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Possible relationship between *IGF-1/SnaBI* genotypes and milk yield of Holstein-Friesian cows

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The correlation between polymorphisms in the *IGF-1* gene and production traits in beef cattle is well known. The effect of insulin-like growth factor on the value of milk traits is not yet adequately understood. The aim of the study was to attempt to describe the effect of *IGF-1/SnaBI* substitution on selected milk performance parameters of the Black-and-White variety of Holstein-Friesian cows. Three genotypes were identified: *CC*, *CT* and *TT*. The results showed a correlation between *IGF-1/SnaBI* genotypes and milk yield (highest for *CC* homozygotes and lowest for *CT* heterozygotes). No relationship could be established between the genotype and the quality characteristics of milk.

KEY WORDS: cows, milk yield, *IGF-1*

One of the molecular factors that can potentially influence milk yield in cows is the *IGF-1* gene, which encodes insulin-like growth factor. In cattle the gene is located in the fifth autosomal chromosome (BTA5, 73.5 cM) and contains six exons and five introns [5]. Together with growth hormone (GH), IGF-1 is a part of the somatotropic axis. In addition to IGF-1, IGF-2 and GH, the somatotropic axis contains other factors secreted by the cells of various tissues, such as liver cells, including growth hormone receptor (GHR), growth hormone binding protein (GHBP), insulin-like growth factor binding proteins (IGFBP) and their receptors [12]. The somatotropic axis performs a pleiotropic function and is crucial for the regulation of numerous metabolic and physiological processes, including organ growth and development [10]. Its activity is controlled by the hypothalamus. Components of the somatotropic axis influence the development of mammary gland tissue in cows. Expression of the *IGF-1* gene is highest during late pregnancy and lowest during mammogenesis, lactogenesis, galactogenesis and involution

[17]. Binding of GH to its receptor activates a number of genes, including *IGF-1* [16]. The *IGF-1* gene is mainly expressed in the liver, whose cells synthesize the small (70 amino acids) protein somatomedin C, also known as insulin-like growth factor [1]. IGF-1 exhibits endocrine activity. It is secreted by the liver into the bloodstream, through which it is transported to other tissues [15]. In the postnatal period, IGF-1 and IGF-2 play an important role in organogenesis and also influence foetal development [6]. IGF-2 is a small protein, with only 67 amino acids and a molecular weight of 7471Da. Like IGF-1 it is included among the somatomedins [14]. Both factors are crucial in the growth and development of many organs, but IGF-2 is primarily active in the foetus, and later its role is gradually taken over by IFG-1. Together with growth hormone, IGF-1 regulates metabolic processes and is responsible for postnatal growth and development. Hence these proteins play a key role in mammary gland development and lactogenesis and influence fertility as well [9].

It is important to study the *IGF-1* gene and its impact on milk yield because it is located in the same region of chromosome 5 as at least 73 loci of other genes associated with quantitative traits of milk and meat production [3]. The aim of this study was to describe the effect of the *IGF/SnaBI* substitution on selected milk production traits (milk yield and its protein and fat content) of Black-and-White Holstein-Friesian cows.

Materials and methods

The study was conducted on 735 Holstein-Friesian cows of the Black-and-White variety, from one herd in the Opole Voivodeship. Blood was drawn from the external jugular vein during routine veterinary procedures, and then placed in EDTA tubes. DNA was isolated from the samples using the MasterPure Genomic DNA Purification Kit by Epicenter Technologies, according to the manufacturer's instructions.

The primers used for amplification were taken from Ge et al. [4]. Their sequences were F 5'-ATTACAAAGCTGCCTGCCCC-3' and R 3'-ACCTTACCCGTATGAAAGGAATATACGT-5'.

The PCR mix contained approximately 50 ng of DNA template, 0.06 µl of DreamTaq DNA Polymerase (ThermoScientific™, 5 U/µl), 1.5 µl of 10x DreamTaq Buffer (ThermoScientific™), 300 µl of each dNTP (10 µM), and 0.15 of each primer (100 µM). The reaction was run in an ABi Gene 2700 thermocycler. The temperature profile is shown in Table 1.

The efficiency of the PCR was evaluated by electrophoresis of the product (4 µl) in a 1.5% agarose gel (Syngen) with ethidium bromide in 1x TBE for 20 min at 130 V. The length of the amplified DNA fragments was 249 bp.

The remaining 11 µl of the sample was digested with *SnaBI* restriction endonuclease (ThermoScientific™), which recognized the TAC↓GTA region. The reaction mix con-

Table 1
PCR temperature profile

Stage		Temperature	Time
Initial denaturation		94°C	5 min
Denaturation	31 cycles	94°C	60 s
Primer annealment		62°C	35 s
Elongation		72°C	60 s
Final elongation		72°C	5 min

tained 0.2 µl of the enzyme and 2 µl of 10x Tango buffer. The samples were incubated at 37°C for 4 h. To visualize the genotypes, the digested fragments were electrophoresed on a 3% agarose gel (Syngen) stained with ethidium bromide in 1X TBE for 40 min at 130 V. The genotypes obtained on the gels were viewed under UV light. Three genotypes were obtained: heterozygous *CT* (three fragments: 249 bp, 233 bp and 26 bp), homozygous *TT* (two fragments: 233 bp and 26 bp) and homozygous *CC* (one fragment of 249 bp).

Statistical analyses were performed using the appropriate R packages [11]. An additive relationship matrix was constructed based on the three-generation pedigree using the kinship2 R package. The following linear model was constructed using the lmekin function in the coxme R package:

$$Y = \mu + G + H + YS + \beta_1 A + \beta_2 L + \alpha + e,$$

where:

Y – phenotypic value of each trait

μ – overall mean

G – fixed effect corresponding to the genotype of a given polymorphism

H – fixed effect of herd

YS – fixed effect of the season of calving

$\beta_1 A$ – regression coefficient for the age of the cow

$\beta_2 L$ – regression coefficient for length of lactation

α – random polygenic component accounting for all known pedigree relationships

e – random residual

The analyses performed simultaneously for all three lactations also included the fixed effect of lactation. Bonferroni's correction was applied for multiple comparisons.

Results and discussion

Three genotypes were identified in the herd: *CT*, *TT* and *CC*. The frequencies of each allele and genotype for the whole population are presented in Table 2.

Table 2
Frequency of *IGF-1/SnaBI* alleles and genotypes in the test herd

	Genotype			Allele	
	CT	*TT*	*CC*	C	T
Frequency	0.4832	0.2594	0.2574	0.499	0.501

The data in Table 2 show that the heterozygous genotype (*CT*) is the most common in the population (0.4832). The frequencies of the other two genotypes (*TT* and *CC*) are very similar, 0.2594 and 0.2574, respectively. The frequency of the alleles mirrors that of the genotypes and is similar for *C* and *T*, at 0.499 and 0.501, respectively.

The results for milk yield during 305-day lactation are presented in Table 3. The mean milk yield during the first lactation was 9341.87 kg. The difference between the highest yield (genotype *CC*) and the lowest (genotype *CT*) was 1.72%, or 159.45 kg of milk. Cows with genotype *TT* had intermediate milk yield.

The results for fat and protein content in relation to *IGF-1/SnaBI* genotype are presented in Table 3. The findings were similar for all genotypes in the case of both protein and fat.

Table 3
Milk yield and percentage content of fat and protein in milk depending on *IGF-1/SnaBI* genotype

Genotype	Milk yield during 305-day lactation (kg)		Fat content (%)		Protein content (%)	
	n	mean	n	mean	n	mean
CT	353	9284.51 ±82.67	353	4.11 ±0.02	353	3.38 ±0.01
TT	192	9347.43 ±105.57	192	4.14 ±0.04	192	3.34 ± 0.02
CC	190	9443.96 ±112.67	190	4.13 ±0.04	190	3.34 ± 0.02

Few researchers have previously attempted to study the association between polymorphism in the *IGF-1/SnaBI* gene and milk performance traits. The vast majority of studies focus on how the gene influences meat production traits and cattle growth and development. Polymorphisms in the *IGF-1* gene have been shown to affect such traits as carcass size and weight, fat content in the carcass, thickness of fat tissue, daily weight gain, the suitability of meat for processing, and distribution of fat tissue [8]. Insulin-like growth factor also influences muscle growth and mammary gland development, which suggests that it could affect milk production as well.

The T>C transition occurring in the regulatory region has both direct and indirect effects on production traits [2]. Heterozygotes (*TC*) have been shown to have higher milk yield than homozygotes (*TT* and *CC*), but this was not confirmed in the present study. Moreover, a positive association had been shown between the genotype and the quality characteristics of milk, with the milk of heterozygotes containing more protein and fat than that of homozygotes. No such correlation was found in the present study between the *IGF-1/SnaBI* genotypes and the percentage content of protein and fat.

In an Irish population of Holstein-Friesian cattle, no association was found between milk production traits (milk yield and percentage content of protein) and any of the five *IGF-1* polymorphisms studied. However, the study confirmed a link between the polymorphisms and the percentage content of fat [16]. A study on bulls investigated inherited production potential by assessing the milk yield of heifers sired by them. The results indicated that of the ten *IGF-1* gene polymorphisms analysed, one was associated with an increase in milk yield, two with an increase in percentage content of protein, and one with an increased percentage content of fat in the milk [9]. Polymorphisms occurring in the introns of the *IGF-1* gene have also been shown to be linked to both milk yield and milk content of fat and protein [8]. Three of the mutations studied had a negative impact on milk yield. A>G substitutions were detected in introns 3 and 6, which decreased fat content. Moreover, individuals with a mutation in intron 3 had lower milk yield. The polymorphisms were detected in only 0.4% of the population, which may suggest that they were eliminated by selection focused on the highest possible milk yield.

Polymorphisms occurring in the *IGF-1* gene are associated not only with fat and protein content in milk but also with the proportions of fatty acids [7]. Milk produced by homozygous (*TT)* cows contained more saturated fatty acids, while the milk of heterozygous (*CT*) individuals contained more unsaturated fatty acids.

The results of the study showed a clear association between polymorphisms occurring in the regulatory region of the *IGF-1* gene and milk yield. There was no correlation found between quality traits of milk (fat and protein content) and *IGF-1/SnaBI* genotypes. Nevertheless, it is worth noting that while polymorphic variants of single

genes may have little influence on milk performance, in conjunction with other genes they may have a more beneficial effect [13]. In the era of genomic selection based on the use of predictive equations allowing specific values to be assigned to SNPs, *IGF-1/SnaBI* genotypes should be considered to play a potential role.

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