



Association between *IGF-1* gene polymorphism and milk production traits in Polish Red-and-White cattle

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Gene, which encodes insulin-like growth factor 1 – *IGF-1* in cows, is localized on the chromosome 5 (BTA5) and consists of 6 exons (<http://www.ensembl.org>). The promoter region of bovine *IGF-1* gene 2 polymorphic sites revealed microsatellite repeats (CA)_n (Kirkpatrick 1992) and C/T transition (Ge *et al.* 1997).

Maj *et al.* (2008) found that animals with genotype CC are characterized by higher relative expression of *IGF-1* as well as IGF-1 content in blood serum in relation to others. Moreover, analysis of transcription factors binding sites (TFBS) proved that allele T has the NF-1 binding site identical with the consensus sequence for this TF. Products of the NF-1 genes family differ in their abilities to either activate or repress transcription (Gronostajski 2000). In *IGF-1/SnaBI* polymorphism allele T seems to repress transcription of *IGF-1* gene, as it was proved that IGF-1 concentration from day 35 to 140 postpartum tends to increase (McCarthy *et al.* 2009). Furthermore, during lactation, the circulating concentrations of IGF-1 is one-third greater in cows with good genetic merit for fertility in comparison to cows with poor merit (Cummins *et al.* 2012). It means that selection for milk traits based on IGF-1 concentration is not negatively correlated with reproductive traits.

The study involved 197 cows of Polish Red-and-White breed reared on a farm located in the Opole province. The DNA was isolated from whole blood using DNA purification kit following the manufacturer's instruction.

The polymorphism in the *IGF-1* gene was identified using the ACRS-PCR (artificially created restriction site-polymerase chain reaction) method. The amplification of the *IGF-1* gene was carried out with primes given by Ge *et al.* (2001). PCR reactions were performed in a volume 15 µl containing: 75–85ng DNA, 0.2mmol of dNTP mix, 1xPCR buffer with NH₄, 12pmol of each primer, 2.5 mmol MgCl₂, 0.75 U of *Taq* DNA polymerase, loading dye and PCR grade water.

The following PCR thermal profile was applied: the

initial denaturation – 94°C/5 min followed by 30 cycles of denaturation – 94°C/60 sec, annealing – 58°C/45 sec, elongation – 72°C/60 sec and the final elongation – 72°C/5 min. The *IGF-1* gene fragment was digested with 3U of the *SnaBI* restriction enzyme at 37°C overnight. Restriction fragments were separated in 3% agarose gels stained with ethyidium bromide. The length of individual fragments was compared with the pUC19/*MspI* DNA marker.

For the association analysis following traits were taken into consideration: milk yield (kg), percentage content of fat, protein, lactose and dry matter as well as somatic cell count (SCC). In SCC the full-scale was changed into logarithmic to achieve the normal distribution. Performance data were analyzed for lactations I, II, III, IV+ separately as well as for all lactations together.

Associations between *IGF-1* gene variants and performance traits were evaluated using the Statistica software, ver. 8.0 with GLM multiple factor mixed nested model. The following models were applied for calculations:

• for lactations I, II, III, IV+ :

$$y_{ijklm} = \mu + a_i + b_j + c_k + d_l + f_m + b(\text{HF}) + e_{ijklm}$$

where: y_{ijklm} , analyzed trait; μ , overall mean; a_i , effect of *IGF-1* genotype; b_j , effect of sire; c_k , effect of calving year/season; d_l , effect of study year; f_m , effect of factor nested in genotype (different number of test day records per cow in lactation); $b(\text{HF})$, regression of HF breed genes percentage on analyzed trait; e_{ijklm} , random error.

for all lactations:

$$y_{ijklmn} = \mu + a_i + b_j + c_k + d_l + f_m + g_n + b(\text{HF}) + e_{ijklmn}$$

where, y_{ijklmn} , μ , a_i , b_j , c_k , d_l , f_m , $b(\text{HF})$, e_{ijklmn} , as above; g_n , effect of lactation.

The differences between means were verified with Duncan's test using Statistica software, ver. 8.0. Population parameters were calculated using PowerMarker software (Liu and Muse 2005).

Analyzing polymorphism *IGF-1/SnaBI* in the herd of Polish Red-and-White cattle 3 genotypes were found with following frequency: CC – 0.20, CT – 0.62 and TT – 0.18. Comparing these frequency with expected values deviation from genetic equilibrium was observed ($P < 0.01$). Mean heterozygosity of the herd was 0.61, while PIC – 0.37. Allele frequency in investigated population (C – 0.51, T – 0.49)

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was similar to those observed in Polish Red, Polish Holstein-Friesian and Charolais cattle (Klauzińska *et al.* 2004, Siadkowska *et al.* 2006, Reyna *et al.* 2010, Szewczuk *et al.* 2012).

Association analysis between *IGF-1/SnaBI* variants and investigated milk production traits are listed in Table 1. Statistically significant relationships were found between individual genotypes and milk yield in the second lactation. Cows bearing genotypes *CC* and *CT* characterized higher value of this trait in comparison to cows with genotype *TT* ($P < 0.01$). Similar effect was observed in the first and third lactation but was not confirmed statistically. Associations were also found in somatic cells count calculated for all lactations. Cows with genotype *TT* showed higher value of SCC in relation to other genotypes ($P < 0.01$).

Analyzed polymorphism C-512T in Holstein-Friesian cows as well as in Jersey cattle showed no differences between milk production traits (Hines *et al.* 1998, Grzelak *et al.* 2007, Polasik *et al.* 2010 and Szewczuk *et al.* 2012).

However, Siadkowska *et al.* (2006) confirmed that heterozygous genotype in Polish H-F cattle is favorable for milk yield converted into fat or fat and protein corrected milk ($P < 0.01$). Authors also noticed that genotype *CT* was superior to others in most investigated traits e.g. fat and protein content in milk ($P < 0.01$). Similar results in relation to genotype *CT* were observed by Mehmannaavaz *et al.* (2010) in Iranian Holstein breed. Bulls carrying heterozygous genotype characterized higher estimated breeding values of milk and fat yield compared to homozygous genotypes ($P < 0.1$). What is more, this genotype was associated with higher protein yield, fat and protein content (%) but differences were not confirmed statistically. Bonekdar *et al.* (2010) also proved the influence of *IGF-1/SnaBI* genotypes on milk traits. Genotypes *TT* and *CC* had lower milk fat ($P < 0.1$) and protein ($P < 0.05$) concentration than *CT*. Similar associations were found for

corrected total fat and protein produced over the year ($P < 0.1$).

In our investigations genotype *CT* was also mostly associated with higher fat and protein percentage for lactations analyzed separately and together but these differences were not confirmed by statistical test. For the milk yield heterozygous genotype showed intermediate influence for lactations analyzed separately. In the case of lactations analyzed together *CT* cows had highest milk yield but the difference was low.

In our research we also analyzed somatic cell count as an indicator of the quality of milk. We found that genotypes *CT* and *TT* were unfavorable for this trait. So far only Mullen *et al.* (2011) have investigated variants of the *IGF-1* gene in relation to somatic cell score (SCS). The results showed that polymorphism C-512T in H-F cows was not associated with SCS. However novel substitution localized in the intronic region of *IGF-1* gene were significantly correlated with this trait ($P < 0.1$).

Polymorphism *IGF-1/SnaBI* was also investigated by many authors in beef cattle. Results of their studies proved associations between individual genotypes and growth and carcass traits. Genotype *CC* was favorable i.a. for weight gain, body weight, subcutaneous backfat, feed intake and feed conversion for growth ($P < 0.5$) (Ge *et al.* 20010, Curi *et al.* 2005, Siadkowska *et al.* 2006) but Reyna *et al.* (2010) found that heterozygous genotype – *GC* is also advantageous for weaning and pre-weaning weight gain ($P < 0.5$).

As we mentioned substitution C-512T was studied in relation to reproductive traits as well. Bulls carrying *CC* genotype exhibited an average age at scrotal circumference 28 cm higher than *CT* and *TT* ($P < 0.5$) (Lirón *et al.* 2012). It supports hypothesis that allele *C* is not negatively correlated with reproductive traits.

In conclusion the obtained results showed that

Table 1. Mean values of estimated traits depend on *IGF-1* genotype

Lactation	Genotype	No.	Milk yield [kg]		Fat [%]		Protein [%]		Lactose [%]		Dry matter [%]		SCC	
			X	SD	X	SD	X	SD	X	SD	X	SD	X	SD
I	CT	757	21,82	5,12	4,25	0,95	3,32	0,36	4,93	0,20	13,44	1,30	5,25	1,17
	TT	248	21,76	4,97	4,10	0,86	3,20	0,28	4,90	0,20	13,02	0,94	5,53	1,19
	CC	382	21,83	5,41	4,21	0,97	3,32	0,39	4,91	0,21	13,29	1,21	5,27	1,02
II	CT	685	23,97 ^a	7,98	4,41	1,03	3,42	0,47	4,78	0,25	13,51	1,35	5,68	1,12
	TT	153	21,76 ^{ab}	7,40	4,17	0,91	3,40	0,39	4,80	0,22	13,23	1,17	5,87	1,00
	CC	229	24,39 ^b	7,42	4,38	1,08	3,35	0,37	4,81	0,22	13,42	1,37	5,54	1,11
III	CT	495	25,16	7,99	4,48	1,04	3,36	0,43	4,75	0,25	13,39	1,22	5,76	1,09
	TT	128	23,52	8,85	4,31	0,90	3,47	0,43	4,75	0,23	13,40	1,16	5,92	1,31
	CC	114	27,27	8,89	4,46	1,20	3,22	0,38	4,71	0,24	13,32	1,54	5,55	1,05
IV+	CT	703	24,69	8,33	4,67	1,00	3,38	0,40	4,68	0,24	13,46	1,28	6,27	1,15
	TT	129	27,49	8,85	4,21	1,04	3,30	0,40	4,75	0,27	13,08	1,40	6,14	1,34
	CC	37	23,51	8,84	4,60	1,02	3,15	0,37	4,56	0,27	13,10	1,08	5,93	1,41
Total	CT	2660	23,77	7,48	4,38	1,00	3,36	0,42	4,79	0,25	13,45	1,29	5,72 ^a	1,20
	TT	661	23,24	7,56	4,18	0,92	3,32	0,38	4,82	0,24	13,16	1,14	5,80 ^b	1,23
	CC	770	23,52	7,07	4,32	1,04	3,31	0,38	4,83	0,24	13,32	1,30	5,43 ^{ab}	1,08

N, number of test-day records; ^{a...}, means within columns bearing the same superscript letters differ significantly at $P < 0.01$.

polymorphism *IGF-1/SnaBI* is associated with milk yield and somatic cell count in Polish Red-and-White cattle ($P < 0.01$). Allele *C*, which appeared with frequency 0.51 was favorable for these traits in homo- and heterozygous form. For other investigated traits similar tendency was observed but differences were smaller and could not be confirmed statistically.

SUMMARY

The aim of this study was to analyze polymorphism C–512T in the *IGF-1* gene in relation to milk production traits in Polish Red-and-White cattle. Traits analyzed were: milk yield, content of fat, protein, lactose, dry matter and somatic cell count. Polymorphism analysis showed presence of 2 alleles in the investigated herd with frequency *C* – 0.51, *T* – 0.49. Association analysis showed that cows with *CC* and *CT* genotypes are characterized by higher milk yield in the second lactation comparing to those with *TT* genotype. Moreover, *CC* genotype was favorable for somatic cell count.

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