

Results and Discussion

The PCR reaction resulted in 378 bp-long products. After digestion with *Bgl*I restriction enzyme, the alanine encoding allele A was cleaved into three fragments – of 254 bp, 96 bp and 28 bp. The lysine encoding allele K was visible in gel as two bands – of 282 bp and 96 bp. Of the 246 cows examined, 124 AA, 105 AK and 16 KK genotypes were identified. This gives frequencies of 0.28 and 0.72 for K and A alleles, respectively. The frequencies of alleles depend on different breeds. Earlier, the lysine encoding variant frequency in Holstein-Friesian cattle was reported to range from 0.32 (Berry et al., 2010) and 0.36 (Molee et al., 2012), through 0.55 (Kaupe et al., 2007) to 0.70 (Grisart et al., 2002).

In this paper, the effect of *DGAT1* gene K232A polymorphism was analyzed on four milk production traits. The summary results are presented in Table 1.

Individuals with the lysine encoding allele K were associated with significantly (P<0.01 – 0.05) reduced average daily milk yield but significantly (P<0.01) greater milk fat concentration. Highest (4.17±0.12) milk fat concentration in KK genotype was not caused by the negative energy balance as the level of BCS and changes in BCS during the first month after parturition was not significantly different from other genotypes (Figures 1 and 2) and fat to protein ratio was in the optimal range. The highest milk fat content could be also result of significantly lowest (27.06±0.96) milk production of homozygote KK, but in this case, the statistical model included regression to average milk yield. For this reason, it can be stated, that higher fat content in the KK genotype is caused solely by *DGAT1* K232A polymorphism. Homozygotes AA were characterized by significantly (P<0.01 – 0.05) highest (28.47±0.36) average daily milk yield and significantly (P<0.01 – 0.05) lowest fat to protein ratio (1.19±0.02). The low fat-to-protein ratio indicates positive effect of AA genotype on body condition score changes reflecting energy balance level.

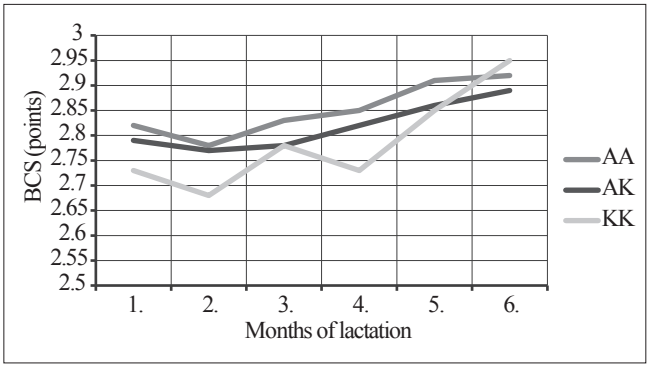

Fig. 1. Effect of genotype on body condition score during first six months of lactation

Table 1
Frequency of genotypes and means and their confidence limits of selected milk production traits in Holstein cows

	Genotype	Milk yield, kg/day	Fat, %	Proteins, %	F/P	BCS	Change of BCS
a	AA (n=124)	28.47±0.36^{B,c}	3.78±0.04^{B,C}	3.19±0.02^{B,c}	1.19±0.02^{B,c}	2.85±0.02	-0.026±0.024
b	AK (n=105)	27.29±0.4^{A}	4.03±0.05^{A}	3.24±0.03^{A}	1.25±0.02^{A}	2.82±0.02	-0.033±0.027
c	KK (n=16)	27.06±0.96^{a}	4.17±0.12^{A}	3.27±0.06^{a}	1.25±0.05^{a}	2.78±0.06	-0.035±0.007

Letter A, a; B, b; C, c means genotypes AA, AK, KK. These letters within rows means bearing the same superscript differ significantly at: small letters – P≤0.05; capitals – P≤0.01
n - number of cows; F/P – fat-to-protein ratio in milk; BCS – body condition score level

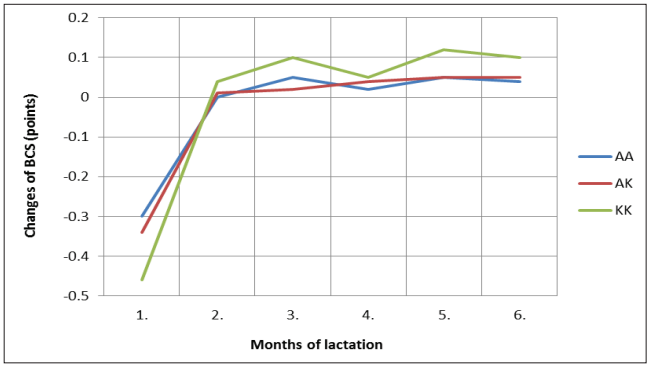

Fig. 2. Effect of genotype on changes of body condition score during first six months of lactation

Our results are in accordance with other research which found that the K allele is generally related to markedly elevated fat content and a less markedly elevated protein content, while its impact on milk yield is negative (Gautier et al., 2007; Thaller et al., 2003; Winter et al., 2002). According to Molee et al. (2012), the genotype KK has the greatest effect on all milk composition content traits, while genotype AA has the greatest effect on yield traits. Banos et al. (2008) also found no significant effect of the polymorphism on body condition score or its changes. Komisarek and Michalak (2008) reported that in cows producing less fat in milk the severity of NEB was reduced.

Several studies (Winter et al., 2002; Strzałkowska et al., 2005) have confirmed that *DGAT1* K232A polymorphism is responsible for variation in milk production traits in cattle but to date no research has focused on detailed observation of *DGAT1* K232A polymorphism influence on milk performances in individual months after parturition. Evaluated milk performance parameter changes during the first six months of lactation in relation to *DGAT1* polymorphism variants are presented in Table 2. Detailed observation of parameters shows that average milk fat content in genotype KK was significantly ($P<0.01 – 0.05$) higher for the six months of lactation on average as well as (Table 1) in individual months after parturition (Table 2). Significantly ($P<0.01 – 0.05$) lower milk fat content in almost all observed months was found for genotype AA. In contrast, in almost all observed months, genotype AA cows excelled in average daily milk production. Fat to protein ratio, as the energy status indicator, were highest in the first month after parturition but did not exceed optimal range. In almost all observed months, fat to protein ratio was highest in the KK genotype but nonsignificantly. In addition, BCS changes were in this genotype no significantly highest (Figure 2).

Table 2
Effect of months of lactation and genotypes on milk production traits

Month	Genotype	Milk yield, kg/day	Fat, %	Proteins, %	F/P
1	AA	26.61±0.86	3.97±0.12$^{b.c}$	3.11±0.05	1.33±0.07
	AK	26.42±0.94	4.2±0.13^{a}	3.17±0.05	1.35±0.08
	KK	26.15±1.28	4.46±0.33^{a}	3.22±0.14	1.36±0.19
2	AA	29.82±0.89	3.72±0.1^{B}	3.06±0.04	1.21±0.04^{b}
	AK	28.98±0.99	3.99±0.12^{A}	3.11±0.05	1.29±0.04^{a}
	KK	28.73±2.41	3.84±0.28	3.11±0.11	1.24±0.09
3	AA	28.95±0.8	3.75±0.1	3.15±0.05	1.18±0.04
	AK	28.01±0.89	3.88±0.11	3.2±0.05	1.21±0.04
	KK	29.09±2.17	4.06±0.28	3.19±0.12	1.17±0.09
4	AA	28.67±0.93^{b}	3.74±0.1$^{B.c}$	3.23±0.05	1.16±0.03^{B}
	AK	26.84±1.02^{a}	4.05±0.12^{A}	3.27±0.05	1.24±0.03^{A}
	KK	26.89±2.48	4.09±0.3^{a}	3.36±0.13	1.22±0.08
5	AA	28.52±0.88	3.75±0.12$^{b.c}$	3.27±0.07	1.15±0.04
	AK	27.14±0.98	3.99±0.13^{a}	3.34±0.07	1.2±0.04
	KK	27.41±2.31	4.22±0.3^{a}	3.35±0.17	1.26±0.1
6	AA	28.27±0.86^{B}	3.77±0.11$^{B.C}$	3.31±0.05	1.14±0.03$^{B.c}$
	AK	26.23±0.95^{A}	4.09±0.12^{A}	3.38±0.05	1.21±0.04^{A}
	KK	25.27±2.56	4.28±0.28^{A}	3.44±0.12	1.25±0.07^{a}

Letter $^{A, a; B, b; C, c}$ means genotypes AA, AK, KK. These letters within rows means bearing the same superscript differ significantly at: small letters – $P\leq0.05$; capitals – $P\leq0.01$

These detailed results also confirm the fact that higher fat content in the KK genotype is caused solely by *DGAT1* K232A polymorphism.

Selected milk production traits were monitored during the years 2009 – 2011. Their development is summarized in Table 3. Our results are consistent with the global trend of increasing milk production with lower content of milk components and reproductive performance degradation because of a more significant decline in body condition score in early lactation. Butler (2003) reached the same conclusions.

In this report, the effect of season of calving was analyzed on milk production traits as well. The results are presented in Table 4. The season of calving significantly affected the average daily milk yield, content of milk components and their ratio. Katok and Yanar (2012) also described the same effect. Significant effect (P<0.01 – 0.05) of investigated seasons on the highest milk yield in early lactation was observed in cows calved from September to November. The milk of these cows contained the highest content of fat and fat to protein ratio. On the other hand, the lowest milk production was found in the cows calved from March to May. The high temperatures negatively affected the performance of dairy cows during these months, which is also confirmed by Gantner et al. (2012). The fat content of milk was significantly (P<0.01) lowest in cows calved in December – February. From monitoring, the effect of calving season on indicators of physical fitness it is evident that the most intensive change in body condition from calving to the first months of lactation was determined in dairy cows calved during the summer months (June, July, and August). However, this result is not statistically significant. Significantly (P <0.01) lower body condition score during the summer months due to reduced appetite in cows was recorded by Balogh et al. (2012). Significantly (P <0.01), dairy cows in the winter months (December, January, February) presented the lowest BCS as well as the lowest ratio of fat / protein in milk.

Conclusion

In conclusion, the selection of the DGAT1 polymorphism in a breeding program may significantly affect not just milk production traits but also level of body condition score and its changes. It is well established that the K allele is generally related to markedly elevate fat content and less distinctively elevated protein content, while its impact on milk yield is negative. In our study, we found that the higher fat content in milk of the KK genotype in Holstein primiparous cows during the first six months of lactation as well as in individual months after calving is caused solely by DGAT1 K232A polymorphism without significant effect of BCS changes. However, further studies are required to describe in detail similar relationships in subsequent parities of dairy cows because of more significant metabolic stress induced by higher age and increasing milk yield.

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Table 3
Effect of calving year on milk production traits

	Year	Milk yield, kg/day	Fat, %	Proteins, %	F/P	BCS	Change of BCS
a	2009	$26.16\pm0.6^{B,C}$	4.04±0.08	$3.28\pm0.04^{B,c}$	1.24±0.03	2.77 ± 0.04^{c}	-0.011±0.04
b	2010	$27.76\pm0.43^{A,C}$	3.97±0.06	3.19 ± 0.03^{A}	1.24±0.02	2.77 ± 0.03^{c}	-0.05±0.028
c	2011	$28.9\pm0.58^{A,B}$	3.96±0.08	3.22 ± 0.04^{a}	1.23±0.02	$2.91\pm0.03^{a,b}$	-0.032±0.038

Letter $^{A, a; B, b; C, c}$ means genotypes AA, AK, KK. These letters within rows means bearing the same superscript differ significantly at: small letters – P≤0.05; capitals – P≤0.01

Table 4
Effect of calving season on milk production traits

	Season	Milk yield, kg/day	Fat, %	Proteins, %	F/P	BCS	Change of BCS
a	March-May	26.9 ± 0.6^{C}	$3.93\pm0.07^{b,C}$	3.21 ± 0.04^{b}	1.22±0.03	2.83 ± 0.03^{D}	-0.02±0.04
b	June-August	27.49 ± 0.51^{c}	$4.06\pm0.06^{a,D}$	$3.27\pm0.04^{a,d}$	1.25±0.02	2.84 ± 0.03^{D}	-0.05±0.03
c	September-November	$28.59\pm0.63^{A,b,d}$	$4.11\pm0.08^{A,D}$	3.23±0.03	1.27 ± 0.03^{D}	2.86 ± 0.04^{D}	-0.02±0.04
d	December-February	$27.44\pm0.56^{b,c}$	$3.87\pm0.07^{B,C}$	3.22 ± 0.03^{b}	1.2 ± 0.03^{C}	$2.75\pm0.03^{A,B,C}$	-0.03±0.04

Letter $^{A, a; B, b; C, c}$ means genotypes AA, AK, KK. These letters within rows means bearing the same superscript differ significantly at: small letters – P≤0.05; capitals – P≤0.01

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