

Association of *DGAT1* and *leptin* with fertility traits in Holstein cows

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The objective of this study was to investigate relationship between the *K232A* mutation in the *DGAT1* gene and the *C963T* mutation in the *Leptin* gene with reproduction traits. The study was conducted on 286 purebred Holstein cows. In *DGAT1* we found a favourable effect of *AA* genotype on age at the first calving ($P < 0.15$) and on conception rate after first service in heifers ($P < 0.15$). In the *Leptin* a positive effect of *CC* genotype on interval from calving to 1st service was observed ($P < 0.05$). A tendency for positive effect of *CC* genotype on age at the first service ($P < 0.16$) was observed as well. In another examined fertility traits we found no association with *DGAT1* and *Leptin* genotypes. Present study brings a new information about studied SNP and such results may be used to assist genetic selection in dairy cattle.

Keywords: single nucleotide polymorphism, fertility, cattle, leptin, *DGAT1*

1 Introduction

During last decades we can observe an increase in milk production which is often associated with decreased health and fertility due to its negative genetic correlation (Pryce et al., 2004). Identification of markers involved to reproduction could help with managing decreasing trend in cattle reproduction. In cattle, the *Leptin* gene (*LEP*) was mapped to chromosome 4 and it consists of three exons. Until now, 1366 mutations have been identified in the *LEP* gene (EMBL-EBI, 2016). The promoter region of *LEP* was firstly described by Liefers et al. (2005). The *C963T* mutation was primarily studied for an association with energy status (Liefers et al., 2005) and reproduction traits (Liefers et al., 2005; Clempson et al., 2011), however the *C963T* mutation seems to be also very important in production traits like milk yield and milk content (Kadlecova et al., 2014).

The *diacylglycerol acyltransferase (DGAT1)* gene is located at 14 bovine chromosome and it is identified to encode acyl-CoA:diacylglycerol acyltransferase, the enzyme that catalyses the last step in triglyceride synthesis. A missense, non-conservative lysine-to-alanine substitution (*K232A*) in this gene has been proved to have a major influence on milk production traits and particularly on fat content in milk (Gautier et al. 2007). Ashwell et al. (2004) suggested possible pleiotropic effects of this polymorphism on reproduction, which was also observed by Rychtarova et al. (2014) in Czech Fleckvieh cattle. However some described associations are contradictory in allele effect (Kaupe et al., 2007; Oikonomou et al., 2009).

Previous studies in *DGAT1* are uncertain and despite numerous studies in the *Leptin* gene, the knowledge about *C963T* mutation is, although it seems to be very promising for cattle breeding, limited. The aim of this study was therefore to analyse the relationship of the *K232A* and *C963T* polymorphism with reproduction traits in Holstein cattle.

2 Material and methods

A total of 287 purebred Holstein cows raised at one farm were involved to the study. These cows were born between 2004 and 2009 and were daughters of 47 bulls. Phenotype information was obtained from the official progeny testing database of the Czech-Moravian Breeders' Corporation. Data of age

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at the 1. service (AFS), age at 1. calving (AFC), number of services per conception (NSC), conception rate after 1. service (CR), interval from calving to 1. service (CFI), days open (DO) and calving interval (CI) were recorded for the first 3 lactations. Genomic DNA was isolated from whole blood and genotypes were determined as described by Kadlecova et al. (2014).

Once genotypes were determined allele frequencies were estimated and deviations from the Hardy-Weinberg equilibrium were tested by χ^2 test. The investigated traits were transformed using natural logarithm to normalize their distribution. Observations of conception rate after 1. service were class variables (1 = yes, 0 = no) and therefore were analysed using a binary model. For CR and NSC was used procedure GLIMIX and for other traits we used procedure MIXED in statistical software SAS 9.4.

The statistical model always included effect of sire, *LEP* and *DGAT1* genotypes. Furthermore to evaluate CR and NSC in heifers we used effect of age at the 1. service; in AFS and AFC we used combined effect of year and season of birth. To evaluate other traits, combined effect of year and season of calving were included to the equation, in CI we used also effect of group of age at calving (3 groups). The differences between mean values of the studied traits were analysed with the Bonferroni multiple comparisons.

3 Results and discussion

The laboratory analysis showed three genotypes in both genes. Genotype and allele frequencies are shown in Table 1.

Table 1 Allele and genotype frequencies (%) in studied genes

	AA	AK	KK	A	K	χ^2
<i>DGAT1</i>	54.36	39.37	6.27	0.74	0.26	0.169
	CC	CT	TT	C	T	χ^2
<i>LEP</i>	37.98	40.42	21.60	0.58	0.42	8.232**

χ^2 = chi-square test; ** P < 0.01;

Table 2 Association of *DGAT1* K232A with fertility traits and its genotype frequency

Trait	AA	AK	KK	F-test value	p-value ¹
AFS ²	417.8 (410.0, 425.8)	422.0 (413.2, 431.0)	406.5 (388.2, 425.6)	0.267	
AFC ²	716.6 ^A (705.3, 728.1)	733.1 ^A (720.1, 746.3)	713.3 (685.9, 741.7)	0.081	^A 0.125
CR – heifers ³	61.3 ±4.0 ^A	48.0 ±4.8 ^A	63.2 ±11.4	0.092	^A 0.107
CR - cows ³	39.6 ±3.5	40.0 ±3.7	33.9 ±7.1	0.728	
NSC - heifers ³	1.81 ±0.12	1.14 ±0.15	1.17 ±0.24	0.165	
NSC - cows ³	2.20 ±0.16	2.31 ±0.16	2.58 ±0.27	0.510	
CFI ²	124.6 (116.4, 133.5)	127.9 (118.9, 137.6)	133.0 (117.9, 150.0)	0.528	
DO ²	188.6 (176.4, 201.8)	183.3 (170.4, 197.1)	201.1 (172.3, 234.6)	0.522	
CI ²	463.0 (443.2, 483.6)	460.1 (439.9, 481.3)	482.3 (448.8, 518.4)	0.383	

¹ P-value after Bonferroni correction. The mean columns marked with the same superscript differ at denoted significance level.

² Values in bracket present 95 % confidence interval

³ LSM ± standard error

The distributions of genotypes were tested for concordance with expected values according to Hardy-Weinberg equilibrium. In the *DGAT1* gene the population followed the Hardy-Weinberg equilibrium, but the genotype frequencies in *LEP* showed significant departure (P<0.05) of the equilibrium.

The effect of *DGAT1* gene (Table 2) and *LEP* gene (Table 3) polymorphism on reproduction traits in heifers and cows were studied.

Table 3 Association of *Leptin* C963T with fertility traits and its genotype frequency

Trait	CC	CT	TT	F-test value	p-value ¹
AFS ²	413.1 ^A (404.2, 422.1)	423.5 ^A (414.9, 432.4)	420.4 (409.4, 431.7)	0.144	^A 0.152
AFC ²	715.2 (701.8, 728.9)	728.8 (715.8, 742.1)	729.9 (713.3, 746.9)	0.174	
CR - heifers ³	58.8 ±4.8	58.1±4.7	47.7 ±6.4	0.337	
CR - cows ³	39.4 ±3.6	39.2 ±3.8	38.8 ±5.0	0.994	
NSC - heifers ³	1.26 ±0.13	1.57 ±0.14	1.06 ±0.18	0.347	
NSC - cows ³	2.92 ±0.16	2.68 ±0.16	2.81 ±0.20	0.882	
CFI ²	121.1 ^a (112.8, 129.9)	128.3 (119.7, 137.6)	135.2 ^a (124.3, 147.0)	0.029	^a 0.030
DO ²	181.6 (168.9, 195.1)	189.9 (176.4, 204.6)	196.1 (177.8, 216.4)	0.374	
CI ²	459.5 (439.7, 480.2)	467.7 (446.6, 489.8)	464.4 (440.1, 490.1)	0.661	

¹ P-value after Bonferroni correction. The mean columns marked with the same superscript differ at denoted significance level.

² Values in bracket present 95 % confidence interval

³ LSM ± standard error

In *DGAT1* an association with AFC was observed in advantage of AA genotype. Genotype AA was also favourable in conception rate in heifers. However a negative effect of K variant of *DGAT1* was described by Kaupe et al. (2007) on maternal non-return rate. Similar results were described also by Rychtarova et al. (2014), where they connect the *KK* genotype with better fertility. Moreover Oikonomou et al. (2009) reported the Alanine variant unfavourable toward NSC and conception rate during 305 days. On the other hand Rychtarova et al. (2014) studied Czech Fleckvieh cattle, therefore due to the different breed the association can be changed. Concerning the production traits, the A variant of *DGAT1* was linked with higher milk yield (Oikonomou et al., 2009; Kadlecova et al., 2014). Based on our results the Alanine variant could help improve fertility in Holstein cattle without compromising milk yield.

In the *Leptin* gene we observed a tendency for significance ($P < 0.16$) in AFS and significant ($P < 0.05$) differences in CFI interval. In both cases the favourable genotype was CC. The C963T mutation was studied by Liefers et al. (2005) where they described a positive influence of T allele on energy balance and dry matter intake. In their work they also described negative effect of T allele on first observed oestrus after parturition. This finding is consistent with our results regarding to CFI. In recent study the TT genotype had significantly longer interval from calving to 1. service which could be caused by delayed observation of oestrus after parturition. In *LEP* we also observed a tendency for significance where heifers with CC genotype were served earlier in comparison to CT genotype. Promoter mutation on *LEP* was also investigated by Clempson et al. (2011), but they found the TT genotype favourable for DO and CI where the interval was shortened about 22 days. Additionally the T allele was also connected to decreased milk yield and higher fat percent in milk (Giblin et al., 2010); however no associations were described in fertility traits in their study.

4 Conclusions

Our results could point the K232A variant in *DGAT1* as a good marker for improving the AFC and conception rate in Holstein heifers. Furthermore we observed an association with CFI and AFS in the *Leptin* promoter region. Presented study brought new information about associations in Holstein cattle in both studied genes. In other studied traits we did not observed significant differences. However because there are some results on the threshold of significance ($P < 0.15$) higher sample number may be beneficial, to reveal more associations in studied polymorphisms. Nevertheless there is numerous study with contradict results which could impede use of this markers in improvement of fertility traits.

Thus further study will be beneficial to clarify association in these mutations with fertility traits in Holstein cattle.

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