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***PIT1-HinfI* gene polymorphism and its associations with milk production traits in polish Black-and-White cattle**

Abstract

Associations between polymorphism localised in the six exon of *PIT1* gene (*PIT1-HinfI*) and milk production traits of Black-and-White cattle were analysed. A total of 900 cows were included in the study. PCR-RFLP method was used. The frequencies of the genotypes and alleles were as follows: 0.054 for AA, 0.377 for AB and 0.569 for BB, and 0.243 for *PIT1*^A and 0.757 – *PIT1*^B. There were no associations between *PIT1-HinfI* polymorphism and milk production traits of the cows.

Key Words: pituitary transcription factor 1, PCR-RFLP, dairy cattle, milk production traits

Zusammenfassung

Titel der Arbeit: **Polymorphismus des PIT1-Hinf-1-Gens und sein Zusammenhang zwischen den Milchleistungsmerkmalen bei schwarzbunten Kühen**

Es wurden die Zusammenhänge zwischen dem Polymorphismus von Exon 6 des *PIT1*-Gens (*PIT1-HinfI*) bei schwarzbunten Kühen analysiert. Die Untersuchungen erfolgten an 900 Kühen. Angewandt wurde die PCR-RFLP-Methode. Die Genotyp- und Allelfrequenz war wie folgt: für AA 0,054, für AB 0,377 und für BB 0,569 sowie für *PIT1*^A 0,243 und 0,757 für *PIT1*^B. Zwischen dem Polymorphismus von *PIT1-HinfI*-Gen und den Milchleistungsmerkmalen bei untersuchten Kühen wurden keine Zusammenhänge festgestellt.

Schlüsselwörter: Hypophysärer Transkriptionsfaktor 1, PCR-RFLP, Milchrind, Milchleistungsmerkmale

Introduction

Pit-1 (official nomenclature – POU1F1) is a member of the POU-family of transcription factors that regulate mammalian development. Pit-1, an approx. 33-kilodalton pituitary-specific protein, contains two domains, termed POU-specific and POU-homeo, which are both necessary for high-affinity DNA binding to promoters of the *GH* and *PRL* genes (HERR et al., 1988; ROSENFELD, 1991). Pit-1 activates *GH* and *PRL* gene expression, in part, through an N-terminal transactivation domain rich in hydroxylated amino acid residues (THEILL et al., 1989). During development, *PIT1* gene expression precedes *GH* and *PRL* gene expression in somatotrophic and lactotrophic cells, respectively, and is the major cell-specific activator of hormone expression from these cell types (SUPOWIT et al., 1992). The inhibition of Pit-1 synthesis markedly decreased both GH and PRL expression and proliferation of somatotrophic and lactotrophic cell lines (CASTRILLO et al., 1991). SCULLY et al., (2000) showed that whereas Pit-1 activates *GH* gene expression in one cell type, the somatotrope, it restricts its expression from another cell type, the lactotrope.

The *PIT1* gene is controlled by several factors that interact with its 5' regulatory region, although autoregulation of the *PIT1* gene itself also occurs as there are two Pit-1 binding sites in the 5' flanking region (CASTRILLO et al., 1991). RHODES et al. (1993) explored the molecular mechanism responsible for activation of the *PIT1* gene

in vivo. They demonstrated that an enhancer element, located more than 10 kb upstream of the transcriptional start site, was essential for pituitary-specific expression of the *PIT1* gene in transgenic mice. RAJAS et al. (1998) characterized 12 kb of genomic DNA upstream of the *PIT1* promoter. They identified a distal region that decreased the basal transcriptional activity of the *PIT1* minimal promoter, indicating that this region behaves as a silencer. This distal regulatory region contains 3 Pit-1 autoregulatory elements.

Bovine *PIT1* cDNA has been sequenced by BODNER et al., (1988). *PIT1* was sublocalized to the centromeric region of bovine chromosome 1, located midway between *TGLA57* and *RM95*. In the bovine *PIT1* gene, the restriction fragment length polymorphism (for the *HinfI* restriction enzyme) was identified (MOODY et al., 1995). Molecular basis of this polymorphism was the silent mutation (G→A) located within exon 6 of the *PIT1* gene (DIERKES et al., 1998).

RENAVILLE et al. (1997) showed that the A allele (for the *PIT1-HinfI* polymorphism) was found to be superior for milk and protein yields and inferior for fat percentage in dairy cattle. ZWIERZCHOWSKI et al. (2002) showed that the allele A of the *Pit1* locus positively affected milk production traits. In beef cattle, ZHAO et al. (2000) reported that *PIT1-HinfI* polymorphism appears to affect growth traits in Angus cattle and may be a candidate gene for use in marker assisted selection (MAS). ZWIERZCHOWSKI et al. (2001) and DYBUS et al. (2003) found no associations between *PIT1-HinfI* and growth performance and carcass traits of beef cattle.

The aim of this study was to estimate the allelic frequencies at the *PIT1-HinfI* locus of the bovine *PIT1* gene and to investigate the relationship of this polymorphism and milk production traits Black-and-White cows.

Materials and Methods

A total of 900 Black-and-White cows, with diverse proportion of HF genes, were genotyped. The cows were kept in five herds in the West Pomerania region of Poland (Table 1).

Table 1
Characteristics of the investigated population of dairy cows (Charakteristik der untersuchten Milchkuhpopulationen)

Herd	n	Number of cows in relation to their HF genes share		Average milk yield in the first 305-day lactation (kg)
		0-50%	50.1-100%	
1 st	116	8 (6.90%)	108 (93.10%)	6233
2 nd	209	40 (19.14%)	169 (80.86%)	4668
3 rd	126	4 (3.17%)	122 (96.83%)	7797
4 th	140	50 (35.71%)	90 (64.29%)	5382
5 th	309	72 (23.30%)	237 (76.70%)	4782
Total	900	174 (19.33)	726 (80.67)	5228

The *PIT1-HinfI* genotypes were analysed using the PCR-RFLP method (SAIKI et al., 1985). The crude DNA was isolated from blood samples using *MasterPure*TM kit

(Epicentre Technologies). The yields were approximately 70-80 µg of DNA/ml of blood. A 451-base pair (bp) fragment of the *PIT1* gene was amplified using forward 5'-AAACCATCATCTCCCTTCTT-3' and reverse 5'-AATGTACAATGTGCCTTCTGAG-3' primers (WOOLLARD et al., 1994). The PCR reaction contained approximately 100 ng of genomic DNA, 15 pmol of each primer, 2 µl 10 x PCR buffer (MBI Fermentas), 1.5 mM MgCl₂, 200 µM dNTP and 0.5 units *Taq*-polymerase in a total volume of 20 µl. The following cycles were applied: denaturation at 94.5°C/5 min, followed by 30 cycles at 94°C/40 sec, primer annealing at 56 °C/40 sec, PCR products synthesis at 72 °C/40 sec, and final synthesis at 72 °C/4 min using a DNA thermal cycler (Perkin Elmer Cetus Corp.). Amplified DNA was digested with *HinfI* (G↓ANTC) enzyme (MBI Fermentas). The digestion products were separated by horizontal electrophoresis (90 volts, 50 minutes) through 2% agarose gels (Gibco BRL) in 1 x TBE and 1.0 µM ethidium bromide.

The data for 305-day milk production in the first, second and third lactation, including production of milk, milk fat and milk protein and proportions of milk fat, milk protein and sum of milk fat and protein, were obtained from the farm documentation. Statistical calculations were performed using procedures of SAS[®]. Differences in the frequencies of *PIT1* genotypes (*AA*, *AB* and *BB*) in analysed herds of cows were tested with the chi-square test of independence. The effect of *PIT1* genotypes on the milk production traits of the cows were analysed using General Linear Model (GLM) procedure. The model used was as follows:

$$Y_{ijklmno} = \mu + G_i + S_j + HF_k + YS_l + H_m + b_1 (x_1 - DD)_n + E_{ijklmno}$$

$Y_{ijklmno}$ – 305-day milk production record at 1st, 2nd and 3rd lactation of cow *o*, μ – the overall mean, G_i – the fixed effect of *PIT1* genotype ($i = 1, \dots, 3$), S_j – the fixed effect of sire, HF_k – percentage of HF genes (fixed effect), YS_l – the fixed effect of year-season of calving class, H_m – the fixed effect of the herd, DD_n – days of milk, b_1 – the linear regression coefficient of days in milk, x_1 – days of milk of cow *o*, $E_{ijklmno}$ – the random error.

Results

The following DNA restriction fragments were obtained for the *PIT1-HinfI* polymorphism: 244 and 207 bp for the *BB* genotype, 451, 244 and 207 for the *AB* and 451 bp (no digestion) for the *AA* (Fig.).

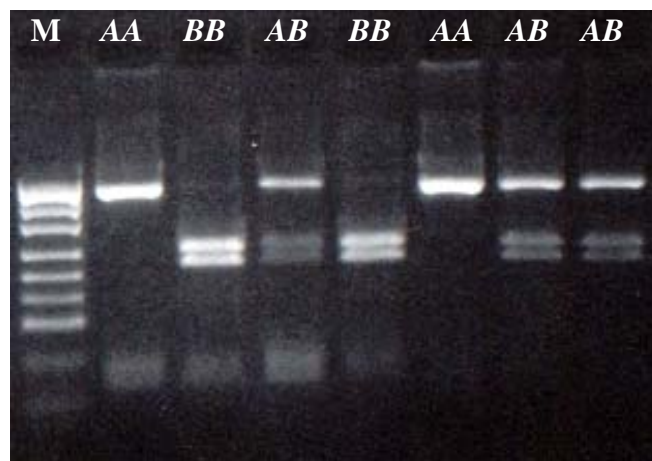


Fig.: Representative results of *PIT1-HinfI* analysis detected by agarose gel electrophoresis (Elektrophoretische Trennung von Restriktionsfragmenten *PIT1-HinfI*)
M – DNA marker (pUC19/*MspI*).

Table 2

Frequencies of genotypes and alleles of the *PIT1-HinfI* (Frequenz der Genotypen und Allele von *PIT1-HinfI*)

Herd	n	Genotypes			Alleles	
		AA	AB	BB	<i>PIT1</i> ^A	<i>PIT1</i> ^B
1 st	116	0.069 (n=8)	0.328 (n=38)	0.603 (n=70)	0.233	0.767
2 nd	209	0.043 (n=9)	0.411 (n=86)	0.546 (n=114)	0.248	0.752
3 rd	126	0.048 (n=6)	0.373 (n=47)	0.579 (n=73)	0.234	0.766
4 th	140	0.048 (n=7)	0.421 (n=59)	0.531 (n=74)	0.261	0.739
5 th	309	0.055 (n=17)	0.369 (n=114)	0.576 (n=178)	0.239	0.761
Total	900	0.052 (n=47)	0.382 (n=344)	0.566 (n=509)	0.243	0.757

The *BB* genotype was the most frequent in all the studied herds (0.531-0.603), followed by the heterozygotic *AB* (0.328-0.421), whereas the *AA* was the least frequent (0.043-0.069). The frequency of the *PIT1*^A ranged from 0.233 to 0.261 (Table 2).

Table 3

Significance of influence of factors covered by statistical model on examined traits (F values). (Varianzanalyse (F-Werte) für verschiedene Effekte die ausgewertete Leistungsmerkmale beeinflussen)

Character	<i>PIT1</i> genotype	Sire	HF genes	Year /Season	Herd	Days in milk
DF (1 st /2 nd /3 rd lactation)	2/2/2	178/151/104	50/44/40	18/18/16	4/4/4	1/1/1
Milk yield (kg):						
1 st lactation	0.27	6.24**	14.35**	5.00**	244.06**	151.49**
2 nd lactation	1.08	4.41**	8.06**	3.98**	84.18**	168.85**
3 rd lactation	0.13	4.08**	8.00**	8.21**	86.10**	135.14**
Fat yield (kg)						
1 st lactation	1.39	6.16**	13.23**	5.30**	247.48**	112.71**
2 nd lactation	2.04	5.08**	7.56**	4.96**	73.03**	122.95**
3 rd lactation	0.59	5.30**	6.39**	8.76**	71.05**	67.36**
Fat content (%)						
1 st lactation	0.79	1.97**	1.88**	4.27**	10.98**	0.44
2 nd lactation	0.94	2.70**	1.78**	2.93**	10.15**	1.30
3 rd lactation	0.49	2.94**	1.67*	2.57*	11.45**	1.23
Protein yield (kg)						
1 st lactation	0.69	7.45**	15.24**	3.98**	316.97**	183.34**
2 nd lactation	2.13	5.70**	7.85**	4.68**	118.62**	186.52**
3 rd lactation	0.13	5.28**	7.72**	11.05**	100.76**	144.95**
Protein content (%)						
1 st lactation	0.20	2.36**	1.84**	1.64	19.60**	12.36**
2 nd lactation	1.73	2.35**	1.67**	5.41**	18.79**	5.41*
3 rd lactation	0.18	1.46*	1.14	2.45*	3.36*	4.67*
Fat/Protein content (%)						
1 st lactation	0.74	2.04**	1.88**	2.62*	9.52**	0.45
2 nd lactation	1.55	2.32**	1.60*	3.88**	11.46**	2.99
3 rd lactation	0.50	2.38**	1.56*	3.13**	8.15**	0.02

* - significance of differences at $P \leq 0,05$; ** significance of differences at $P \leq 0,01$.

The genetic equilibrium in the studied population was not disturbed. The size of certain genotypes of the *PIT1* was not statistically different from the theoretical one. No differences in genotype and allele frequencies were found between herds (*AA* - $\chi^2_{4;0,05} = 1,07$, *AB* - $\chi^2_{4;0,05} = 2,11$, *BB* - $\chi^2_{4;0,05} = 0,89$).

Table 4

Mean and standard deviation of milk production traits in cows carrying different *PIT1-HinfI* genotypes. (Mittelwerte und Standardabweichungen der Milchleistungsmerkmale bei Kühen mit verschiedenen Genotypen *PIT1-HinfI*)

Lactation	Genotype	n	Milk yield (kg)	Fat		Protein		F&P (%)
				kg	%	kg	%	
I	AA	47	5190 (1286.0)	214.4 (59.2)	4.134 (0.507)	163.4 (44.4)	3.156 (0.227)	7.290 (0.595)
	AB	344	5212 (1426.6)	214.4 (63.9)	4.118 (0.431)	164.5 (47.5)	3.150 (0.194)	7.268 (0.548)
	BB	509	5242 (1379.1)	217.9 (61.8)	4.152 (0.438)	166.0 (46.7)	3.157 (0.193)	7.309 (0.542)
	Total	900	5228	216.4	4.138	165.3	3.154	7.292
II	AA	31	5755 (1432.9)	240.7 (70.0)	4.178 (0.627)	187.2 (49.3)	3.247 (0.227)	7.425 (0.735)
	AB	242	5673 (1459.0)	236.3 (67.6)	4.142 (0.516)	183.3 (50.9)	3.206 (0.202)	7.348 (0.611)
	BB	327	5643 (1348.0)	235.4 (66.7)	4.156 (0.531)	182.5 (44.6)	3.222 (0.233)	7.378 (0.631)
	Total	600	5661	236.0	4.151	183.1	3.217	7.368
III	AA	17	6100 (1458.2)	259.6 (80.7)	4.225 (0.720)	194.8 (54.1)	3.178 (0.216)	7.403 (0.761)
	AB	165	5974 (1681.7)	247.0 (83.6)	4.094 (0.511)	191.1 (56.2)	3.177 (0.183)	7.271 (0.599)
	BB	184	6018 (1357.3)	248.2 (67.0)	4.103 (0.555)	192.7 (46.0)	3.196 (0.206)	7.299 (0.655)
	Total	366	6002	248.2	4.104	192.1	3.187	7.291

F&P – fat and protein

Table 4 shows the influence of the *PIT1-HinfI* polymorphism on milk production traits in the B&W cows.

Discussion

Frequencies of *PIT1-HinfI* alleles obtained in this study were similar to the frequencies obtained earlier for Black-and-White cattle. Higher frequency of the *PIT1^A* (0.32) was observed in study carried out by DIERKES et al. (1998). Somewhat higher frequency of the *PIT1^A* (0.26) was observed in the studies of MOODY et al. (1995) and KLAUZIŃSKA et al. (1999); 0.25 in the studies carried out by ZWIERZCHOWSKI et al. (2002) and OPRZADEK et al. (2003). Slightly lower frequency of the *PIT1^A* (0.18, 0.15 and 0.15) was observed by RENAVILLE et al. (1997), WOOLLARD et al. (1994) and HORI-OSHIMA and BARRERAS-SERRANO (2002), respectively.

Pit-1 transcription factor is a component of the GH cascade, also called “somatotrophic axis”. It has been described as the critical cell-specific transcription factor responsible for activating expression of the prolactin (*PRL*) and growth hormone (*GH*) genes in the anterior pituitary gland. Because the *PRL* and the *GH* are essential for mammary

gland development and milk yield, the *Pit-1* gene has a potential to explain genetic variations in dairy traits (ZWIERZCHOWSKI et al., 2002).

RENAVILLE et al. (1997) showed that the allele *A* of the *PIT1* gene was found to be superior for milk and protein yields and inferior for fat percentage. A canonical transformation revealed that *Pit-1* had three actions, one linked to milk yield traits and angularity, a second linked to body depth and rear leg set, and a third linked to lower fat yields and to higher angularity. These authors also showed that RFLP in *PIT1* gene is a promising new possibility to select for increased protein yield and, to a lesser extent, milk yield through selection for the allele *A*. PARMENTIER et al. (1999) demonstrated significant superiority of the allele *A* for milk and protein yield, but an inferiority for fat yield. HORI-OSHIMA and BARRERAS-SERRANO (2002) found that the animals with the *AA* genotype for *Pit-1/HinfI* polymorphism had higher milk yield. Similar results were published by ZWIERZCHOWSKI et al. (2003) who showed that both genotypes with allele *A* at the *PIT1* locus positively affected all milk production traits studied. The *AB* genotype was superior for the milk yield and for daily yield of all milk components, while genotype *AA* was shown to positively affect their concentrations. Recently, DE MATTOS et al. (2004) found that the heterozygous *HinfI* (*AB*) sires were superior in relation to the *HinfI* *BB* sires for milk fat production ($P < 0.05$).

In our study, no associations between RFLP in *PIT1* gene and milk production traits were found for B&W cattle. Bearing in mind the above mentioned results, it is difficult to indicate which allele of *PIT1-HinfI* polymorphism should be favoured in the improvement of production traits of Black-and-White cattle.

References

- BODNER, M.; CASTRILLO, J.L.; THEILL, L.E.; DEERINCK, T.; ELLISMAN, M.; KARIN, M.:
The pituitary-specific transcription factor GHF-1 is a homeobox-containing protein. *Cell*, **55** (1988), 505-518
- CASTRILLO, J.L.; THEILL, L.E.; KARIN, M.:
Function of the homeodomain protein GHF1 in pituitary cell proliferation. *Science*, **253** (1991), 197-199
- DE MATTOS, K. K. ; DEL LAMA, S.N.; MARTINEZ, M.L.; FREITAS, A.F.:
Association of bGH and *Pit-1* gene variants with milk production traits in dairy Gyr bulls. *Brazilian Journal of Agricultural Research*, **39** (2004), 147-150
- DIERKES, B.; KRIEGESMANN, B.; BAUMGARTNER, B.G.; BRENING, B.:
Partial genomic structure of the bovine *PIT1* gene and characterization of a *HinfI* transition polymorphism in exon 6. *Animal Genetics*, **29** (1998), 405
- DYBUS, A.; KMIEĆ, M.; SOBEK, Z.; PIETRZYK, W.; WIŚNIEWSKI, B.:
Associations between polymorphism of growth hormone releasing hormone (*GHRH*) and pituitary transcription factor 1 (*PIT1*) genes and production traits of Limousine cattle. *Arch. Tierz., Dummerstorf* **46** (2003), 527-534
- HERR, W.; STURM, R.A.; CLERC, R.G.; CORCORAN, L.M.; BALTIMORE, D.; SHARP, P.A.; INGRAHAM H.A.; ROSENFELD, M.G.; FINNEY, M.; RUVKUN, G.:
The POU domain: a large conserved region in the mammalian *pit-1*, *oct-1*, *oct-2*, and *Caenorhabditis elegans unc-86* gene products. *Genes and Development*, **2** (1988), 1513-1516
- HORI-OSHIMA, S.; BARRERAS-SERRANO, A.:
Relationships between DGAT1 and *Pit-1* genes polymorphism and milk yield in Holstein cattle. *Journal of Animal Science*, **81**, Suppl. 1 (2002), 252
- KLAUZIŃSKA, M.; ZWIERZCHOWSKI, L.; SIADKOWSKA, E.; SZYMANOWSKA, M.; GROCHOWSKA, R.; ŻURKOWSKI, M.:
Comparison of selected gene polymorphisms in Polish Red and Polish Black-and-White cattle. *Animal Science Papers and Reports*, **18** (2000), 107-116
- MOODY, D.E.; POMP, D.; BARENDSE, W.:

- Restriction fragment length polymorphism in amplification products of the bovine *PIT1* gene and assignment of *PIT1* to bovine chromosome 1. *Animal Genetics*, **26** (1995), 45-47
- OPRZADEK, J.; FLISIKOWSKI, K.; ZWIERZCHOWSKI, L.; DYMNICKI, E.:
Polymorphism at *loci* of leptin (*LEP*), *Pit1* and *STAT5A* and their association with growth, feed conversion and carcass quality in Black-and-White bulls. *Animal Science Papers and Reports*, **21** (2003), 135-145
- PARMENTIER, I.; PORTETELLE, D.; GENGLER, N.; PRADI, A.; BERTOZZI, C.; VLEURICK, L.; GILSON, R.; RENAUVILLE, R.:
Candidate gene markers associated with somatotropic axis and milk selection. *Domestic Animal Endocrinology*, **17** (1999), 139-148
- RAJAS, F.; DELHASE, M.; DE LA HOYA, M.; VERDOOD, P.; CASTRILLO, J.L.; HOOGHE-PETERS, E.L.:
Nuclear factor 1 regulates the distal silencer of the human *PIT1/GHF1* gene. *Biochemical Journal*, **333** (1998), 77-84
- RENAVILLE, R.; GENGLER, N.; VRECH, A.; PRANDI, A.; MASSART, S.; CORRADINI, C.; BERTOZZI, C.; MORTIAUX, F.; BURNY, A.; PORTETELLE, D.:
Pit-1 gene polymorphism, milk yield, and conformation traits for Italian Holstein-Friesian bulls. *Journal of Dairy Science*, **80** (1997), 3431-3438
- RHODES, S.J.; CHEN, R.; DIMATTIA, G.E.; SCULLY, K.M.; KALLA, K.A.:
A tissue-specific enhancer confers Pit-1 dependent morphogen inducibility and autoregulation on the Pit-1 gene. *Genes and Development*, **7** (1993), 913-932
- ROSENFELD, M.G.:
POU-domain transcription factors: powerful developmental regulators. *Genes and Development*, **5** (1991), 897-907
- SAIKI, R.K.; SCHARF, S.; FALOONU, F.; MULLIS, K.; HORN, G.T.; ERLICH, H.A.; ARNHEIM, N.:
Enzymatic amplification of β -globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science*, **230** (1985), 1350-1354
- SAS. 2001. SAS Inst. Inc., Carry, NC.
- SCULLY, K.M.; JACOBSON, E.M.; JEPSEN, K.; LUNYAK, V.; VIADIU, H.; CARRIERE, C.; ROSE, D.W.; HOOSHMAND, F.; AGGARWAL, A.K.; ROSENFELD, M.G.:
Allosteric effects of Pit-1 DNA sites on long-term repression in cell type specification. *Science*, **290** (2000), 1127-1131
- SUPOWIT, S.C.; RAMSEY, T.; THOMPSON, E.B.:
Extinction of prolactin gene expression in somatic cell hybrids is correlated with the repression of the pituitary-specific trans-activator GHF-1/Pit-1. *Molecular Endocrinology*, **6** (1992), 786-792
- THEILL, L.E.; CASTRILLO, J.-L.; WU, D.; KARIN, M.:
Dissection of functional domains of the pituitary-specific transcription factor GHF-1. *Nature*, **342** (1989), 945-948
- WOOLLARD, J.; SCHMITZ, C.B.; FREEMAN, A.E.; TUGGLE, C.K.:
Rapid communication: *HinfI* polymorphism at the bovine *Pit-1* locus. *Journal of Animal Science*, **72** (1994), 3267
- ZHAO, Q.; DAVIS, M.E.; HINES, H.C.:
Association of two *Pit-1* gene polymorphisms with growth rate in beef cattle. *Journal of Animal Science* **78** (2000) Suppl.1, 77
- ZWIERZCHOWSKI, L.; KRZYŻEWSKI, J.; STRZAŁKOWSKA, N.; SIADKOWSKA, E.; RYNIEWICZ, Z.:
Effects of polymorphism of growth hormone (GH), Pit-1, and leptin (LEP) genes, cow's age, lactation stage and somatic cell count on milk yield and composition of Polish Black-and-White cows. *Animal Science Papers and Reports* **20** (2002), 213-227
- ZWIERZCHOWSKI, L.; OPRZADEK, J.; DYMNICKI, E.; DZIERZBICKI, P.:
An association of growth hormone, α -casein, β -lactoglobulin, leptin and *Pit-1 loci* polymorphism with growth rate and carcass traits in beef cattle. *Animal Science Papers and Reports* **19** (2001), 65-77

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