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Full Length Research Paper

Analysis of two Pit-1 gene polymorphisms: Single nucleotide polymorphisms (SNPs) distribution patterns in Podolica cattle breed

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Pit-1 is a pituitary-specific transcription factor responsible for pituitary development and hormone expression in mammals. Pit-1 is a member of the POU domain containing proteins, a group of transcriptional regulators with a critical role in cell differentiation and proliferation. It was shown that this group of proteins control the transcription of the growth hormone (GH), the prolactin (PRL), the thyroid-stimulating hormone *β*-subunit (TSH-*β*), the GHRH receptor genes and the Pit-1 gene itself. In this study, the Pit-1/*Hinf*I and Pit-1/*Taq*I *loci* were investigated using PCR-RFLP approach in a sample of 104 Podolica cattle. All the possible genotypes for both single nucleotide polymorphisms (SNPs) were identified. The allelic frequencies at Pit-1/*Taq*I *locus* were 0.76 (*G*) and 0.24 (*A*), while those at Pit-1/*Hinf*I *locus* were 0.70 (*B*) and 0.30 (*A*). Combined genotypic frequencies and possible haplotypes frequencies were also reported. Moreover, some population genetic indexes, namely: gene heterozygosity (He), gene homozygosity (Ho), effective allele numbers (N_e), fixation index (F_{IS}) and polymorphism information content (PIC) were calculated.

Key words: POU1F1 gene, Podolica breed, PCR-RFLP.

INTRODUCTION

Pit-1 (official nomenclature POU1F1) is a pituitary-specific transcription factor responsible for pituitary development and hormone expression in mammals (Cohen et al., 1997). Pit-1 is a member of the POU domain which contain proteins and a group of transcriptional regulators that have a critical role in differentiation and proliferation of cells (Mangalam et al., 1989). It was shown that they control transcription of the growth hormone (GH), prolactin (PRL) (Nelson et al., 1988; Mangalam et al., 1989), the thyroid-stimulation hormone *β*-subunit (TSH-*β*) (Simmons et al., 1990; Steinfelder et al., 1991), the GHRH receptor genes (Lin et al., 1992) and the Pit-1 gene itself (Rhodes et al., 1993).

The inhibition of Pit-1 synthesis leads to a marked decrease in expression of PRL and GH and in proliferation of cell lines producing PRL and GH (McCormick et al. 1990). Mutations in the Pit-1 gene lead to the absence of growth hormone and to pituitary hypoplasia in mice (Li et al., 1990) and to congenital hypothyroidism, dwarfism and prolactin deficiency in humans (Pfaffle et al., 1992).

The bovine Pit-1 gene is organized in six exons coding a polypeptide chain of 291 amino acids (~33 kD). The Pit-1 gene, sequenced by Bodner et al. (1988), was sublocalized to the centromeric region of bovine chromosome 1 (BTA1) and located midway between TGLA57 and RM95 (Moody et al., 1995).

Association studies have shown that Pit-1 is related to growth rate, carcass and milk production traits in domestic animals. Pit-1 was found to be related to birth weight (Yu et al., 1996), weaning weight, average daily gain and backfat thickness (Yu et al., 1995), as well as lean to fat ratio (Stancekova, et al., 1999) in pigs. In cattle, Pit-1 was found to be associated with body weight, average daily gains (Renaville et al., 1997a; Carrijo et al., 2008) and milk production traits (Renaville et al., 1997b; de Mattos et al., 2004; Xue et al., 2006). On the other hand, many studies reported no associations between Pit-1 and production traits of animals (Di Stasio et al., 2002; Zwierzchowski et al., 2001; Dybus et al., 2004;

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Table 1. Name and sequence of the Pit-1 primers, PCR product size and amplified regions.

Primer name	Primer sequences	Product size	Amplified region	references
Pit1E2_F	5'-CTTACCAGTCCCGTCTATT-3'	165 bp	Exon 2	Pan et al. (2008)
Pit1E2_R	5'-TTCTTACCTGCCATCACG-3'			
Pit1E6_F	5'-AAACCATCATCTCCCTTCTT-3'	451 bp	Intron 5 and exon 6	Wollard et al. (1994)
Pit1E6_R	5'-AATGTACAATGTGCCTTCTGAG-3'			

Zhao et al., 2004).

To date, *Hinf*I polymorphism has been reported in exon 6 of the bovine Pit-1 gene by PCR-RFLP technique (Woollard et al., 1994). This single nucleotide polymorphism (SNP) was found in the coding region of the bovine Pit-1 gene. It is a substitution A→G (NM_174579: c.1178A): the loss of the *Hinf*I site in allele *B* is a silent mutation (Dierkes et al., 1998). Moreover, four intronic polymorphisms were also reported: two located in intron 3, one in intron 4 and the last in intron 5 (Zhao et al., 2004). Recently, a new silent mutation in exon 2 was discovered by Pan et al. (2008) and also reported by Huang et al. (2008): this SNP is a substitution G→A at position 545 (NM_174579: c.545G) and it is detectable by PCR-RFLP method using the *Taq*I restriction enzyme.

No previous study have investigated the distribution of Pit-1 gene polymorphisms in Podolica cattle breed. The aim of the current study was to reveal the distribution pattern of known variants at bovine Pit-1/*Hinf*I and Pit-1/*Taq*I *loci* using PCR-RFLP approach in Podolica breed by estimating haplotype, genotype, allele frequencies and some population genetic indexes.

MATERIALS AND METHODS

One-hundred and four unrelated Podolica cattle (47 female and 57 male) were included in the study. The animals were from seven different farms located in southern Italy.

The Podolica breed is derived from *Bos primigenius podolicus* (forebears of the modern *Bos taurus*), and has spread throughout an area that mainly covers the inland territories of southern Italy. It has been present in Italy for a very long time and represents yet another example of successful biological adaptation to a hostile environment. The breed numbers 100,000 heads, 25,000 of which are listed in the Italian Herd Book of ANABIC (National Association of Italian Beef-Cattle Breeders). One of the outstanding characteristics of this cattle is its exceptional ability to adapt to particularly difficult environments, as well as its extraordinary capacity to utilize food resources that would not otherwise be used. The Podolica was long used mainly in a work capacity and only secondarily for beef and dairy products. In fact, its milk is ideal for producing the famous "caciocavallo" cheese. Subsequently, with the rise and spread of agricultural mechanization, the selective trend of this breed became geared more towards beef production and, to a lesser extent, towards dairy production, particularly in certain areas (Dario et al., 2009)

Determination of Pit-1 polymorphisms

Individual blood samples (approximately 10 ml per animals) for DNA genotyping were collected from 104 Podolica cattle on K_3-EDTA tubes and stored at -25°C. Genomic DNA was isolated from whole blood using NucleoSpin Blood Kit (Macherey-Nagel). After genomic DNA isolation, the individuals were genotyped for the Pit-1 gene polymorphisms by PCR-RFLP technique.

The sequences of primers used for amplification of the two regions of Pit-1 gene and the size of the PCR amplicons are reported in Table 1. The 165 bp gene fragment including part of the exon 2 was amplified using thirty-five amplification cycles at the following conditions: 94°C/30 s, 57°C/30 s and 72°C/30 s. The 451 bp gene fragment, harbouring part of the intron 5 and part of exon 6, was amplified using thirty-six amplification cycles at the following conditions: 95°C/1 min, 54°C/1 min and 72°C/2 min. Both amplicons were electrophoretically separated on 2% agarose gel stained with ethidium bromide.

RFLP analysis was conducted to detect the polymorphisms. The 165 bp PCR product was digested with *Taq*I restriction endonuclease (Fermentas, 2 h, 65°C) and analysed on a 3% agarose gel stained with ethidium bromide, in TBE buffer. The 451 bp fragment was digested with *Hinf*I restriction endonuclease (TaKaRa, 4h, 37°C) and analysed on a 2% agarose gel stained with ethidium bromide in TBE buffer. The *Taq*I cuts the 165 bp amplification product into 138 and 27 bp fragments for allele *G,* while allele *A* remains uncut. The following DNA restriction fragments were expected: 138 and 27 bp for the GG genotype; 165, 138 and 27 bp for the GA genotype and 165 bp for the AA genotype. The *Hinf*I cuts the 451 bp PCR product into 244 and 207 bp fragments for the *B* allele, while allele *A* remains uncut. The possible genotype patterns were: 244 and 207 bp for the BB genotype; 451, 244 and 207 bp for the AB genotype and 451 bp for the AA genotype.

Statistical analysis

The allele frequencies for both SNPs were calculated by simple allele counting (Falconer and Mackay, 1996). The differences of the observed and expected frequencies of genotypes were tested using a Chi-square test in order to verify if the population was in Hardy-Weinberg equilibrium.

Population genetic indexes, namely: gene heterozygosity (He), gene homozygosity (Ho), effective allele numbers (N_e) and fixation index (F_{IS}) were performed by POPGENE32 software version 1.32 (Yeh et al., 2000). Moreover, polymorphism information content (PIC) was calculated according to Botstein et al. (1980). Haplotype estimation was performed by ARLEQUIN software version 3.11 (Excoffier et al., 2005). This software estimates the frequency of haplotypes present in the population by maximum likelihood methods (Excoffier and Slatkin, 1995).

RESULTS

Two SNPs located in the bovine Pit-1 gene were detected by PCR-RFLP technique. In the studied Podolica cattle population, both *loci* were found to be polymorphic. Three genotypic patterns were produced as

Table 2. Frequencies of genotypes, alleles, combined genotypes and haplotypes in the sample of Podolica breed for both considered SNPs.

Genotype frequency (%)		Allele frequency		Combined genotype (%)		Haplotype frequency	
AA	6.73	A	0.24	AAAA	4.81	Haplotype (c.545G>A; c.1178A>G)	
GA	34.62	G	0.76	AABB	1.92	[A;A]	0.192
GG	58.65			GAAA	4.81	[A;B]	0.048
				GAAB	25.00	[G;A]	0.111
AA	14.42	A	0.30	GABB	4.81	[G;B]	0.649
AB	31.73	B	0.70	GGAA	4.81		
BB	53.85			GGAB	6.73		
				GGBB	47.11		

a result of the *Taq*I restriction enzyme. Two (GG), one (AA) and three (GA) band patterns could be distinguished on the gel, which are the products of two alleles (*G* and *A*). Similarly, three different genotypic patterns were produced after *Hinf*I enzymatic digestion: BB genotype (two bands); AA (one band) and AB (three bands).

The observed frequencies of *G* and *A* alleles at Pit-1/*Taq*I *locus* were 0.76 and 0.24, respectively. The expected frequencies of the three genotypes, calculated according to the Hardy-Weinberg equilibrium, were 57.70% (GG) 36.52% (GA) and 5.78% (AA). The observed genotypic frequencies were 58.65% (GG) 34.62% (GA) and 6.73% (AA) (Table 2). A Chi-square test was performed to evaluate if the population was in Hardy-Weinberg equilibrium. The calculated χ^2 value was 0.28 (d.f. = 1), indicating Hardy-Weinberg equilibrium in the population (P = 0.59).

The observed frequencies of *B* and *A* alleles at Pit-1/*Hinf*I *locus* were 0.70 and 0.30, respectively. The expected frequencies of the three genotypes calculated according to the Hardy-Weinberg equilibrium, were 48.60% (BB) 42.23% (AB) and 9.17% (AA) respectively. As shown in Table 2, the most frequent genotype in the population was BB genotype (53.85%) followed by AB (31.73%) and AA (14.42%). The calculated χ^2 value was 6.43 (d.f. = 1), indicating Hardy-Weinberg disequilibrium in the population (P < 0.05). Comparison between the observed and the expected numbers of genotypes at Pit-1/*Hinf*I *locus* showed an excess of BB and AA animals and consequently a deficiency of heterozygotes.

As reported in Table 2, the combined analysis of the two considered *loci* showed that the GGBB genotype was the most frequent in the studied population (47.11%), followed by the double heterozygous (25.00%): these data were consistent with the result obtained considering the high frequencies of GG and BB genotypes, separately. No animal genotyped as AAAB were found in the sample of Podolica cattle. The remaining six genotypes showed a frequency ranging from 6.73 (GGAB) to 1.92% (AABB). Consequently, on the basis of the analysis of the possible haplotypes, the most frequent haplotype in the population was GB (0.649), while the AB haplotype had the lowest frequency (0.048) (Table 2).

In the present population, Ho, He, N_e, F_{IS} and PIC are shown in Table 3. F_{IS} is a measure of the deviation of genotypic frequencies from panmictic frequencies in terms of heterozygous deficiency or excess. Negative F_{IS} values indicate heterozygote excess and positive values indicate heterozygote deficiency when compared with Hardy-Weinberg equilibrium expectations. As reported in Table 3, a slight excess of homozygosity (positive F_{IS} value) was found for Pit-1/*Hinf*I *locus* (F_{IS} = 0.249). On the other hand, the F_{IS} value observed for Pit-1/*Taq*I *locus* suggest a condition of equilibrium in the population as confirmed by the results of χ^2 test used to verify the Hardy-Weinberg equilibrium. The PIC is a parameter indicative of the degree of informativeness of a marker. The PIC value may range from 0 to 1. In the studied population, PIC values were 0.298 and 0.332 for Pit-1/*Taq*I and Pit-1/*Hinf*I *locus*, respectively. According to the classification of PIC (low polymorphism if PIC value < 0.25, median if 0.25 < PIC value < 0.50 and high if PIC value > 0.50), both *loci* possessed middle genetic diversity.

The observed N_e (1.575 and 1.731 for Pit-1/*Taq*I and Pit-1/*Hinf*I *locus*, respectively) and the PIC values, indicates a good level of genetic variability in Podolica breed at the considered *loci*.

DISCUSSION

Table 4 illustrates the Pit-1/*Taq*I and Pit-1/*Hinf*I allelic frequencies in Podolica breed and in different bovine breeds as observed by other authors. Genetic polymorphism at the two considered *loci* has not been previously reported for Podolica breed and no data concerning both *loci* on the same animals were found in literature. In Podolica breed, the Pit-1/*Hinf*I allele frequencies were intermediate between those reported in Angus (Zhao et al., 2004) and in Limousine breed by Dybus et al. (2003). Moreover, it is important to underline that in dairy breed, the frequency of *A* allele decreased and in Zebuine breeds, a very strong reduction of this value was observed. The Pit-1/*Taq*I *G* allele was predominant in Podolica breed as reported in other cattle

Table 3. Genetic indexes calculated at the two considered loci.

	Gene homozygosity (Ho)	Gene heterozygosity (He)	Effective allele numbers (N_e)	Polymorphic information content (PIC)	Fixation index (F_{IS})
PIT1/*Taq*I c.545G>A	0.654	0.346	1.575	0.298	0.052
PIT1/*Hinf*I c.1178A>G	0.683	0.317	1.731	0.332	0.249

Table 4. Allele frequencies of the two considered SNPs in Podolica breed and in different breeds as observed by other authors.

Breed	PIT-1/*Taq*I		PIT-1/*Hinf*I		References
	A	*G*	*A*	*B*	
Italian Holstein-Friesian	-	-	0.19	0.81	Renaville et al. (1997b)
Holstein	-	-	0.15	0.85	Wollard et al. (1994)
Piemontese	-	-	0.25	0.75	Di Stasio et al. (2002)
Limousine	-	-	0.27	0.73	Dybus et al. (2003)
Qinchuan	-	-	0.23	0.77	Zhang et al. (2009)
Angus	-	-	0.33	0.67	Zhao et al. (2004)
Angus	0.18	0.82	-	-	Pan et al. (2008)
Belgian-Blue	-	-	0.53	0.47	Renaville et al. (1997°)
Canchim*	-	-	0.20	0.80	Carrijo et al. (2008)
Nanyang	-	-	0.46	0.54	Xue et al. (2006)
Nanyang	0.20	0.80	-	-	Pan et al. (2008)
Gyr	-	-	0.05	0.95	de Mattos et al. (2004)
Indian zebuine	-	-	0.06	0.94	Mukesh et al. (2007)
Polish Black and White	-	-	0.24	0.76	Dybus et al. (2004)
Qinchuan	0.32	0.69	-	-	Pan et al. (2008)
Jiaxian Red	0.12	0.88	-	-	Pan et al. (2008)
Chinese Holstein	0.00	1.00	-	-	Pan et al. (2008)
Luxi	0.30	0.70	-	-	Pan et al. (2008)
Jinnan	0.16	0.84	-	-	Pan et al. (2008)
Guyuan	0.15	0.85	-	-	Pan et al. (2008)
Podolica	0.24	0.76	0.30	0.70	Present work

*Mean value calculated among animals belonging to two different lineages

breeds (Pan et al., 2008). The fixation of the *G* allele found in Chinese Holstein may be due to the dairy selection; from this point of view, further studies should be carried out concerning the Pit-1/*Taq*I polymorphism in other dairy breeds in order to compare the allele frequencies and to look for a possible relationship with the different productive attitude. Finally, it is important to underline that the studied SNPs were both silent mutations, so these two SNPs could be investigated also as useful instruments for population studies.

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REFERENCES

Bodner M, Castrillo JL, Theill LE, Deerinck T, Ellisman M, Karin M (1988). The pituitary-specific transcription factor GHF-1 is a homeobox-containing protein. Cell 55: 505-518.

Botstein D, White RL, Skalnick MH, Davies RW (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphism. Am. J. Hum. Genet. 32: 314-331.

Carrijo SM, Alencar MM, Toral FLB (2008). Association of PIT1 genotypes with growth traits in Canchim cattle. Sci. Agr. 65: 116-121.

Cohen LE, Wondisford FE, Radovick S (1997). Role of *Pit-1* in the gene expression of growth hormone, prolactin and thyrotropin. Endocrinol. Metab. Clin. North Am. 25: 523-540.

Dario C, Selvaggi M, Carnicella D, Bufano G (2009). STAT5A/*Ava*I polymorphism in Podolica bulls and its effect on growth performances traits. Livest Sci 123: 83-87.

De Mattos KK, Del Lama SN, Martinez ML, Freitas AF (2004). Association of bGH and *Pit-1* gene variants with milk production traits

in dairy Gyr bulls. Pesqui. Agropecu. Bras. 39: 147-150.
Dierkes B, Kriegesmann B, Baumgartner BG, Brening B (1998). Partial genomic structure of the bovine *Pit-1* gene and characterization of a *Hinf*I transition polymorphism in exon 6. Anim. Genet. 29: 405-405.
Di Stasio L, Sartore S, Albera A (2002). Lack of association of GH1 and Pou1f1 gene variants with meat production traits in Piemontese cattle. Anim. Genet. 33: 61-64.
Dybus A, Kmieć M, Sobek Z, Pietrzyk W, Wiśniewski B (2003) Associations between polymorphism of growth hormone releasing hormone (*GHRH*) and pituitary transcription factor 1 (*PIT1*) genes and production traits of Limousine cattle. Arch Tierz 46: 527-34.
Dybus A, Szatkowska I, Czerniawska-Piątkowska E, Grzesiak W, Wójcik J, Rzewucka E, Zych S (2004). *PIT1-Hinf*I gene polymorphism and its associations with milk production traits in polish Black-and-White cattle. Arch. Tierzt. 47: 557-563.
Excoffier L, Laval G, Schneider S. (2005). Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evol. Bioinformatics Online 1: 47-50.
Excoffier L, Slatkin M (1995). Maximum-likelihood estimation of molecular haplotype frequencies in a diploid population. Mol. Biol. Evol. 12: 921–927.
Falconer DS, Mackay TFC (1996). Introduction to Quantitative Genetics, 4th edition. Longman Group Ltd., Essex, UK.
Huang W, Maltecca C, Khatib H (2008). A proline-to-histidine mutation in POU1F1 is associated with production traits in dairy cattle. Anim. Genet. 39: 554-557.
Li S, Crenshaw EB III, Rawson EJ, Simmons DM, Swanson LW, Rosenfeld MG (1990). Dwarf locus mutants lacking three pituitary cell types result from mutations in the POU-domain gene Pit-1. Nature 347: 528-533.
Lin C, Lin SC, Chang CP, Rosenfeld MG (1992). Pit-1 dependent expression of the receptor for growth hormone releasing factor mediates pituitary cell growth. Nature 360: 765-768.
Mangalam HJ, Albert VR, Ingraham HA, Kapiloff M, Wilson L, Nelson C, Elsholtz H, Rosenfeld MG (1989). A pituitary POU-domain protein, *Pit-1*, activates both growth hormone and prolactin promoters transcriptionally. Gene Dev. 3: 946-958.
McCormick A, Brady H, Theill LE, Karin M (1990). Regulations of the pituitary-specific homeobox gene GHF1 by cell autonomous and environmental cues. Nature 345: 829-832.
Moody DE, Pomp D, Barendse W (1995). Restriction fragment length polymorphism in amplification products of the bovine *Pit-1* gene and assignment of *Pit-1* to bovine chromosome 1. Anim. Genet. 26: 45-47.
Nelson C, Albert VR, Elsholtz HP, Lu LI, Rosenfeld MG (1988). Activation of cell-specific expression of rat growth hormone and prolactin gene by a common transcription factor. Science 239: 1400-1405.
Pan C, Lan X, Chen H, Guo Y, Shu J, Lei C, Wang X. (2008). A *Taq*I PCR-RFLP Detecting a novel SNP in exon 2 of the Bovine *POU1F1* gene. Biochem. Genet. 46: 424-432.
Pfaffle RW, DiMattia GE, Parks JS, Brown MR, Wit JM, Jansen M, Van der Nat HJ, Van den Brande L, Rosenfeld MG, Ingraham HA (1992). Mutation of the POU-specific domain of Pit-1 and hypopituitarism without pituitary hypoplasia. Science 257: 1118-1121.
Renaville R, Gengler N, Parmentier I, Mortiaux F, Massart S, Bertozzi C, Burny A, Portetelle D (1997a). *Pit-1* gene *HinfI* RFLP and growth traits in double-muscled Belgian Blue Cattle. J. Anim. Sci. 75(Suppl.1): 146.
Renaville R, Gengler N, Vrech E, Prandi A, Massart S, Corradini C, Bertozzi C, Mortiaux F, Burny A, Portetelle D (1997b). *Pit-1* gene polymorphism, milk yield and conformation traits for Italian Holstein-Friesian bulls. J. Dairy Sci. 80: 3431-3438.
Rhodes SJ, Chen R, DiMattia GE, Scully KM, Kalla KA, Lin SC, Yu VC, Rosenfeld MG (1993). A tissue-specific enhancer confers Pit-1-dependent morphogen inducibility and autoregulation on the Pit-1 gene. Gene Dev. 7: 913-932.
Simmons DM, Voss JW, Ingraham HA, Holloway JM, Broide RS, Rosenfeld MG, Swanson LW (1990). Pituitary cell phenotypes involve cell-specific Pit-1 mRNA translation and synergistic interactions with other classes of transcription factors. Gene Dev. 4: 695-711.
Stancekova K, Vasicek D, Peskovicova D, Bulla J, Kubek A (1999). Effect of genetic variability of the porcine pituitary-specific transcription factor (Pit-1) on carcass traits in pigs. Anim. Genet. 30: 313-315.
Steinfelder HJ, Hauser P, Nakayama Y, Radovick S, McClaskey JH, Taylor T, Weintraub BD, Wondisford FE (1991). Thyrotropin-releasing hormone regulation of human TSHß expression: role of a pituitary-specific transcription factor (Pit-1/GHF-1) and potential interaction with a thyroid hormone-inhibitory element. Proc. Natl. Acad. Sci. USA 88: 3130-3134.
Xue K, Chen H, Wang S, Cai X, Liu B, Zhang CF, Lei CZ, Wang XZ, Wang YM, Niu H (2006). Effect of genetic variations of the POU1F1 gene on growth traits of Nanyang cattle. J. Genet. Genomics 33: 901-907.
Yeh FC, Yang R, Boyle TJ, Ye Z, Xiyan JM (2000). PopGene32, Microsoft Windows-based freeware for population genetic analysis, version 1.32. Mol. Biol. Biotechnol. Centre, Univ. Alberta, Edmonton, Alberta, Canada.
Yu TP, Rothschild MF, Tuggle CK, Haley C, Archibald A, Marklund L, Anderson L (1996). Pit-1 genotypes are associated with birth weight in three unrelated pig resource families. J. Anim. Sci. 74(Suppl. 1): 22.
Yu TP, Tuggle CK, Schmitz CB, Rothschild MF (1995). Association of Pit-1 polymorphism with growth and carcass traits in pigs. J. Anim. Sci. 73: 1282-1288.
Woollard J, Schmitz CB, Freeman AE, Tuggle CK (1994). Rapid communication: *HinfI* polymorphism at the bovine *Pit-1* locus. J. Anim. Sci. 72: 3267-3267.
Zhang C, Liu B, Chen H, Lan X, Lei C, Zhang Z, Zhang R (2009). Associations of a *Hinf*I PCR-RFLP of *POU1F1* gene with growth traits in Qinchuan cattle. Anim. Biotechnol. 20: 71-74.
Zhao Q, Davis ME, Hines HC (2004). Associations of polymorphisms in the *Pit-1* gene with growth and carcass traits in Angus beef cattle. J. Anim. Sci. 82: 2229-2233.
Zwierzchowski L, Oprzydek J, Dymnicki E, Dzierzbicki P (2001). An association of growth hormone, κ-casein, ß-lactoglobulin, leptin and Pit-1 loci polymorphism with growth rate and carcass traits in beef cattle. Anim. Sci. Pap. Rep. 19: 65-78.