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THE INFLUENCE OF FATTY ACID SYNTHASE POLYMORPHISM ON MILK PRODUCTION TRAITS IN POLISH HOLSTEIN-FRIESIAN CATTLE

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ABSTRACT

Fatty acid synthase gene (*FASN*) was indicated as gene linked to milk production traits in cattle, especially milk fat yield and fatty acid composition. Fatty acid synthase coded by this gene is strictly involved in fat metabolism, so it is supposed that variability of *FASN* gene could influence these traits. The aim of this study was to analyze the correlation between the polymorphism of fatty acid synthase gene and milk production traits in Polish Holstein-Friesian cattle. PCR-RFLP method was used for genotyping. The major allele for analyzed locus was $FASN_{g.17924G}$ (f=0.63). The influence of the $FASN_{g.17924A>G}$ polymorphism on milk production traits was analyzed. It was found that there was a significant correlation between the $FASN_{g.17924A>G}$ polymorphism and selected production traits: milk yield ($p \leq 0.05$), fat yield ($p \leq 0.05$) and protein yield ($p \leq 0.05$). Further analysis of *FASN* gene polymorphism should be performed for the purpose of identifying of quantitative trait nucleotide (QTN) for Marker Assisted Selection.

Key words: *FASN*, fatty acids, milk, polymorphism, dairy cattle.

INTRODUCTION

Identification of genetic markers for milk and meat production traits is the main aim of the studies on quantitative trait loci (QTL) (Rothschild and Soller, 1997). The attempts are usually made to indicate candidate genes located within or very close to QTL described in the genome. The next step is to indicate the differences in DNA sequence that can be described as quantitative trait nucleotides(QTN). Phenotypic effects are produced as a result of QTNs occurrence (Switonski, 2008). A significant QTL was identified on the bovine chromosome 19 (BTA19) region containing fatty acid synthase gene (*FASN*). Because of that and due to the fatty acid synthase function, the *FASN* gene is considered as a potential candidate gene for some milk production quality traits (Morris *et al*., 2007).

Fatty acid synthase (FASN) is a complex homodimeric enzyme that catalyzes *de novo* biosynthesis of long-chained fatty acids in mammals. FASN takes part in lipogenesis in adult individuals and plays a very important role during embryonic development (Chirala *et al*., 2003). The bovine *FASN* gene was mapped to chromosome 19 (BTA19) at q22 band (Roy *et al*., 2001). Several QTLs linked to fat content in milk have been described within the aforementioned chromosome (Taylor *et al*., 1998; Biochard *et al*., 2003). The studies on the bovine *FASN* gene structure have revealed occurrence of several single nucleotide polymorphisms (SNPs) linked to the fat content and fatty acids composition in milk (Roy *et al*., 2006) and meat (Zhang *et al*., 2008).

The aim of this study was to determine the genotype and allele frequencies of $FASN_{g.17924A>G}$ and the relationships between the *FASN* genotypes and milk production traits in Polish Holstein-Friesian cattle.

MATERIALS AND METHODS

This study was performed on a total group of 109 Polish Holstein-Friesian cows, kept on one of the West Pomeranian cattle farms. Only animals with at least three complete lactations were considered. Whole peripheral blood was collected from the jugular vein to the test tubes containing an anticoagulant (K_3EDTA). This study was performed in May and June 2010.

DNA isolation was performed using commercial MasterPure™ DNA Purification Kit for Blood (Epicentre Biotechnology, WI, USA). Genetic analyses of the *FASN* polymorphism were made by PCR-RFLP method. On the basis of the *FASN* gene sequence (GenBank, AF285607) and with the use of the Primer3 software (Rozen and Skaletsky, 2000), the following primers were designed:
Forward: 5`-GACCTTGACACGGCTCAACT-3`
Reverse:5`-GGGCACAGCATGAGGTTTAG-3`

PCR amplifications were performed in 15 µl volume of reaction mixture containing: ~60 ng of genomic DNA, 15 picomoles of each primer, 1x*Taq* DNA Polymerase buffer with $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$, 0.2 mM dNTP, 0.4 U *Taq* DNA Polymerase (Fermentas UAB, Vilnius, Lithuania) and nuclease-free

deionized water up to 15 µl. Amplifications were performed in Biometra (TPersonal) thermocycler according to the following program: initial denaturation at 94°C for 5 min, followed by 35 cycles (denaturation at 94°C for 30 s, primer annealing at 60°C for 45 s and an extension at 72°C for 45 s) and final extension at 72°C for 5 min.

PCR amplification efficiency and specificity were evaluated by agarose gel electrophoresis of 4 µl of PCR products and the remaining volume was submitted to the restriction analysis with 3 U of *Aci*I enzyme (Fermentas UAB). The obtained fragments were separated in a 3% agarose gel (Prona Agarose, Basica LE GQT) in 1×TBE buffer. DNA was stained with ethidium bromide (AppliChem Gmb). The results of electrophoresis were observed in UV light (Vilber Lourmat) and photographed.

Data regarding milk production traits were obtained from the farm records. An analysis of the relationship between the *FASN* genotype and studied traits (milk yield, fat and protein yield, fat and protein content in milk) was performed using the General Linear Model (GLM).

The analytical model utilized was:

$Y_{ijklmn} = \mu + G_i + S_j + YS_k + \alpha(w_l - w) + \beta(HF_m - HF) + e_{ijklmn}$

where:

Y_{ijklm} - analyzed trait*; μ – overall mean; G_i – effect of genotype; S_j – random effect of sire; YS_k – effect of calving year-season; α – regression coefficient for calving age; w_l – calving age of cow l; w – average calving age; β – regression coefficient for the percentage of Holstein-Friesian genes in cow genotype; HF_m – percentage of Holstein-Friesian genes in the genotype of cow m; HF – average percentage of Holstein-Friesian genes in cow genotype; e_{ijklmn} – random error.*

RESULTS

A 228-base pair sequence was digested with *Aci*I enzyme. $FASN^{AA}$, $FASN^{AG}$ and $FASN^{GG}$ genotypes were obtained. In the present study, $FASN^{A}$ was a minor allele and its frequency was 0.37. In the analyzed group, heterozygotic genotype was the most frequent (0.52), while the frequencies of $FASN^{AA}$ and $FASN^{GG}$ were 0.11 and 0.37, respectively. There were statistically significant differences in milk yield in the first lactation depending on the *FASN* genotype. The $FASN^{AA}$ cows produced more milk (+873 kg) than did the $FASN^{AG}$ individuals ($p \leq 0.05$). Similar tendency was observed in the next two lactations. The highest milk yield was observed in the $FASN^{AA}$ individuals (12.566 kg – second lactation and 12.599 kg – third lactation), however the differences observed in milk yield were not statistically significant.

The $FASN^{AA}$ individuals were characterized by highest milk and protein yield. Cows with that genotype produced 36 kg fat more and 27 kg protein more in the first lactation than did the $FASN^{AG}$ individuals ($p \leq 0.05$). In the subsequent lactations, the differences in fat and protein yield were not statistically significant. The detailed data regarding cattle yield are listed in Table 1.

Table 1. Average values and standard deviations (in parenthesis) of the analyzed traits depending on the $FASN_{g.17924A>G}$/*Aci*I genotype

Lactation	Genotype	n	Milk yield	Fat		Protein	
			(kg)	(kg)	%	(kg)	%
I	$FASN^{AA}$	12	9060 (323)*	387 (15)*	4.28 (0.08)	306 (10)*	3.39 (0.05)
	$FASN^{AG}$	57	8187 (200)*	351 (9)*	4.31 (0.06)	279 (6)*	3.41 (0.02)
	$FASN^{GG}$	40	8708 (261)	363 (9)	4.22 (0.08)	290 (7)	3.35 (0.03)
II	$FASN^{AA}$	12	12566 (491)	517 (12)	4.14 (0.12)	416 (14)	3.32 (0.07)
	$FASN^{AG}$	57	11018 (251)	471 (12)	4.29 (0.06)	364 (8)	3.30 (0.02)
	$FASN^{GG}$	40	11386 (319)	463 (13)	4.11(0.09)	373 (9)	3.29 (0.03)
III	$FASN^{AA}$	12	12599 (401)	491 (14)	3.93 (0.15)	413 (13)	3.28 (0.05)
	$FASN^{AG}$	57	11919 (247)	489 (12)	4.12 (0.08)	388 (8)	3.26 (0.03)
	$FASN^{GG}$	40	12191 (323)	480 (14)	3.97 (0.09)	387 (9)	3.19 (0.03)

*$p \leq 0.05$

DISCUSSION

In the recent years, many reports regarding QTLs in farm animals linked to e.g. fat deposition, milk yield, fat and protein yield and fatty acids composition, have been explored and reported (Abe et al., 2008; Daetwyler et al., 2008; Morris et al., 2010). This warrants more exhaustive research on the *FASN* gene variability as an important genetic marker for cattle. In the present study, the $FASN^{A}$ frequency (0.37), was similar to that (0.31) obtained by Morris *et al*. (2007) for Holstein-Friesian cattle. Higher $FASN^{A}$ frequencies were reported in the following breeds: Holstein-Friesian – 0.53 (Schennink *et al*., 2009) and Angus – 0.62 (Zhang *et al*., 2008). Significantly lower frequencies were obtained in

Jersey breed – 0.13 (Morris *et al*., 2007) and Korean breed – 0.15 (Oh *et al*., 2011) which is indicative of extensive genetic variability of this marker in different breeds however, within HF there seems to be similar trend

Genotypic distribution obtained in the present study was as follows: $FASN^{AA}$ – 0.11, $FASN^{AG}$ – 0.52 and $FASN^{GG}$ – 0.37. Schennink *et al*. (2009) and Zhang *et al*. (2008) reported a similar frequencies (0.50 and 0.51, respectively), whereas that showed by Oh *et al*. (2011) was 0.25. A very high $FASN^{GG}$ frequency was observed in Korean cattle – 0.73 (Oh *et al*., 2011). Zhang *et al*. (2008) have observed more frequent occurrence of the $FASN^{AA}$ homozygote – 0.36, compared to the $FASN^{GG}$ homozygote – 0.13.

Previous studies on *FASN g.17924A>G* polymorphism have been focused on its influence on fatty acids composition in milk and meat. The result of the *FASN g.17924A>G* polymorphism is a single amino acid exchange of threonine for alanine. Zhang *et al*. (2008) indicated that the aforementioned exchange is localized only a few amino acids from the fatty acid synthase region considered as a hypothetical substrate binding site. In fact, this polymorphism may significantly influence the substrate-binding process, because of its possible affection on the structure of substrate binding site. Moreover, this amino acid substitution can probably result in decreased hydrolysis activity of FASN thioesterease domain. Schennink *et al*. (2009) have observed an influence of polymorphic variants on the changes of milk fat amount in Dutch Holstein-Friesian cattle. Previous studies have indicated statistically highly significant differences in the saturated fatty acids (SFA) content and statistically significant differences in the monounsaturated fatty acids (MUFA) content as well as in total milk fat content. Milk collected from the $FASN^{AA}$ cattle was characterized by the highest fat content and an increased myrystic acid content. An analysis of the milk collected from the $FASN^{GG}$ individuals indicated less fat content and a higher MUFA proportion (Schennink *et al*., 2009). Also, in the present study, milk obtained from the $FASN^{AA}$ cattle was characterized by higher fat content.

The *FASN* gene polymorphism analysis in Angus cattle (Zhang *et al*., 2008) and Korean cattle (Oh *et al*., 2011) indicate strong relationship between the $FASN_{g.17924A>G}$ polymorphic variants and fatty acids content in meat. The $FASN^{GG}$ genotype in both Korean and Angus cattle was linked to an increased oleic acid (main MUFA fraction) and decreased palmitic acid (main SFA fraction) content. Furthermore, an increased MUFA/SFA ratio was observed. Bhuiyan *et al*. (2009) have confirmed that $FASN_{g.17924A>G}$ polymorphism affects on fatty acids composition in Korean cattle.

The $FASN^{AA}$ genotype is linked to an increased fat content and decreased MUFA proportion. So, the products from the $FASN^{AA}$ individuals have a lower value for human nutrition. This relationship is completely opposite to that of $FASN^{GG}$ genotype – a decreased fat content but an increased MUFA proportion. So, it can be concluded that as a result of the potential breeding selection for the amount of produced fat, its value for human nutrition purposes can decrease. Therefore, it is worth considering whether the action taken in connection with the human nutritional requirements should be focused on an increased amount of fat or its improved quality. On the contrary this information may also be applied to identify $FASN^{AA}$ for higher fat and protein content in the countries where quantity of milk, fat as well as protein content is important rather than quality due to wide spread malnutrition and with potential food security threats.

The present study indicated that there were the relationships between the $FASN_{17924A>G}$ polymorphism and selected milk production traits. On the basis of the results obtained in the present study and elsewhere, it can be concluded that the bovine chromosome 19, especially in the *FASN* gene region, should be a subject of the further research on the genetic marker for the bovine production traits. A detailed analysis of other SNPs localized within this gene, could possibly allow for indicating quantitative trait nucleotide, which could be used in marker assisted selection.

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