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Association between *IGF1R* / i16 / *Taq*I and *IGF1* / *SnaB*I polymorphisms and milk production traits in Polish Holstein-Friesian cows*

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The insulin-like growth factor (IGF) signalling pathway plays a crucial role in the regulation of growth and development of mammals. The strong mammary insulin-like growth factor receptor type 1 (*IGF1R*) mRNA expression during lactation suggests a role for peripheral insulin-like growth factor I (IGF-I) in maintenance of lactation. The aim of the study was to identify the frequencies of alleles and genotypes of *IGF1/Sna*BI polymorphism and two known polymorphisms within intron 16 of the *bIGF1R* gene (SNP ID rs41960582 and rs41960583) as well as to determine associations between these polymorphisms and milk production traits in Polish Holstein-Friesian cows. The frequency of *IGF1/Sna*BI allele A occurred similar to that of allele B (0.55 and 0.45, respectively). No effect was found of *IGF1* genotype on milk production traits. In the case of *IGF1R* polymorphisms detected by *Taq*I digestion (frequency of allele *A* = 0.61 and 0.39 of allele *B*), no significant effects of the *IGF1R/Taq*I polymorphism on the fat and protein yield and milk fat content was identified as well as no association between *BB* genotype and milk yield (P≤0.05). The high frequencies of all genotypes for both polymorphisms enabled the analysis of association between genotype combinations and milk traits. Cows with the $IGF1R^{BB}/IGF1^{AB}$ genotype combination (9% of the examined population) yielded more milk, fat and protein compared to other combinations (P≤0.05).

KEYWORDS: cattle /insulin-like growth factor / milk production traits / SNP / QTL

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Various studies have shown that a number of single genes associated with mammary or muscle growth, development and function are excellent candidates for linkage relationships with quantitative traits of economic importance. Among them, a somatotropic axis (SA) contains the most promising candidates [Parmentier *et al.* 1999].

The insulin-like growth factor (IGF) signalling pathway, a part of SA, involves the coordinated function of two ligands (IGF-I and IGF-II), three cell surface receptors (IGF-IR, IGF-IIR and IR), and at least six high-affinity binding proteins (IGFBPs) combined so that all together play a crucial role in normal growth and development [Liu *et al.* 1993]. Gene knockout experiments in mice have demonstrated that the IGF-I axis is required for normal growth [Baker *et al.* 1993].

Insulin-like growth factor I (IGF-I) is a 70 amino acid, single chain polypeptide encoded by a single gene [Fotsis *et al.* 1989]. Brief summary of the current state of knowledge about insulin-like growth factors (IGFs) was given by Szewczuk *et al.* [2009]. In cattle the *IGF1* gene was mapped to chromosome 5 [Bishop *et al.* 1991]. However, transcripts derived from exons 1 and 2 are alternatively spliced onto exon 3 and finally the mature IGF-I is encoded only by parts of exons 3 and 4 [Adamo *et al.* 1991, Kim *et al.* 1991]. *IGF1* gene shows several polymorphisms as earlier described by Zych *et al.* [2007]. Moreover, Gao *et al.* [2009] identified three transitions in exon 2 in native beef cows, including Chinese Simmental, Nanyang and Luxi Yellow cattle. Reyna *et al.* [2010] described *IGF1*/*Nru*I polymorphism located in intron 4. Numerous authors analysed the association between the polymorphisms within *IGF1* gene and production traits in dairy and beef cattle [Hines *et al.* 1998, Ge *et al.* 2001, Curi *et al.* 2005, Pereira *et al*. 2005, Siadkowska *et al.* 2006, Akis *et al*. 2010, Mehmannavaz *et al.* 2010, Szewczuk *et al.* 2011].

In *Bos taurus* the IGF-IR is encoded by a single gene located on chromosome 21 [Moody *et al.* 1996]. The human *IGF1R* gene consists of twenty-one exons spanning over 310 kb of genomic DNA (GenBank acc. No AY332722) and is expressed in almost all tissues and cell types during embryogenesis and postnatal growth [Bondy *et al.* 1990]. Structure of the bovine *IGF1R* gene (*bIGF1R*) is not yet completely established. The Human Genome Sequencing Center (HGSC) at Baylor College of Medicine (Houston, Texas, United States) also works on squencing and annotating the genome of *Bos taurus*. Bovine Genome Project includes among others the Whole Genome Shotgun (WGS) libraries [Zimin *et al.* 2009]. WGS sequence of *Bos taurus* chromosome 21 (based on Btau 4.2 acc. No. AC_000178.1; total length about 71,6 Mb pairs) contains coding region of the *bIGF1R* gene and consists of all 21 exons separated by sometimes long introns, spanning more than 301,2 kbp in length. The exon / intron organization of *bIGF1R* gene seems to be similar to its human equivalent. Occurrence and sequence length of promoter(s), 5'UTR and 3'UTR are still unknown.

Moody *et al.* [1996] by digestion of 625-bp PCR product with *Taq*I restriction enzyme revealed a polymorphism in alleles *A* and *B*. However, they concluded that

usefulness of this polymorphism may be limited by the low *B* allele frequency and its presence in only *Bos indicus* cattle. After a decade, as an indirect result of WGS libraries, more than 220 single nucleotide polymorphisms (SNPs) were identified in the *Bos taurus IGF1R* gene (http://www.ncbi.nlm.nih.gov/snp) released by the HGSC of the Baylor College of Medicine. However, there is no additional evidence for the frequency and validation data of each submitted SNP and the effects of these polymorphisms on beef or milk production traits with regard to *Bos taurus* were not yet investigated.

The aim of this study was to evaluate the frequencies of alleles and genotypes of *IGF1*/*Sna*BI polymorphism and dinucleotide polymorphism within intron 16 of the *bIGF1R* gene (SNP ID rs41960582 and rs41960583) in Polish Holstein-Friesian (HF) cows and to determine associations between these polymorphisms and milk production traits.

Material and methods

The study involved 201 Polish Holstein-Friesian (HF) cows kept on a farm located in the West Pomeranian province. The DNA was isolated using MasterPure™ DNA Purification Kit (EPICENTRE TECHNOLOGIES) according to the manufacturer's instructions.

The polymorphism of the *IGF1* gene and its receptor was identified using the Amplification Created Restriction Site (ACRS) and Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) methods, respectively. The amplification of the promoter fragment of the *IGF1* gene was carried out with primer sequences designed by Ge *et al.* [2001]. The fragment of the *IGF1R* gene, containing complete sequences of exon 16 and 17, was amplified with the original primer sequences designed using the Primer3 software on the basis of the GenBank acc. No. NO007319 sequence (Tab. 1).

Table 1. Primer sequences used for amplification of the selected gene fragments

Gene	Primer	Primer sequence	Fragment
IGF1	IGF677F	5'-ATTACAAAGCTGCCTGCCCC-3'	249 bp
	IGF897R	5'-ACCTTACCCGTATGAAAGGAATATACGT-3'*	
IGF1R	e16-17IRf	5' GAGATTCCAGAGCCAGCCATA 3'	878 bp
	e16-17IRr	5' TATAGAGGACCGAGAACCAAGC 3'	

*Primer IGF897R introduced a *Sna*BI restriction site.

PCR was carried out in a mixture containing the DNA template, the *Taq* DNA polymerase (FERMENTAS™), buffer with $(NH_4)_2SO_4$, $MgCl_2$, dNTP mix (FERMENTAS™) and pair of primers (OLIGO, IBB of the Polish Academy of Sciences, Warsaw). The whole reaction mixture was made up with nuclease-free deionized water (EPICENTRE TECHNOLOGIES™) to a final volume of 20 µl.

The following PCR thermal profile was applied: the initial DNA template denaturation at 94°C for 5 min followed by 33 cycles of denaturation of DNA template at 94°C for 50 s, annealing at 59.5°C (T_a) for 60 s, extension at 72°C for 50 s, and the final elongation at 72°C for 7 min. The PCR was carried out in the BIOMETRA™ thermal cycler. In regard to the gene encoding IGF-I, a specific PCR product of 249 bp was obtained. For the *IGF1R* gene the PCR product of 878 bp was achieved.

The *IGF1* gene fragment was digested with 5 units of the *Eco*105I (*Sna*BI) endonuclease (10 U/µl, TAC↓GTA; MBI FERMENTAS/ABO, Gdansk, Poland) at 37°C for 3 h. In the case of the *IGF1R* gene fragment, the obtained PCR product was digested over 2 h at 65°C with 5 units of the *Taq*I restriction enzyme (10 U/µl, T↓CGA; MBI FERMENTAS/ABO, Gdansk, Poland). In order to verify the obtained result, 10 µl of the product was separated by electrophoresis in 2% agarose gel (BASICA PRONA Agarose™) stained with ethidium bromide. The gels were treated with electric field in the presence of 1×TBE buffer, and then examined under UV light (312 nm) in a VILBER LOURMAT™ transilluminator The length of the products obtained was compared with the pUC19/*Msp*I molecular mass marker (FERMENTAS™). Selected samples of particular *IGF1R*/*Taq*I genotypes were sequenced (IBB, Polish Academy of Sciences, Warsaw).

The association of the milk, milk protein, and milk fat yield as well as protein and fat content of milk with the polymorphism of the selected genes was analysed based on the data obtained from the official milk recording. The differences between genotypes were evaluated with Duncan's test (STATISTICA 9.0 PL, software package) – Statsoft Inc. [2009]. Statistical computations were performed using a General Linear Model (GLM). The following model was used:

$$Y_{ijkl} = \mu + G_i + s_j + LACk + CS_l + \beta\,(x_l - A_l) + e_{ijkl}$$

where:

Y_{ijkl} – analysed trait;
μ – overall mean;
G_i – fixed effect of *IGF1* genotype or combination (i=1, …3 or 1,…9);
s_j – random effect of sire (j=1, …112);
$LACk$ – fixed effect of lactation (k=1, 2);
CS_l – fixed effect of calving season (l=1, 2);
β – linear regression coefficient on calving age;
x_l – calving age;
A_l – mean calving age;
e_{ijkl} – random error.

All procedures carried out with the use of animals were approved by the Local Ethics Commission, Permission (No 25/09).

Results and discussion

In the case of the two polymorphisms within intron 16 of the *IGF1R* gene (determined as *IGF1R* / i16 / *Taq*I) three distinct patterns (designed as *AA*, *AB*, and *BB* genotypes) were observed in PCR-RFLP analysis. Digestion of the 878-bp PCR product with the restriction enzyme *Taq*I resulted in two DNA bands (545 and 333 bp) for homozygote *AA* and three (387, 333 and 158 bp) for homozygote *BB* (Fig. 1.). The first cut sequence plays a role of an internal control of digestion. Both polymorphisms are recognized by second cut of the *Taq*I restrictase. However, there is no possibility to detect each polymorphism separately. Both C/T transitions were originally identified in Herefords as an indirect result of Bovine Genome Project. SNPs are marked at positions 492 and 491 within GenBank GQ487665 sequence for rs41960582 and rs41960583, respectively). Results of genotyping *IGF1R* / i16 / *Taq*I polymorphism in Polish HF cows are shown in Table 2.

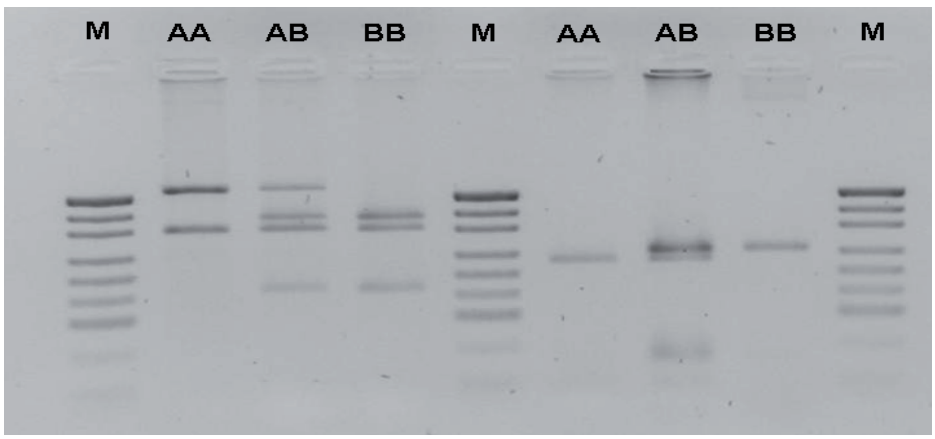

Fig. 1. Agarose gel electrophoresis presenting different genotypes of polymorphisms within intron 16 of the bovine *IGF1R* gene and in the 5'-noncoding region of the *IGF1* gene. Lanes 1, 5 and 9: M – pUC19/*Msp*I DNA length marker. Lanes 2 – 4: *IGF1R*/*Taq*I polymorphism. Lanes 6 – 8: *IGF1*/*Sna*BI polymorphism.

Table 2. Numbers and frequencies of the *IGF1R*/*Taq*I and *IGF1/Sna*BI genotypes and alleles in Polish HF cows examined

*IGF1R/Taq*I genotype				Total	Allele	
	AA	*AB*	*BB*		*A*	*B*
Number	71	104	26	201	0.6119	0.3881
Frequency	0.3532	0.5174	0.1294	1.0000		
*IGF1/Sna*BI genotype				total	allele	
	AA	*AB*	*BB*		*A*	*B*
Number	53	113	35	201	0.5448	0.4552
Frequency	0.2637	0.5622	0.1741	1.0000		

In Polish HF cattle about 52% individuals were found to carry *AB* genotype; frequencies of *AA* and *BB* genotypes were also relatively high and reached 35% and 13%, respectively. Theoretically, the two polymorphisms may occur as natural eight variants of haplotypes: five for *AA* genotype (*CC*/*CC*, *TT*/*TT*, *CC*/*TT*, *CT*/*TT*, *CC*/*CT*), two for *AB* (*CT*/*CC* and *TT*/*CT*), and only one for *BB* genotype (*TT*/*CC*). To determine the sequence of *AB* genotype (*CT*/*CC* or *TT*/*CT*), 36 random heterozygous samples (24 for Polish HF, 4 for Limousine, 4 for Hereford and 4 for Angus were sequenced (genotyping data for beef cattle were withdrawn from our unpublished studies). It proved to be less complicated than we had expected. Each cow occurred heterozygous (*CT*) at the first SNP (rs41960583) and homozygous (*CC*) at the second SNP (rs41960582) with no reference to the breed. In addition, another 24 dairy cows carrying *AA* genotype were sequenced. All of them were double homozygous (*CC*/*CC*) (Fig. 2). In light of this, occurrence of rs41960582 SNP in Polish HF cows can be considered doubtful or extremely rare.

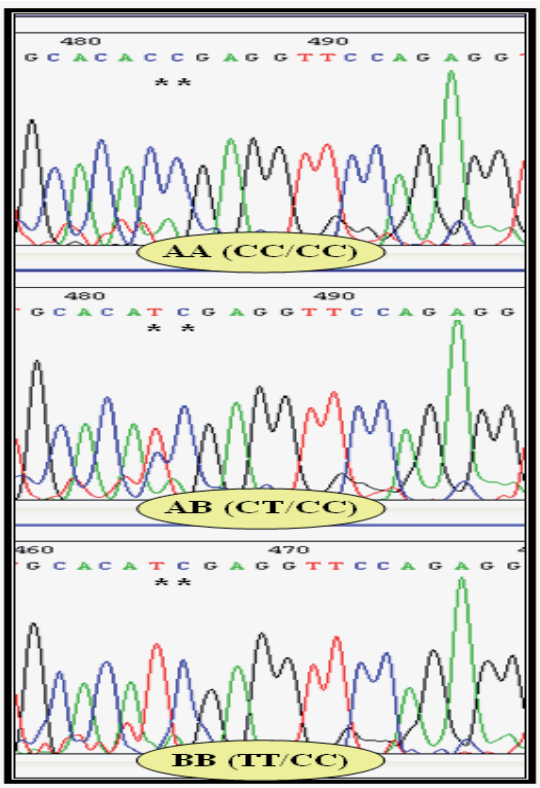

Fig. 2. Identification of two polymorphic sites (rs41960583 and rs41960582) within intron 16 of *bIGF1R* by direct sequencing analysis. Asterisk (*) – mutation sites.

IGF1R/*Taq*I polymorphism earlier described by [Moody *et al.* 1996] in *Bos indicus* cattle and analysed by Curi *et al.* [2005] and Akis *et al.* [2010], is not clearly understandable. Comparing the sequences of primers designed by Moody *et al.* [1996] with NCBI reference sequence AC_000178.1 revealed that primers flanking the 625 bp fragment of bovine *IGF1R* gene are located in the exon 12 (forward primer) and 13 (reverse primer) and that polymorphism mentioned is located within intron 12. The results presented here clearly show that *IGF1R*/*Taq*I polymorphism within intron 16 and polymorphism *IGF1R*/*Taq*I detected by Moody *et al.* [1996] are totally different mutations. We proposed, therefore, more appropriate nomenclature of *IGF1R*/*Taq*I polymorphisms including exact localization (for example *IGF1R*/i16/*Taq*I in present study). This will allow to interprete better the results of investigations forthcoming in the field of *IGF1R* gene polymorphism.

For the *IGF1*/*Sna*BI polymorphism (the C/T transition at position 512 bp 5' to the ATG codon of the first exon), the following restriction patterns were identified: two restriction fragments (of 223 and 26 bp) for the *AA* genotype, three restriction fragments (of 249, 223 and 26 bp) for the *AB* genotype and one restriction fragment of non-digested PCR product (249 bp) for the *BB* genotype – Figure 1. The frequency of genotypes and alleles for the *IGF1/Sna*BI polymorphism are shown in Table 2.

In the analysed herd, frequency of *AB* genotype was the highest, whereas that of *BB* – the lowest (0.5622 and 0.1741, respectively). The frequencies of both alleles were similar and amounted to 0.55 and 0.45 for the *A* and *B* allele, respectively. High frequency of *A* allele (0.55) was also found by Hines *et al.* [1998] and Siadkowska *et al.* [2006] – 0.52 – in HF cattle as well as by Li *et al.* [2004] (0.56) and Ge *et al.* [2001] (0.639) in Angus cattle. Somewhat lower frequencies of *A* allele (0.55 and 0.47) were reported by Klauzińska *et al.* [2004] in Polish Red and Black-and-White cows, respectively, by Mehmannavaz *et al.* [2010] – 0.438 – in the Iranian Holstein bulls and Bonakdar *et al*. [2010] in HF cows – 0.463. In several *Bos indicus* breeds low frequencies of allele *A* (0.20-0.38) were observed – Curi *et al.* [2005], Pereira *et al*. [2005] and Akis *et al.* [2010].

Table 3. Means and standard errors (in parentheses) for the analysed milk production traits in Polish HF cows with the *IGF1R* gene variants

Polymorphism	Genotype	Cows number	Milk yield per lactation (kg)	Fat		Protein	
				kg	%	kg	%
IGF1R/*Taq*I	*AA*	71	7507[a] (156.91)	304 (7.00)	4.07 (0.06)	253 (5.33)	3.36[a] (0.02)
	AB	104	7560 (125.70)	304 (5.07)	4.05 (0.04)	252 (3.89)	3.34[b] (0.02)
	BB	26	7909[a] (243.94)	313 (10.05)	3.96 (0.07)	256 (7.42)	3.25[ab] (0.05)
	Total	201					

[ab]Means within columns bearing the same superscript letters differ significantly at P≤0.05.

Association was investigated between two analysed polymorphisms and milk production traits in cattle. The mean values of the analysed milk production traits for the individual with different *IGF1R*/ i16/*Taq*I genotypes are presented in Table 3.

In the analysed herd, the advantageous (P≤0.05) effect of the *BB* genotype on milk yield per lactation (+ 402 kg) was found compared to the *AA* genotype. The milk of cows of *AA* and *AB* genotypes was characterized by higher (P≤0.05) protein content (%) compared to the milk of *BB* cows. No significant effect of the *IGF1R*/*Taq*I polymorphism on the fat and protein yield and milk fat content was found. No literature reports were identified concerning effects of *IGF1R* / i16 / *Taq*I polymorphism on milk production traits in cattle. Only few studies regarding the *IGF1R* / *Taq*I polymorphism located within intron 12 have been found. According to Akis *et al.* [2010], the *IGF1R* / i12 / *Taq*I polymorphism does not improve the meat and milk production traits in cattle. Also Curi *et al.* [2005] claim that the mentioned polymorphism has significant effect neither on the cattle body weight gain and composition, nor on the quality of carcass.

Associations between the *IGF1*/*Sna*BI genetic variants and the analysed milk production traits are presented in Table 4. No significant effects of the polymorphism in question were identified on the milk, protein and fat yield as well as on milk protein and fat content. However, cows of the *AB* genotype yielded more milk per lactation (7679 kg) than those of remaining two genotypes (~7450 kg). The mean fat and protein yields were at a similar level irrespective of the cows' genotype.

Table 4. Means and standard errors (in parentheses) for the analysed milk production traits in Polish HF cows with the *IGF1* gene variants

Polymorphism	Genotype	Cows number	Milk yield per lactation (kg)	Fat		Protein	
				kg	%	kg	%
IGF1/*Sna*BI	*AA*	53	7472 (135.72)	306 (6.69)	4.10 (0.06)	251 (4.52)	3.36 (0.02)
	AB	113	7679 (123.73)	304 (5.06)	3.99 (0.04)	254 (3.86)	3.31 (0.02)
	BB	35	7461 (270.10)	307 (10.77)	4.14 (0.05)	252 (8.88)	3.38 (0.03)
	Total	201					

In several studies, the effect of *IGF1/Sna*BI SNP has generally been tested in relation to beef production traits. Little is known about its effect on milk production traits in cattle. In the present study as well as in that by Hines *et al*. [1998] no differences were found between *IGF1*/*Sna*BI polymorphism effect and dairy production traits in Holsteins. Similar findings were reported by Grzelak *et al*. [2007] in Jersey cattle. However, in most analysed traits the cows of the heterozygous *AB* genotype appeared superior to those of remaining genotypes. As shown by Siadkowska *et al.* [2006], heterozygous cows yielded daily more fat (+20 g) and protein (+14.5 g) than those of the *AA* genotype (P≤0.01). The *AB* genotype also appeared favourable for fat and

protein content of milk. No differences were found between genotypes in the daily milk yield. However, when milk yield was converted into fat (FCM) or fat and protein corrected milk (VCM), the *AB* genotype appeared superior to other genotypes (P≤0.05 for FCM and P≤0.01 for VCM). Moreover, the heterozygous bulls had the estimated breeding values of milk yield (EBVM) and estimated breeding values of fat yield (EBVF) higher than homozygous (P<0.1) – Mehmannavaz *et al.* [2010]. In contrast, Akis *et al*. [2010] suggested no relationship between *IGF1/Sna*BI polymorphism and economic traits of interest in EAR and SAR cattle probably due to the respectively low carcass weight (280-300 kg in adults) and milk parameters (maximum milk production 1000-1500 kg) as well as low frequency of *AA* genotype.

The high frequencies of all genotypes for both polymorphisms enabled the analysis of association between genotype combinations and milk performance traits of cows (Tab. 5). The *BB*/*BB* combination was excluded from the analysis due to the low sample size (only one individual).

Table 5. Means and standard errors (in parentheses) for the analysed milk production traits in Polish HF cows with variants of the *IGF1R*/*IGF1* combined genotypes

Combination *IGF1R*/*Taq*I / *IGF1*/*Sna*BI	N	Milk yield per lactation (kg)	Fat		Protein	
			kg	%	kg	%
AA/AA	23	7576^{a} (226.73)	305 (10.83)	4.04 (0.10)	253 (7.48)	3.34 (0.04)
AA/AB	39	7529^{b} (198.29)	305 (9.57)	4.06 (0.07)	255 (7.11)	3.36 (0.03)
AA/BB	9	7236^{c} (719.61)	295 (25.86)	4.14 (0.11)	245 (22.70)	3.40^{a} (0.04)
AB/AA	23	7454^{d} (179.06)	315 (9.77)	4.22^{a} (0.09)	254 (6.19)	3.40^{b} (0.03)
AB/AB	57	7613^{e} (184.81)	297^{a} (6.79)	3.94^{a} (0.06)	250 (5.46)	3.31 (0.03)
AB/BB	24	7535^{f} (281.01)	310 (11.54)	4.14 (0.07)	253 (9.30)	3.37 (0.03)
BB/AA	7	7186^{g} (427.94)	277^{b} (13.91)	3.87 (0.10)	236 (12.77)	3.29 (0.04)
BB/AB	18	8299^{abcdefg} (260.28)	331^{ab} (11.10)	4.00 (0.09)	267 (8.22)	3.24^{ab} (0.07)
BB/BB	1	5946 (-)	241 (-)	4.05 (-)	200 (-)	3.37 (-)
Total	201					

$^{ab...}$Means within columns bearing the same superscript letters differ significantly atP≤0.05.

The *BB*/*AB* genotype combination determined higher milk yield per lactation compared to following combinations: *BB*/*AA* (+ 1113 kg), *AA*/*BB* (+ 1063 kg), *AB*/*AA* (+ 845 kg), *AA*/*AB* (+ 770 kg), *AB*/*BB* (+ 764 kg), *AA*/*AA* (+ 723 kg) and *AB*/*AB* (+ 686 kg) (p≤0.05). However, in the case of *BB*/*AA* and *AA*/*BB* combinations, low numbers of individuals and high values of SE could affect the result of statistical analysis. The

significant (P≤0.05) effect of the *BB*/*AB* genotype combination on the fat yield was found in comparison to the *BB*/*AA* and *AB*/*AB* combinations (+54 kg and + 34 kg, respectively). The mean fat content was highest in the milk of cows with the *AB*/*AA* genotype combination, and the lowest for the combination *AB*/*AB* (P≤0.05). Cows with the *BB*/*AB* combination had the lowest mean milk protein content (3.24%) and those with the *AA*/*BB* and *AB*/*AA* combinations – the highest (3.40%) – P≤0.05. In the case of the protein yield, no significant differences between genotypes were observed, although the cows with the *BB*/*AB* genotype combination showed the highest mean value. Taking into account the above-mentioned relations, it can be concluded that cows with the *BB*/*AB* combination (9% of the examined population) yielded most milk, fat and protein.

The detection and mapping of genetic markers linked to quantitative trait *loci* (QTL) can be used to enhance genetic improvement of livestock populations. Strong QTL for meat [Li *et al.* 2004] and milk [Smaragdov *et al.* 2006] production traits were assigned to *Bos taurus* chromosome 5 (BTA5), where the *IGF1* gene was mapped. The higher mRNA expression of paracrine / autocrine IGF-I derived in P1 promoter in the mammary gland during mammogenesis when compared with decrease in lactation indicates a potential proliferative role of IGF-I system throughout development. During involution, IGF-I expression increased again. The high mammary *IGF1R* mRNA expression during lactation suggests a role for peripheral IGF-I in maintenance of lactation [Plath-Gabler *et al.* 2001]. The results obtained in the present study provide a valuable reference for further candidate gene research and marker-assisted selection. The relatively small sample size used in this study may lack the effective power of detecting the possible association between *IGF1*/*IGF1R* and the milk traits. In addition, both polymorphisms are not the causative mutation. Rather, *IGF1*/*Sna*BI and *IGF1R*/*Taq*I polymorphisms may be considered as a genetic markers possibly linked to other polymorphism(s) located closely in the same or another gene. More tests are needed among random populations to verify the associated effects of both polymorphisms as the potential genetic markers.

REFERENCES

1. ADAMO M.L., BEN-HUR H., LeROITH D., BERTS JR C.T., 1991 – Transcription initiation in the two leader exons of the rat *IGF1* gene occurs from dispersed versus localized sites. ***Biochemical and Biophysical Research Communications*** 176, 887-893.
2. AKIS I., OZTABAK K., GONULALP I., MENGI A, UN C., 2010 – IGF-1 and IGF-1r gene polymorphisms in East Anatolian Red and South Anatolian Red cattle breeds. ***Russian Journal of Genetics*** 46(4), 497-501.
3. BAKER J., LIU J.P., ROBERTSON E.J., EFSTRATIADIS A., 1993 – Role of insulin-like growth factors in embryonic and postnatal growth. ***Cell*** 75, 73-82.
4. BISHOP M.D., TAVAKKOL A., THREADGILL D.W., SIMMEN F.A., SIMMEN R.C.M., DAVIS M.E., WOMACK J.E., 1991 – Somatic cell mapping and restriction fragment length polymorphism analysis of bovine insulin-like growth factor I. ***Journal of Animal Science*** 69, 4306-4311.

5. BONAKDAR E., RAHMANI H.R., EDRISS M.A., TABATABAEI B.E.S., 2010 – IGF-I gene polymorphism, but not its blood concentration, is associated with milk fat and protein in Holstein dairy cows. ***Genetics and Molecular Research*** 9(3), 1726-1734.
6. BONDY C.A., WERNER H., ROBERTS C.T., LEROITH D., 1990 – Cellular pattern of insulin-like growth factor I (IGF-I) and type I IGF receptor gene expression in early organogenesis: comparison with IGF-II gene expression. ***Molecular Endocrinology*** 4, 1386-1398.
7. CURI R.A., OLIVEIRA H.N., SILVEIRA A.C., LOPES C.R., 2005 – Association between IGF-I, IGF-IR and GHRH gene polymorphisms and growth and carcass traits in beef cattle. ***Livestock Production Science*** 94, 159-167.
8. FOTSIS T., MURPHY C., GANNON F., 1989 – Nucleotide sequence of insulin-like growth factor I (*IGF-I*) and its *IGF-IA* precursor. ***Nucleic Acids Research*** 18, 676.
9. GAO X., SHI M., XU X., LI J., REN H. XU S., 2009 – Sequence variations in the bovine IGF-I and IGFBP3 genes and their association with growth and development traits in Chinese Beef Cattle. ***Agricultural Sciences in China*** 8 (6), 717-722.
10. GE W., DAVIS M.E., HINES H.C., IRVIN K.M., SIMMEN R.C.M., 2001 – Association of genetic marker with blood serum insulin-like growth factor-I concentration and growth traits in Angus cattle. ***Journal of Animal Science*** 79, 1757-1762.
11. GRZELAK T., KMIEĆ M., WOJDAK-MAKSYMIEC K., KULIG H., KOWALEWSKA-ŁUCZAK I., 2007 - *IGF1/Sna*BI gene polymorphism in Jersey cows. ***2nd Polish Congress of Genetics***, SGGW Warsaw, 18-20 September. In Polish, summary in English
12. HINES H.C., GE W., ZHAO Q., DAVIS M.E., 1998 - Association of genetic markers in growth hormone and insulin-like growth factor I loci with lactation traits in Holsteins. ***Animal Genetics*** 29 (suppl. 1), 63.
13. KIM S.W., LAJARA R., ROTWEIN P., 1991 – Structure and function of a human insulin-like growth factor-I gene promoter. ***Molecular Endocrinology*** 5, 1964-1972.
14. KLAUZIŃSKA M., ŻURKOWSKI M., SIADKOWSKA E., SZYMANOWSKA M., GROCHOWSKA R., ZWIERZCHOWSKI L., KLEWIEC J., 2004 – Analysis of genetic structure in Polish Red and Polish Black-and-White cattle using twelve marker ***loci*** potentially related to milk or meat production traits. *Animal Science Papers and Reports* 22 (2), 153-171.
15. LI C., BASARAB J., SNELLING W.M., BENKEL B., MURDOCH B., HANSEN C., MOORE S.S., 2004 – Assessment of positional candidate genes *MYF*5 and *IGF-I* for growth on bovine chromosome 5 in commercial lines of *Bos taurus*. ***Journal of Animal Science*** 82, 1-7.
16. LIU J.P., BAKER J., PERKINS A.S., ROBERTSON E.J., EFSTRATIADIS A., 1993 – Mice carrying null mutations of the genes encoding insulin-like growth factor I (*Igf-1*) and type 1 IGF receptor (*Igf1r*). ***Cell*** 75, 59-72.
17. MEHMANNAVAZ Y., AMIRINIA C., BONYADI M., TORSHIZI R.V., 2010 – Association of *IGF-1* gene polymorphism with milk production traits and paternal genetic trends in Iranian Holstein bulls. ***African Journal of Microbiology Research*** 4(1), 110-114.
18. MOODY D.E., POMP D., BARENDSE W., 1996 – Linkage mapping of the bovine insulin-like growth factor-1 receptor gene. ***Mammalian Genome*** 7(2), 168-169.
19. PARMENTIER I., PORTETELLE D., GENGLER N., PRANDI A., BERTOZZI C., VLEURICK L., GILSON R., RENAVILLE R., 1999 – Candidate gene markers associated with somatotropic axis and milk selection. ***Domestic Animal Endocrinology*** 17, 139-148.
20. PEREIRA A.P., DE ALENCAR M.M., DE OLIVEIRA H.N., REGITANO L.C.D., 2005 – Association of GH and IGF-1 polymorphisms with growth traits in a synthetic beef cattle breed. ***Genetics and Molecular Biology*** 28, 230-236.

21. PLATH-GABLER A., GABLER C., SINOWATZ F., BERISKA B., SCHAMS D., 2001 – The expression of the IGF family and GH receptor in the bovine mammary gland. ***Journal of Endocrinology*** 168, 39-48.
22. REYNA X.F., MONTOYA H.M., CASTRELLÓN V.V., RINCÓN A.M., BRACAMONTE M.P., VERA W.A., 2010 – Polymorphisms in the IGF1 gene and their effect on growth traits in Mexican beef cattle. ***Genetics and Molecular Research*** 9(2), 875-83.
23. SIADKOWSKA E., ZWIERZCHOWSKI L., OPRZĄDEK J., STRZAŁKOWSKA N., BAGNICKA E., KRZYŻEWSKI J., 2006 – Effect of polymorphism in IGF-1 gene on production traits in Polish Holstein-Friesian cattle. ***Animal Science Papers and Reports*** 24(3), 225-237.
24. SMARAGDOV M.G., PRINZENBERG E.M., ZWIERZCHOWSKI L., 2006 – QTL mapping in cattle: theoretical and empirical approach. ***Animal Science Papers and Reports*** 24(2), 69-110.
25. STATSOFT, INC.: STATISTICA (data analysis software system) 2009 – version 9.0 PL (www.statsoft.com)
26. SZEWCZUK M., ZYCH S., CZERNIAWSKA-PIĄTKOWSKA E., 2009 – The evolution of opinion on the subject of insulin-like growth factors. In Polish, summary in English. ***Postępy Biochemii*** 55 (3), 329-336.
27. SZEWCZUK M., ZYCH S., CZERNIAWSKA-PIĄTKOWSKA E., 2011 – Association between *IGF1*/*Tas*I polymorphism and milk traits of Polish Holstein Friesian cows. ***Archiv fuer Tierzucht*** 54, 10-17.
28. ZIMIN A.V., DELCHER A.L., FLOREA L., KELLEY D.R., SCHATZ M.C., PUIU D., HANRAHAN F., PERTEA G., VAN TASSELL C.P., SONSTEGARD T.S., MARÇAIS G., ROBERTS M., SUBRAMANIAN P., YORKE J.A., SALZBERG S.L., 2009 – A whole-genome assembly of the domestic cow, *Bos taurus*. ***Genome Biology*** 10(4), R42.
29. ZYCH S., SZEWCZUK M., CZERNIAWSKA-PIĄTKOWSKA E., SZATKOWSKA I., 2007 - A new ACRS-SNP in the 5' flanking region of the bovine insulin-like growth factor 1 (*IGF1*) gene (Brief report). ***Archiv fuer Tierzucht*** 50, 531-32.