Arch. Tierz., Dummerstorf **46** (2003) 6, 527-534

[1] Department of Genetics and Animal Breeding, Agriculture University of Szczecin, Poland
[2] Department of Genetics and Animal Breeding, Agriculture University of Poznań, Poland
[3] AGRO-Sokołów 2, OHZ Chotyniec

ANDRZEJ DYBUS[1*], MAREK KMIEĆ[1], ZBIGNIEW SOBEK[2], WITOLD PIETRZYK[1] and BOGDAN WIŚNIEWSKI[3]

Associations between polymorphisms of growth hormone releasing hormone (*GHRH*) and pituitary transcription factor 1 (*PIT1*) genes and production traits of Limousine cattle

Abstract

Associations between polymorphism of the bovine growth hormone releasing hormone (*GHRH*) and pituitary transcription factor 1 (*PIT1*) genes and production traits of Limousine cattle were analysed. A total of 130 calves were included in the study. PCR-RFLP method was used for genotyping. The frequencies of genotypes and alleles of *PIT1* and *GHRH* were as follows: 0.0692 – *AA*, 0.4077 – *AB*, 0.5231 – *BB*, and 0.2731 for $PIT1^A$, 0.7269 for $PIT1^B$; 0.0154 – *AA*, 0.1692 – *AB*, 0.8154 – *BB*, and 0.1 for $GHRH^A$, 0.9 for $GHRH^B$. Associations between polymorphism and production traits of Limousine calves were found. Statistically significant differences ($P \leq 0.01$) between individuals of different *GHRH* genotypes were found in relation to height at sacrum (cm) and height at withers (cm) at 210th day of age. The calves with *AA* genotype of *GHRH* were shorter (-8,14 and -8,33 cm) than *AB* and *BB* individuals ($P \leq 0.01$). The small number of calves with the *AA* genotype did not enable important conclusions.

Key Words: growth hormone releasing hormone, pituitary transcription factor 1, PCR-RFLP, Limousine cattle, production traits

Zusammenfassung

Titel der Arbeit: **Zusammenhänge zwischen Polymorphismus von Somatoliberin-Genen (*GHRH*) und hypophysärem Transkriptionsfaktor 1 (*PIT1*) sowie den Leistungsmerkmalen bei Limousine-Rindern**

Zusammenhänge zwischen dem Polymorphismus von Somatoliberin-Genen (*GHRH*) sowie dem hypophysären Transkriptionsfaktor 1 (*PIT1*) und den Leistungsmerkmalen von Limousine-Rindern wurden ausgewertet. Untersucht wurden 130 Kälber. Zur Genotypbestimmung wurde die PCR-RFLP-Methode angewandt. Folgende Genotyp- und Allel-Frequenzen wurden ermittelt: *PIT1 und GHRH:* 0,0692 – *AA*, 0,4077 – *AB*, 0,5231 – *BB*, 0,2731 und für Allel $PIT1^A$, 0,7269 für $PIT1^B$; 0,0154 – *AA*, 0,1692 – *AB*, 0,8154 – *BB*, und 0.1 für das Allel $GHRH^A$, 0.9 f+r $GHRH^B$. Zwischen dem untersuchten Polymorphismus und den Leistungsmerkmalen der Limousine-Kälber wurden Zusammenhänge festgestellt. Signifikante Unterschiede (bei $p \leq 0,01$) zwischen den Tieren mit verschiedenen *GHRH*-Genotypen wurden bei Kreuzbeinhöhe (cm) sowie Widerristhöhe (cm) am 210. Lebenstag festgestellt. Die Kälber mit dem Somatoliberin-Genotyp AA (*GHRH*) waren kleiner (entsprechend um -8,14 und -8,33 cm) als die Tiere mit den Genotypen *AB* und *BB*. Die geringe Zahl der Kälber mit dem Genotyp *AA* gestattet nicht, weitergehende Schlüsse zu ziehen..

Schlüsselwörter: Wachstum-Releasing-Hormon, hypophysärer Transkriptionsfaktor 1, PCR-RFLP, Limousine, Leistungsmerkmale

Introduction

Growth hormone (GH)-releasing factor (GRF, GHRH) is the hypothalamic peptide that specifically stimulates both synthesis and secretion of pituitary GH. After reaching the pituitary, GRF binds to its specific receptors on somatotrophs, and generates bursts of GH secretion episodically (FROHMAN et al., 1992). GUILLEMIN et al. (1982) have isolated a 44-amino acid peptide with GH-releasing activity from a human

pancreatic tumor that had caused acromegaly. After the isolation of hGRF, the GRFs from other species were described. The sequence of bovine GRF (1-44-NH_2) differs from human GRF by only five residues (ESCH et al., 1983).

MAYO et al. (1985) isolated and characterised the entire structure of the human gene encoding GHRH. The gene consists of five exons separated by interval introns and spanning 10 kb. In cattle, MOODY et al. (1995) identified a restriction fragment length polymorphism (RFLP) within PCR amplification product of the bovine *GHRH* gene. The bovine *GHRH* gene was sequenced and found to be 91 and 77% homologous to portions of exon 3 of the human and murine *GHRH* cDNA sequences, respectively. Linkage analysis determined that *GHRH* was linked to CSSM30 on bovine chromosome 13 (BARENDSE et al., 1994).

Pit-1 (official nomenclature – POU1F1) is a member of the family (POU) transcription factors that regulate mammalian development. Pit-1, an approx. 33-kilodalton pituitary-specific protein, contains two protein domains, termed POU-specific and POU-homeo, which are both necessary for high-affinity DNA binding on 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 the somatotroph and lactotroph, respectively, and is the major cell-specific activator of hormone expression from these cell types (NELSON et al., 1988; FOX et al., 1990). 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.

OHTA et al. (1992) found that the human *PIT1* gene spanned more than 14 kb and was divided into six exons ranging from 61 bp (exon 5) to 225 bp (exon 3); the five introns ranged in size from 0.7 kb (intron 4) to more than 7.5 kb (intron 2). The *PIT1* gene is controlled by several factors that interacts 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 *Hinf*I restriction enzyme) was identified (MOODY et al., 1995). Molecular basis of this polymorphism was the point mutation (G→A) located within exon 6 of the *PIT1* gene (DIERKES et al., 1998). Additionally, ZHAO et al. (2000) detected an SSCP polymorphism in intron 5 of this gene.

RENAVILLE et al. (1997) showed that *A* allele (for the *PIT1-Hinf*I polymorphism) was found to be superior for milk and protein yields and inferior for fat percentage in dairy cattle. In beef cattle, ZHAO et al. (2000) reported that *PIT1-Hinf*I polymorphism

appears to affect growth traits in Angus cattle and may be a candidate gene for use in MAS. Recently, ZWIERZCHOWSKI et al. (2001) showed no associations between *PIT1-Hinf*I and growth performance and carcass traits of beef cattle.
The aim of this study was to estimate the allelic frequencies at the bovine *GHRH-Hae*III and *PIT1-Hinf*I loci and to investigate the relationship of those polymorphisms and production traits of Limousine calves.

Materials and Methods

A total of 130 limousine calves were genotyped. The calves were born between 1998-2001, and were offspring of 4 bulls and 80 cows. Crude DNA was isolated from blood samples using MasterPure^{TM} kit (Epicentre Technologies). The PCR-RFLP method was used for the polymorphism located in the *GHRH* gene. The following primer sequences were designed on the basis of the nucleotide sequence of the *GHRH* gene (GenBank U29611) and Primer3 software (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3.cgi/) namely:

GHRHF – 5'-TTCCCAAGCCTCTCAGGTAA-3'
GHRHR – 5'-GCGTACCGTGGAATCCTAGT-3'

A 297-base pair (bp) fragment of the *GHRH* gene was amplified. The PCR reaction contained 100 ng of genomic DNA, 15 pmol of each primer, 2 µl 10 x PCR buffer (MBI Fermentas), 2.0 mM $MgCl_2$, 200 µM dNTP and 0.5 units *Taq*-polymerase in a total volume of 20 µl. The following cycles were applied: denaturation - 94 °C/5 min, followed by 30 cycles - 94 °C/40 sec, primer anneling - 60 °C/40 sec, PCR products synthesis - 72 °C/40 sec, and final synthesis - 72 °C/4 min using a DNA thermal cycler (Perkin Elmer Cetus Corp.). Amplified DNA was digested by mixing 15 µl of PCR product with 5 units of *Hae*III (GG↓CC) enzyme (MBI Fermentas). The digestion products were separated by horizontal electrophoresis (90 volts, 50 minutes) through 3% agarose gels (Gibco BRL) in 1 x TBE and 1.0 µM ethidium bromide.
A 451-base pair (bp) fragment of the *PIT1* gene was amplified using forward 5'-AAACCATCATCTCCCTTCTT-3' and reverse 5'-AATGTACAATGTGCCTTCT GAG-3' primers (WOOLLARD et al., 1994). The PCR reaction contained 100 ng of genomic DNA, 15 pmol of each primer, 2 µl 10 x PCR buffer (MBI Fermentas), 1.5 mM $MgCl_2$, 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 anneling 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 *Hinf*I (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.
Data for production traits of calves, including body weight in 3, 210 and 365 day of life, height at sacrum, height at withers, chest girth in 3, 210 and 365 day of life and average daily gain for 3-210 and 3-365 day of life were obtained from the farm documentation. Statistical calculations were performed using procedures of SAS®. Distribution frequencies of the two alleles were compared by Chi-square test. The

effect of studied genotypes on the production traits of calves were analysed using GLM procedure. The used model was as follows:

$$Y_{ijklm} = \mu + G_i + S_j + YS_k + P_l + E_{ijklm}$$

where:

Y_{ijklm} – analysed trait; μ – the overall mean, G_i – the fixed effect of *GHRH* and *PIT1* genotypes ($i = 1,...3$), S_j – the fixed effect of sire, YS_k – the fixed effect of year-season, P_l – effect of the sex, E_{ijklm} – the random error.

Results

The following DNA restriction fragments were obtained for the *GHRH-Hae*III polymorphism: 242 and 55 bp for the *AA* genotype, 242, 194, 55, 48 bp for the *AB* genotype, and 194, 55, 48 bp for the *BB* (Fig. 1).

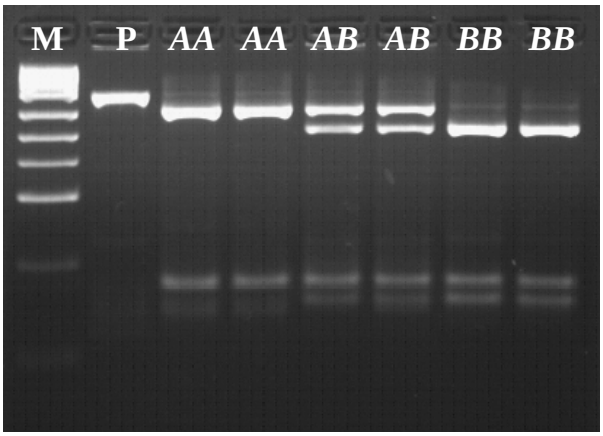

Fig. 1: Representative results of *GHRH-Hae*III analysis detected by agarose gel electrophoresis M – DNA marker pUC19/*Msp*I, P – PCR product (Elektrophoresebild des Polymorphismus *GHRH-Hae*III M – Massenmarker DNA (pUC19/*Msp*I), P –PCR-Produkt)

In case of *PIT1-Hinf*I polymorphism the following DNA restriction fragments were obtained: 244 and 207 bp for the *BB* genotype, 451, 244 and 207 for the *AB* and 451 bp (no digestion) for the *AA* – Fig. 2.

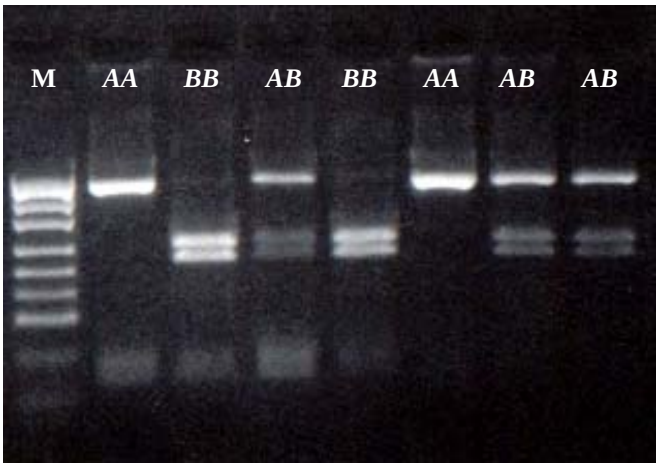

Fig. 2: Representative results of *PIT1-Hinf*I analysis detected by agarose gel electrophoresis M – DNA marker (pUC19/*Msp*I) (Elektrophoresebild des Polymorphismus *PIT1-Hinf*I M – Massenmarker DNA (pUC19/*Msp*I))

Table 1 shows frequencies of genotypes and alleles of the *GHRH-Hae*III and *PIT1-Hinf*I obtained in this study.

Table 1
Frequency of genotypes and alleles of the *GHRH* and *PIT1* genes in Limousine cattle (Frequenz von Genotypen und Allelen von *GHRH* und *PIT1* beim Limousine-Rind)

Polymorphism	Genotypes			Alleles	
	AA	*AB*	*BB*	$GHRH^A$	$GHRH^B$
*GHRH-Hae*III	0.0154 (n=2)	0.1692 (n=22)	0.8154 (n=106)	0.1	0.9
	AA	*AB*	*BB*	$PIT1^A$	$PIT1^B$
*PIT1-Hinf*I	0.0692 (n=9)	0.4077 (n=53)	0.5231 (n=68)	0.27	0.73

The analysis of relationships between the polymorphism at the *GHRH* and *PIT1* genes and production traits of Limousine calves found that the level of the analysed traits was significantly influenced by the sire, year/season, sex and genotype ($P < 0.01$ and $P < 0.05$) — Table 2. All those factors were included in the statistical model which was used to analyse the relationships between the *GHRH* and *PIT1* genes polymorphism and production traits.

Table 2
Significance of influence of factors covered by statistical model on examined traits (F values) (Variationsanalyse (F-Werte) für verschiedene Effekte, die die ausgewerteten Leistungsmerkmale beeinflussen)

Character	*PIT1/GHRH* genotype		Sire	Year/ Season	Sex
DF	2	2	3	18	1
Body weight (kg) at 3 day of age	0.80	1.34	4.94**	2.05*	3.02
Height at sacrum (cm) at 3 day of age	0.41	0.77	0.77	1.37	5.16*
Height at withers (cm) at 3 day of age	1.66	0.62	20.33**	1.98*	1.45
Chest girth (cm) at 3 day of age	1.89	1.24	2.35	1.88*	4.26*
Body weight (kg) at 210 day of age	1.22	1.26	2.12	1.95*	6.09*
Average daily gains (g) from 3rd to 210th day of age	0.63	1.60	3.03*	1.72*	3.36
Height at sacrum (cm) at 210 day of age	0.13	5.54**	1.00	1.32	4.93*
Height at withers (cm) at 210 day of age	0.11	5.29**	0.45	1.28	3.81
Chest girth (cm) at 210 day of age	0.69	0.08	1.44	1.95*	6.67*
Body weight (kg) at 365 day of age	1.36	0.17	1.56	2.71**	13.10**
Average daily gains (g) from 3rd to 365th day of age	0.23	0.90	2.50	1.55	9.56**
Height at sacrum (cm) at 365 day of age	1.20	1.57	2.63	2.28*	11.26**
Height at withers (cm) at 365 day of age	2.18	1.10	0.08	3.21**	6.86*
Chest girth (cm) at 365 day of age	1.46	0.08	0.92	2.51**	13.49**

* - significance of differences at $P \leq 0.05$; ** - significance of differences at $P \leq 0.01$.

Table 3 shows the influence of the *GHRH* and *PIT1* genes polymorphisms on production traits in Limousine cattle.

Table 3
Mean values of production traits in calves with different *GHRH* and *PIT1* genotypes (Mittelwerte von Leistungsmerkmalen bei den Kälbern mit verschiedenen *GHRH*- und *PIT1*- Genotypen)

Traits	*PIT1-Hinf*I			*GHRH-Hae*III		
	AA	*AB*	*BB*	*AA*	*AB*	*BB*
Height at sacrum (cm)						
at 3rd day of age	75.33	75.68	76.03	74.00	76.27	75.78
at 210th day of age	112.00	112.00	112.32	104.00^{AB}	112.14^{A}	112.33^{B}
at 365th day of age	115.67	117.68	118.64	116.00	116.30	118.53
Height at withers (cm)						
at 3rd day of age	75.00	74.83	75.62	74.00	74.82	75.34
at 210th day of age	109.11	108.66	108.93	101.00^{AB}	108.59^{A}	109.03^{B}
at 365th day of age	112.00	116.28	116.80	120.00	115.00	116.59
Chest girth (cm)						
at 3rd day of age	78.67	78.45	79.53	78.50	78.09	79.24
at 210th day of age	156.56	153.10	154.35	152.50	154.59	153.90
at 365th day of age	164.33	167.88	171.15	173.00	170.30	169.66
Body weight (kg)						
at 3rd day of age	35.44	36.85	37.01	35.50	37.91	36.64
at 210th day of age	236.33	244.17	248.00	223.00	249.00	245.35
at 365th day of age	351.33	371.40	381.28	387.00	382.10	375.80
Average daily gains (g)						
from 3rd to 210th day of age	976.67	989.08	1006.97	893.00	1020.27	994.84
from 3rd to 365th day of age	866.33	858.00	881.23	948.00	920.20	864.33

Values in lines with the same index differ significantly; capitals - $P \leq 0.01$, small letters - $P \leq 0.05$.

Discussion

Frequencies of *GHRH-Hae*III alleles obtained in this study were 0.1 ($GHRH^A$) and 0.9 ($GHRH^B$). Higher frequency of the $GHRH^A$ (0.70) in Angus breed, and lower (0.07) in Hereford was observed by MOODY et al. (1995). In case of *PIT1-Hinf*I polymorphism frequency of the $PIT1^A$ allele obtained in this study (0.27) were higher than observed in study carried out by ZWIERZCHOWSKI et al. (2001) for Limousine cattle – 0.22. In the other cattle breeds, the following frequencies of $PIT1^A$ allele were observed by ZWIERZCHOWSKI et al. (2001): 0.22 – Charolaise, 0.25 – Simmental, 0.27 – Hereford and 0.35 – Red Angus; MOODY et al., (1995): 0.45 – Angus, 0.26 – Holstein, 0.21 – Hereford, 0.18 – Gelbvieh, 0.10 – Brahman; KLAUZIŃSKA et al. (1999): 0.26 – Polish Black&White; RENAVILLE et al. (1997): 0.18 - Holstein.

RENAVILLE et al. (1997) showed that the *A* allele was found to be superior for milk and protein yields, inferior for fat percentage, and superior for body depth, angularity, and rear leg set, which is difficult to explain. 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. In beef cattle, ZHAO et al. (2000) reported that *PIT1-Hinf*I polymorphism appears to affect growth traits in Angus cattle and may be a candidate gene for use in MAS. Recently, ZWIERZCHOWSKI et al. (2001) showed no associations between *PIT1-Hinf*I and growth performance and carcass traits of beef cattle.

In humans, different mutations of the *PIT1* gene have been reported in patients with familial pituitary hypoplasia (PFÄFFLE et al., 1992) or with sporadic combined pituitary hormone deficiency (RADOVICK et al., 1992). Mutations in the *Pit1* gene are responsible for the dwarf phenotypes in mice (LI et al., 1990). In our study, no associations between *RFLP* in *PIT1* gene and production traits of Limousine calves

were found. A small number of animals with the *AA* genotype (n=8) makes it impossible to draw important conclusions.

ZIMMERMAN et al. (1993) described congenital gigantism due probably to central hypersecretion of GRF (GHRH). Normal at birth (4.4 kg, 53 cm), the male patient was 182 cm tall with a weight of 99.4 kg at the age of 7 years.

In this study, statistically significant differences ($P \leq 0.01$) between individuals of different *GHRH* genotypes were found only in reference to height at sacrum (cm) and height at withers (cm) at 210th day of age. The calves with *AA* genotype of *GHRH* were shorter (-8,14 and -8,33 cm) than *AB* and *BB* individuals ($P \leq 0.01$). The small number of calves (n=2) with the *AA* genotype did not allow drawing conclusions.

Bearing in mind the results obtained in this study, it should be stressed that the usefulness of *PIT1-Hinf*I and *GHRH-Hae*III polymorphisms for the improvement of production traits of Limousine cattle seems questionable. The obtained results, however, should be verified by further investigations on a greater number of animals.

References

BARENDSE, W.; ARMITAGE, S.M.; KOSSAREK, L.M.; SHALOM, A.; KIRKPATRICK, B.W.; RYAN, A.M.; CLAYTONI, D.; LI, L.; NEIBERGS, H.L.; ZHANG, N.; GROSSE, W.M.; WEISS, J.; CREIGHTON, P.; MCCARTHY, F.; RON, M.; TEALE, A.J.; FRIES, R.; MCGRAW, R.A.; MOORE, S.S.; GEORGES, M.; SOLLER, M.; WOMACK, J.E.; HETZEL, D.J.S.:
A genetic linkage map of the bovine genome. Nature Genetics, **6** (1994), 227-235

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.

DIERKES, B.; KRIEGESMANN, B.; BAUMGARTNER, B.G.; BRENING B.:
Partial genomic structure of the bovine *PIT1* gene and characterization of a *Hinf*I transition polymorphism in exon 6. Animal Genetics, **29** (1998), 405

ESCH, F.C.; BOHLEN, P.; LING, N.C.; BRAZEAU, P.; GUILLEMIN R.:
Isolation and characterization of the bovine hypothalamic growth hormone-releasing factor. Biochemical and Biophysical Research Communications, **117** (1983), 772-779

FOX, S.R.; JONG, M.T.; CASANOVA, J.; YE, Z.S.; STANLEY, F.; SAMUELS, H.H.:
The homeodomain protein, Pit-1/GHF-1, is capable of binding to and activating cell-specific elements of both the growth hormone and prolactin gene promoters. Molecular Endocrinology, **4** (1990), 1069-1080

FROHMAN, L.A.; BOWNS, T.R.; CHOMCZYNSKI P.:
Regulation of growth hormone secretion. Frontiers in Neuroendocrinology, **13** (1992), 344-405

GUILLEMIN, R.; BRAZEAU, P.; BOHLEN, P.; ESCH, F.; LING, N.; WEHRENBERG, W.B.:
Growth hormone releasing factor from a human pancreatic tumor that caused acromegaly. Science, **218** (1982), 585-587

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

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

LI, S.; CRENSHAW, E.B.; RAWSON, E.J.; SIMMONS, D.M.; SWANSON, L.W.; ROSENFELD, M.G.:
Dwarf locus mutants lacking three pituitary cell types results from mutations in the POU-domain gene *Pit-1*. Nature, **347** (1990), 528-533

MAYO, K.E.; CERELLI, G.M.; LEBO, R.V.; BRUCE, B.D.; ROSENFELD, M.G.; EVANS, R.M.:
Gene encoding human growth hormone-releasing factor precursor: structure, sequence, and chromosomal location. Proceedings of the National Academy of Sciences USA, **82** (1985), 63-67

MOODY, D.E.; POMP, D.; BARENDSE, W.:
Restriction fragment length polymorphism in amplification products of the bovine *growth hormone-releasing hormone* gene. Journal of Animal Science, **73** (1995), 3789

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

NELSON, C.; ALBERT, V.R.; ELSHOLTZ, H.P.; LU, L.I.W.; ROSENFELD, M.G.:
Activation of cell-specific expression of rat growth hormone and prolactin genes by a common transcription factor. Science, **239** (1988), 1400-1405

OHTA, K.; NOBUKUNI, Y.; MITSUBUCHI, H.; OHTA, T.; TOHMA, Y.; JINNO, Y.; ENDO, F.; MATSUDA, I.:
Characterization of the gene encoding human pituitary-specific transcription factor, PIT1. Gene, **122** (1992), 387-388

PFÄFFLE, R.W.; DIMATTIA, G.E.; PARKS, J.S.; BROWN, M.R.; WIT, J.M.; JANSEN, M.; VAN DER NAT, H.; VAN DEN BRANDE, J.L.; ROSENFELD, M.G.; INGRAHAM, H.A.:
Mutation of the POU-specific domain of Pit-1 and hypopituitarism without pituitary hypoplasia. Science, **21** (1992), 1118-1121

RADOVICK, S.; NATIONS, M.; DU, Y.; BERG, L.A.; WEINTRAUB, D.B.; WONDISFORD, F.E.:
A mutation in the POU-homeodomain of Pit-1 responsible for combined pituitary hormone deficiency. Science, **21** (1992), 1115-1118

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

STATISTICAL ANALYSIS SYSTEM INSTITUTE:
SAS/STAT user's guide, version 8.2, SAS Institute Inc., Carry, NC, USA. , 2001

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

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: *Hinf*I 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), 77

ZIMMERMAN, D.; YOUNG, W.F. JR.; EBERSOLD, M.J.; SCHEITHAUER, B.W.; KOVACS, K.; HORVATH, E.; WHITAKER, M.D.; EBERHARDT, N.L.; DOWNS, T.R.; FROHMAN, L.A.:
Congential gigantism due to growth hormone-releasing hormone excess and pituitary hyperplasia with adenomatous transformation. Journal of Clinical Endocrinology and Metabolism, **76** (1993), 216-222

ZWIERZCHOWSKI, L.; OPRZĄDEK, 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

Received: 2003-02-27 Accepted: 2003-10-22

Corresponding Author
ANDRZEJ DYBUS, PhD
Department of Genetics and Animal Breeding, Agriculture University of Szczecin,
Doktora Judyma 6, 71-460 Szczecin, POLAND E-Mail: A.Dybus@biot.ar.szczecin.pl