SNPs analyses of the bovine LEP and PIT-1 genes by multiplex PCR-RFLP method and their effect on milk performance traits in Slovak Simmental cattle

Analýza SNPs v bovinných génoch LEP a PIT-1 metódou multiplex PCR-RFLP a ich vplyv na ukazovatele produkcie mlieka u slovenského simentálskeho dobytka

Anna TRAKOVICKÁ, Nina MORAVČÍKOVÁ*, Tomáš MINAROVIČ, Alica NAVRÁTILOVÁ

Slovak University of Agriculture in Nitra, Department of Animal Genetics and Breeding Biology, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic
*nina.moravcikova1@gmail.com

Abstract

The aim of this study was detection of polymorphisms in leptin (LEP) and pituitary specific transcription factor (Pit-1) genes and their evaluation as a genetic markers affected milk performance traits in population of Slovak Simmental cows. The analyzed SNP of LEP gene is located in intron 2 on bovine chromosome 4 and SNP of Pit-1 gene in exon 6 on bovine chromosome 1. The total numbers of blood samples were taken from 288 samples of Slovak Simmental cows. Bovine genomic DNA was isolated by phenol-chloroform extraction method and used in order to estimate LEP/*Sau*3AI and Pit-1/*Hin*fI genotypes by means of MULTIPLEX PCR-RFLP method with using *Sau*3AI restriction enzyme for LEP and *Hin*fI restriction enzyme for Pit-1. In the population of Slovak Simmental cattle were detected genotype AA (390 and 32 bp), genotype AB (390, 303, 88 and 32 bp), genotype BB (303, 88 and 32 bp) for LEP gene and genotype AA (260 bp), genotype AB (260, 190 and 70 bp), genotype BB (190 and 70 bp) for Pit-1 gene. The predominant alleles were A with observed frequency 0.8385 and B with 0.7743 for LEP and Pit-1, respectively. In both analyzed polymorphisms were frequent animals with dominant homozygous genotype. Frequency of SNP LEP/*Sau*3AI AA genotype was 0.7014 and SNP Pit-1/HinfI BB genotype was 0.6007. The statistical analysis show significant effect only between LEP/*Sau*3AI genotype and milk yield in standard length of lactation (P≤0.05), with A as a desirable allele. In genotype comparison produced AA genotype cows significantly more milk, with difference amounting 457.9 and 293.9 kg and therefore this animals might have potentially positive effect on milk yield. The other results from the statistical association analysis between LEP/*Sau*3AI

and Pit-1/*Hin*fI genotypes and milk production parameters – milk, protein and fat yield (kg) in standard length of lactation were not significant.

Keywords: cattle, genetic polymorphism, leptin, multiplex PCR-RFLP, specific pituitary transcription factor

Detailed abstract in native language

Cieľom našej práce bola detekcia jednonukleotidových polymorfizmov v génoch kódujúcich leptín a špecifický transkripčný faktor hypofýzy a hodnotenie ich vplyvu ako potenciálnych genetických markerov pre produkciu mlieka v populácii kráv slovenského simentálskeho plemena. Okrem environmentálnych vplyvov sa podieľajú na výške produkcie mlieka a hodnotách jej ukazovateľov aj genetické predispozície a preto je vhodné, začleniť ich v podobe genetických markerov do tradičných selekčných metód v chovoch mliekového dobytka. Jedným z významných hormónov nielen pre mliekovú úžitkovosť ale aj pre produkciu mäsa a reprodukciu je leptín. Leptín je bielkovina, syntetizovaná najmä v tukovom tkanive, ovplyvňujúca viacero procesov v organizme. Je zapojený do udržiavania energetickej rovnováhy prostredníctvom regulácie príjmu krmiva, v riadení reprodukčných funkcií a imunitnej odpovede. Okrem toho sa jeho účinok prejavuje aj v placente, mliečnej žľaze, kostrových svaloch, mozgu a hypofýze. Hlavnou funkciou leptínu je informovať centrálny nervový systém o hromadení tuku a udržiavaní energetickej rovnováhy v tele. Zároveň sa podieľa aj na vývine mliečnej žľazy, kedy reguluje jej rast a funkciu pomocou interakcie s prolaktínom. U prežúvavcov je produkovaný aj bunkami mliečnej žľazy počas gravidity a v priebehu laktácie. Gén kódujúci leptín bol lokalizovaný na bovinnom chromozóme 4. Nami sledovaný polymorfizmus LEP/*Sau*3AI bol identifikovaný vo vnútri sekvencie druhého intrónu, kde vyvoláva zámenu dusíkatých báz (C za T). Na aktivácii exresie génov rastového hormónu, prolaktínu a tyreotropínu sa podieľa špecifický transkripčný faktor Pit-1. Tento transkripčný faktor je zodpovedný u prežúvavcov aj za vývoj hypofýzy a riadenie expresie sekrečných hormónov. Pit-1 obsahuje dve domény, ktoré sú nevyhnutné pre zabezpečenie vysokej afinity DNA pri väzbe promótorov génov rasového hormónu a prolaktínu. Gén Pit-1 bol zmapovaný na bovinnom chromozóme 1. Jednonukleotidový polymorfizmus Pit-1/*Hin*fI vzniká v exóne 6 ako tichá mutácia a spôsobuje zámenu dusíkatých báz (G za A). Na vyhodnotenie genotypovej štruktúry populácie, vznikajúcej ako dôsledok týchto polymorfizmov, bolo použitých celkovo 288 krvných vzoriek. Genómová DNA bola izolovaná metódou fenol-chloroformovej extrakcie. Jednotlivé alely a genotypy LEP/*Sau*3AI a Pit-1/*Hin*fI polymorfizmov sme identifikovali pomocou multiplex PCR-RFLP metódy, kedy boli využité reštrikčné enzýmy *Sau*3AI pre LEP gén a *Hin*fI pre Pit-1 gén. Po reštrikčnom štiepení PCR produktov LEP génu sme zistili prítomnosť genotypov AA (390 a 32 bp), AB (390, 303, 88 a 32 bp) a BB (303, 88 a 32 bp). Podobne boli všetky tri genotypy identifikované aj pre Pit-1 gén – AA (260 bp), AB (260, 190 a 70 BP) a BB (190 a 70 bp). V analyzovanej populácii simentálskych kráv bola dominantná v prípade LEP génu alela A s frekvenciou 0, 8385 a pre Pit-1 gén to bola alela B s frekvenciou 0,7743. Pri obidvoch sledovaných polymorfizmoch prevažovali v populácii dominantní homozygóti. Frekvencia výskytu jedincov s genotypom LEP/Sau3AI AA bola 0,7014 a Pit-1/HinfI bola 0,6007. Štatistická analýza preukázala signifikatné rozdiely iba v prípade hodnotenia asociačného vzťahu medzi

LEP/*Sau*3AI genotypmi a produkciou mlieka za obdobie normovanej laktácie. Pri porovnávaní vplyvu genotypov sme zistili, že kravy s AA homozygotným genotypom produkovali preukazne viac mlieka, pričom rozdiel tvoril 457,9 and 293,9 kg. Na základe týchto údajov je možné povedať, že takéto zvieratá majú potenciálne pozitívny vplyv na produkciu mlieka a po zhodnotení ostatných kritérií úžitkovosti by bolo vhodné zaradiť ich do ďalšej plemenitby. Výsledky ďalšieho štatistického hodnotenia vzťahu medzi LEP/*Sau*3AI a Pit-1/*Hin*fI genotypmi a ukazovateľmi mliekovej úžitkovosti neboli signifikantné.

Kľúčové slová: genetický polymofizmus, hovädzí dobytok, leptín, multiplex PCR-RFLP, špecifický transkripčný faktor hypofýzy

Introduction

Genetic and environmental factors are known to influence production traits in dairy cattle. Selection of animals with higher production or better reproductive performance is of great significance to breeders and consumers. Current technologies enable scientists to improve on the accuracy and efficiency of traditional selection methods by applying genetic markers trough marker-assisted selection. Therefore, genetic polymorphisms that are significantly associated with certain traits of interest are very useful. Polymorphisms detection in genes related to production traits and the identification of the allele which results in a phenotype of interest can allow for marker assisted selection (Zhao et al., 2004; Gutiérrez-Gil et al., 2008). In the last years the understanding of the genetic basis of mammary gland development and function received an increased attention, because the improvement of milk production should not compromise animal's health (Carsai and Balteanu, 2010). The variations in milk production cannot be attributed just to one gene because the secretion activity of mammary gland is controlled by a cascade of hormones, transcription factors, enzymes, affected by mutations over the years, which are probably the cause of these variations.

Leptin is a protein produced primarily by white adipose tissue and involved in regulation of feed intake, feeding behaviour, energy expenditure, growth and body composition, as well as immune system functions and several aspects of reproduction (Houseknecht et al., 1998). Nutrition is a key determinant of productive potential in cattle and other mammals. Therefore, responses of the productive system to changes in nutrition and metabolic status influence reproductive and economic efficiency of food-producing species in variety of context (Wiliams et al., 2002). As the hormone leptin is involved in regulation of nutritional status and reproductive function this hormone is an interesting protein to investigate during the periparturient period in dairy cattle (Liefers et al., 2005). Leptin binds to a receptor mainly localized on Neuropeptid – Y – neurons, which in hypothalamus also appear to play a key role in the integration of feeding behaviour with internal signals of body energy status (Wayne et al., 1995). Leptin is supposed to be the signal to the reproductive system that enough total energy, in from of fat, is present to support the added energy demands of a successful conception and pregnancy. Leptin may help regulate ovarian development and steroidogenesis and serve as either a primary signal initiating puberty or as a permissive regulator of sexual maturation (Lindersoon et al., 1998). Leptin is supposed necessary by the mammary growth, development and function, when mammary fat cell leptin expression requires prolactin which then cooperates with leptin to influence mammary activity (Feuermann et al., 2006). The

ruminant mammary epithelial cells also synthesize leptin during pregnancy and during established lactation (Leury et al., 2003). In dairy cattle, the increase in milk yield has been accompanied by a more negative energy balance during early lactation and a decrease in fertility (Liefers et al., 2005). During pregnancy leptin levels are high and they decline rapidly towards parturition. Eliminating the energetic costs of lactation by preventing milk delivery in cows caused an increase in plasma leptin levels together with an increase in energy balance (Block et al., 2001). This indicates that the fall in circulating leptin levels towards and during lactation is due to the energetic coast of milk production (Brogan et al., 1999). The leptin (LEP) gene is highly conserved across species, and is located on bovine chromosome 4q32. Its DNA sequence has more than 15,000 base pair and contains three exons, which are separated by two introns (Stone et al., 1996). The coding region of the LEP gene (501 nucleotide length) is contained in exon 2 and 3, which are separated by introns of approximately 2 kb. The LEP gene promoter regions spans are approximately 3 kb (Liefers et al., 2002). The *Sau3AI* polymorphism detected inside the intron 2 as a cytosine to thymine transition results in amino acid change (arginine to cysteine) at position 2059 of the protein chain. The polymorphism in LEP gene was associated with milk performance (Liefers et al., 2002; Madeja et al., 2004; Javanmard, 2010), increased perinatal mortality in dairy (Brickell et al., 2010), calf birth and weaning weights in beef and dairy (Almeida et al., 2003; Nkrumah et al., 2005) and reproductive performance in dairy cattle (Nkrumah et al., 2005).

Bovine Pit-1, a 291 amino acid protein with DNA binding POU domain (De Mattos et al., 2004), is a specific pituitary transcription factor that is responsible for pituitary development and hormone secreting gene expression in mammals (Cohen et al., 1997). Pit-1 is the cellular specific transcription factor for activating expression of growth hormone, prolactin and thyrotropin β-subunit genes in anterior pituitary gland (Tuggle and Trenkle, 1996) but also is a regulatory factor in differentiation and proliferation of cells of pituitary gland (Hoggard et al., 1993). An approximately 33 kDa 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 growth hormone and prolactin genes (Rosenfeld, 1991). The gene encoding Pit-1 was chosen as a candidate gene to investigate its association with lactation performance, growth and carcass traits in several cattle breeds (Renaville et al., 1997; Woollard et al., 1994; Moody et al., 1995). The Pit-1 gene was located in centromeric region of bovine chromosome 1 (Moody et al., 1995). The Pit-1 gene is controlled by several factors that interact with its 5' regulatory the Pit-1 gene itself also occurs as there are two Pit-1 bindings site in the 5' flanking region. Pit-1 is also involved expression of gene coding for thyrotropin releasing hormone (TSH) (Radovick et al., 1992), a key hormone involved in thyroid gland activity. The inhibition of Pit-1 synthesis leads to a marked decrease of growth hormone, prolactin and TSH synthesis (Beigi Nassiri et al., 2010) and therefore is considered a highly valuable genetic marker for improving milk production (Renaville et al., 1997). In the bovine Pit-1 gene, the restriction fragment length polymorphism (HinfI restriction enzyme) was detected (Moody et al., 1995). Molecular basis of this polymorphism was the silent mutation (G→A) located within the exon 6 of the Pit-1 gene (Diekers et al., 1998). HinfI polymorphism of Pit-1 gene was associated with growth (Yang et al., 2010, Carrijo et al., 2008, Zhao et al., 2004), milk composition and production (Renaville et al., 1997; Dybus et al., 2003; De Mattos et al., 2004) and reproduction (Edriss et al., 2009) traits.

The objective of our study was to establish LEP and Pit-1 genes allele and genotype frequencies and significance of association between these polymorphisms and milk production traits in standard length of lactation – milk protein and fat yield in Slovak Simmental cattle.

Materials and Methods

Samples were collected from total 288 cows of Slovak Simmental breed. Genomic DNA for genotyping was extracted from whole blood samples with standard phenol – chloroform extraction method (Miller et al., 1988). DNA concentrations were estimated by spectrophotometer measuring the optical density at wave length of 260 nm.

A 422 bp fragment of LEP gene and 260 bp fragment of Pit-1 gene containing analyzed polymorphic site were amplified by multiplex PCR using forward and reverse primers according to Liefers et al. (2002) and Ozdemir (2012) and analyzed with using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. DNA was amplified in a total volume of 25 µl containing: 1 x PCR buffer (NH4)2SO4, 2 mM MgCl2, 200 µM of dNTPs, 0.8 µM of LEP primers, 0.8 µM of Pit-1 primers,1 U Tag DNA polymerase and 50 ng genomic DNA template. PCR amplification was carried out in C1000TM thermal cycler (Biorad). PCR conditions were: at 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 62°C for 30 s and 72°C for 30 s. After 30 cycles, reactions were completed by extension at 72°C for 5 min. The PCR products for each sample were digested with 1 µl of FastDigest Sau3AI (LEP) and HinfI (Pit-1) restriction enzymes at 37°C in time 10 min. The digestion products were separated by horizontal electrophoresis in 3% agarose gels in 0.5 x TBE (140 V for 45 min) stained with GelRed (Biotium) prior to visualization under UV light.

Table1 Primer sequences of LEP and Pit-1 locus

Locus	Primer sequence
LEP/*Sau*3AI[1]	F 5´ -TGG AGT GGC TTG TTA TTT TCT TCT- 3´
	R 5´-GTC CCC GCT TCT GGC TAC CTA ACT- 3´
PitI/*Hin*fI [2]	F 5´ -ACT CGC TAT TAC ACA ATA GGA GAG CCT- 3´
	R 5´-TCC TGC CAA CTC CTC ACC TCC C - 3´

F – Forward, R – Reverse; [1] Liefers et al. (2002), [2] Ozdemir (2012)

The allele and genotype frequencies of LEP and LEPR genes were estimated by direct counting. The effects of genotypes were determined milk production traits – milk, protein and fat yield in standard length of lactation. Significance of the genotype effects were estimated using the approximated T-statistic provided by SAS Enterprise Guide 4.2.

Results and Discussion

The amplified PCR products of LEP (422 bp) and Pit-1 (260 bp) genes were digested with restriction enzymes *Sau*3AI and *Hin*fI and visualized on 3% agarose gele. Figure 1 show representation results from multiplex PCR-RFLP analysis and PCR products

size and the restriction patterns of observed genotypes of detected genes. In case of LEP gene the digested AA PCR product exhibited two fragments of 390 and 32 bp, whereas the BB genotype gave three fragments 303, 88 and 32 bp (32 bp bands not detected on the gel). For Pit-1 gene the digested AA PCR product exhibited one fragment of 260 bp. For the BB genotype was exhibited 190 and 70 bp.

Table 2 Frequency of allele and genotypes of LEP/*Sau*3AI and Pit-1/*Hin*fI

Cows (n=288)	Genotype			Allele	
		LEP/*Sau*3AI			
	AA	AB	BB	A	B
Number	202	79	7	0.8385	0.1615
Frequency	0.7014	0.2743	0.0243		
		Pit-1/*Hin*fI			
	AA	AB	BB	A	B
Number	15	100	173	0.2257	0.7743
Frequency	0.0521	0.3472	0.6007		

Table 2 shows frequencies of the detected genotypes of both genes. The observed frequencies of A and B alleles of LEP gene were in population of 288 cows 0.8385 and 0.1615, respectively. The A allele was predominant. The observed frequencies of LEP/Sau3AI genotypes were 0.7014 (n=202), 0.2743 (n=79), 0.0243 (n=7) for AA, AB and BB genotype, respectively. The most frequent genotype in population of cows was AA. Our findings were similar to results reported for Holstein-Friesian cows (Liefers et al., 2002), Black-and-White cows (Kulig, 2005), Holstein bulls (Javanmard et al., 2010) different meat producing breeds of cattle (Passos et al., 2007) and Slovak spotted cows (Moravčíková et al., 2012).

Dominant allele of Pit-1 gene was B, with frequency 0.7743. The number of individuals with three genotypes of Pit-1/HinfI were observed with frequencies 0.0521 (AA), 0.3472 (AB) and 0.6007 (BB). The most frequent Pit-1/HinfI genotype in analyzed cow's population was BB. The high frequency of predominant B allele was confirmed in other studies of different cattle breed populations. Woolard et al. (1994) identified similarly allelic frequency for the allele A 0.10 and B 0.85 in group of 214 Holstein dairy cattle. In population of 130 Limousine calves found Dybus et al. (2003) high frequency of B allele (0.7269) that the most frequent was BB genotype. In contrary Ozdemir (2012) reported as a dominant in Anatolian Red cattle AB genotype and in Holstein cattle BB genotype. Generally, in the dairy cattle population is frequency of B allele and genotype BB highest.

Table 3 Basic statistical variation measurements of milk production traits of milk yield in standard length of lactation

Trait	$\bar{x}$	s	x_{min}	x_{max}	n
Milk yield (kg)	5651.74	1479.48	1688.00	9436.00	246
Fat yield (kg)	227.12	62.32	52.00	386.00	246
Protein yield (kg)	190.34	49.20	58.00	313.00	246

Table 4 Means and standard deviations of milk production traits in cows of different LEP/*Sau*3AI and Pit-1/*Hin*fI genotypes

Genotype	n	Traits (means±standard deviations)					
		Milk yield, kg	N	Protein yield, kg	N	Fat yield, kg	N
LEP/Sau3AI							
AA	202	5792.5±1436.1[a]	174	194.5±47.49	174	232.3±59.78	174
AB	79	5334.6±1547.1[a]	65	181.1±51.68	65	216.4±66.57	65
BB	7	5098.6±1536.8[a]	7	173.2±59.96	7	198.5±72.92	7
PitI/HinfI							
AA	15	5163.8±1402.5	15	175.0±45.42	15	206.3±58.50	15
AB	100	5798.0±1469.9	89	196.3±49.57	89	233.4±60.77	89
BB	173	5611.6±1489.6	142	188.2±49.15	142	225.4±63.48	142

[a] significance of difference at $P \leq 0.05$

Table 3 shows the average values of milk, protein and fat yield in standard length of lactation of analyzed cow's population. In subsequent analyses were used only animals with relevant data of production parameters. A result from the association analysis shows significant effect ($P \leq 0.05$) only between LEP/*Sau*3AI genotypes and milk yield. In standard length of lactation produced cows with AA genotype significantly more milk (kg). In comparison with AB and BB genotype were the difference amounting 457.9 and 293.9 kg, respectively. The allele A seems to increase potentially milk yield and therefore animals with AA genotype are useful in dairy cattle breeding program. Similarly were the yields of milk fat and protein higher in homozygous AA cows, but no significant. Significant of differences in comparing average value of these parameters was also affected by number of individuals, when the differences were very low. Our findings are contrary to other association studies, generally is associated with higher milk performance traits the B allele or heterozygous AB genotype. Liefers et al. (2002) reported that heifers with the *Sau*3AI AB genotype produce 1.32 kg/day more milk compared with *Sau*3AI AA genotype. They suggested that B allele could yield a higher milk production without negatively affecting energy balance and fertility. Heravi et al. (2006) evaluated the association of genetic differences in the leptin gene and milk yield, reproduction, body condition score and plasma glucose level in Holstein cows. A significant association was detected between the AB genotype and 305-d milk yield. The results demonstrated that the B allele can yield a higher 305-d milk production with a trend to better reproductive performance. Javanmard et al. (2010) found association between LEP Sau*3AI* polymorphism and milk fat. In evaluated population animal with AA had lower milk fat production than AB genotype. In other our study of Slovak Spotted and Pinzgau cattle populations (Moravčíková et al., 2012) was also reported no significant effect of LEP gene on milk production traits, similarly produced cows with AA genotype in long-life milk production more milk compared to AB and BB cows. The results of statistical analysis show statistically non significant differences between the mean values of milk, protein and fat yield in standard length of lactation and different Pit-1/*Hin*fI genotypes (Table 4). Milk, protein and fat yield were higher in cows with heterozygous AB genotype, but the differences were very low. Also in the study De Mattos et al. (2004) was heterozygous AB genotype superior for fat milk production in relation to homozygous BB genotype. Viorica (2006) reported in Simmental cattle associations between allele A and better milk performance, that

genotypes favourable for selections were AA and AB. In the study Edriss et al. (2009) affected genotype BB significant negatively fat and protein yield and positively birth weight with comparison AA and AB genotype. Similarly in other studies was demonstrated relationship of Pit-1/*Hin*fI polymorphisms and growth traits. Yang et al. (2010) reported in population associations analyse of different cattle breed with growth traits as a dominant allele B and genotype BB with trend to higher body weight and body size. Oprządek et al. (2003) found by evaluation growth, feed conversation and carcass quality significant effect of Pit1/*Hin*f1 gene polymorphism only on carcass dimension, when the most frequent was BB. Combination of molecular and statistical analyses of genes polymorphisms associations with production traits can be strong tools in future breeding dairy cattle programs. Milk performance traits of dairy cattle are in relationship with different factors of body condition, energy balance or animals health and therefore is LEP or Pit-1 interesting as genetic markers in marker assisted selection. In case of Slovak Simmental cattle is necessary to verify this relationship in further associations analysis with more animals in evaluated cattle population.

Conclusion

Single nucleotide polymorphisms in bovine genes encoding leptin and specific pituitary transcription factor in population of 288 Slovak Simmental cows were detected. Genotyping was carried by the multiplex PCR-RFLP analysis. In population of cows were dominant of LEP/*Sau*3AI polymorphism allele A (0.8385) and genotype AA (0.7014). In case of Pit-1/*Hin*fI polymorphism was frequent the B allele (0.7743) and dominant BB genotype (0.6007). Animals with homozygous AA (LEP/*Sau*3AI) and BB (Pit-1/*Hin*fI) genotype had higher milk, protein and fat yield in standard length of lactation, but the results were statistically significant only between LEP/*Sau*3AI and milk yield. Until now has been confirmed potential effect of polymorphism in Pit-1 and LEP genes on cattle production performance in many studies. The relationships with reproduction, milk production and growth traits were found for LEP gene. In contrary to our findings results from associations analyses suggested for milk performance traits as preferable allele B and genotype AB. Analyses of Pit-1 genotype effect on production shows potential positively effect of B allele occurrence on growth and negatively on milk production performance. Preferably average values for milk, growth and reproduction parameters had animals with heterozygous genotype AB and therefore were favourable for selection in breeding dairy or beef cattle programs. Based on these findings it can be said that the genotype AB (LEP/*Sau*3AI) and BB (Pit-1/*Hin*fI) had potential positive effect on evaluated parameters and participation of cows with these allele in genotype in future selection breeding program can increase milk performance traits.

Acknowledges

This work was supported by the Slovak Research and Development Agency under the contract No. APVV-0636-11 and by the project VEGA No. 1/0486/13.

References

Almeida, S. E. M, Almeida, E. A., Moraes, J.C.F., Weimer, T. A., (2003) Molecular markers in the LEP gene and reproductive performance of beef cattle. Journal of Animal Breeding and Genetics, 120, 106-113.

Beigi Nassiri, M. T., Biranvand, Z., Hartatik, T., Fayazil, J., Tavakoli, S., (2010) The Study of PIT1 Gene Polymorphism in the Najdi Cattle Using PCR-RFLP Method. Journal of Animal and Veterinary Advances, 9, 2001-2003.

Block, S. S., Butler, W. R., Ehrhardt, R. A., Bell, A. W., Van Amburgh, M. E., Boisclair, Y. R., (2001) Decreased aoncentration of plasma leptin in periparturient dairy cows is caused by negative energy balance. Journal of Endocrinology, 171 (2), 339-348.

Brogan R. S., Mitchell., S. E., Trayhurn, P., Smith, M. S., (1999) Suppression of leptin during lactation: contribution of the suckling stimulus versus milk production. Endocrinology, 140 (6), 2621-2627.

Carrijo, S. M., De Alencar, M. M., Toral, F. L. B., Reagitano, L. C., (2008) Association of PIT1 genotypes with growth traits in Canchim cattle. Scientia Agricola, 65, 116-121.

Carsai, T. C., Balteanu, V. A., (2010) The polymorphism of some key genes involved in some lactation traits. Animal Science, 8, 45-49.

Cohen, L. E., Wondisford, F. E., Radovick, S., (1997) Role of Pit- 1 in the gene expression of growth hormone, prolactin and thyrotropin. Endocrinology and Metabolism Clinics of North America, 25, 523-540.

De Mattos, K. K., Del Lama, S. N., Martinez, M. L., Freitas, A. F., (2004) Association of bGH and Pit- 1 gene variants with milk production traits in dairy Gyr bulls. Pesquisa Agropecuária Brasileira, 39, 147-150.

Diekers, B., Kriegesmann, B., Baumgartner, B. G., Brening, B., (1998) Partial genomic structure of the bovine Pit-1 gene and characterization of a HinfI transition polymorphism in exon 6. Animal Genetics, 29, 405.

Dybus, A. – Kmieć, M. – Sobek, Z. – Pietrzyk, W. – Wiśniewski, B., (2003) Associations between polymorphisms of growth hormone releasing hormone (GHRH) and pituitary transcription factor 1 (PIT1) genes and production traits of Limousine cattle. Archiv Tierzucht, 46, 527-534.

Edriss, M. A., Edriss, V., Rahmani, H. R., (2009) Associations of Pit-1 gene polymorphism with birth weight, milk and reproduction traits in Isfahan Holstein cows. Archiv Tierzucht, 52, 445-447.

Feuermann, Y., Mabjeesh, S. J., Niv-Spector, L., Levin, D., Shamay, A., (2006) Prolactin affects leptin action in the bovine mammary gland via the mammary fat pad. Journal of ndocrinology, 191, 407-413.

Gutiérrez-Gil, B., Wiener, P., Nute, G. R., Burton, D., Gill, J. L., Wood, J. D., Williams, J. L., (2008) Detection of quantitative trait loci for meat quality traits in cattle. Animal Genetics, 39 (1), 51-61.

Heravi Moussavi, A., Ahouei, M., Nassiry, M. R., Javadmanesh, A., (2006) Association of leptin polymorphism with production, reproduction and

plasma glucose level in Iranian Holstein Cows. Asian Australian Journal of Animal Science, 19, 627-631.

Hoggard, N., Callaghan, K., Levy, A., Davis, J. R., (1993) Expression of Pit-1 and related proteins in diverse human pituitary adenomas. Journal of Molecular Endocrinology, 11, 283-290.

Houseknecht, K. L., Baile, C. A., Matteri, R. L., Spurlock, M. E., (1998) The biology of leptin: a review. Journal of Animal Science, 76, 1405-1420.

Javanmard, A., Khaledi, K., Asadzadeh, N., Solimanifarjam, A. R., (2010) Detection of polymorphisms in the bovine leptin (LEP) gene: association of single nucleotide polymorphism with breeding value of milk traits in Iranian Holstein Cattle. Journal of Molecular genetics, 2, 10-14.

Kulig H., (2005) Association between leptin combined genotypes and milk performance traits of Polish Black-and-White cows. Archiv Tierzucht, 48 (6), 547-554.

Leury, B. J., Baumgard, L. H., Block, S. S., Segoale, N., Ehrhardt, R. A., Rhoads, R. P., Bauman, D. E., Bell, A. W., Boisclair, Y. R., (2003) Effect of insulin and growth hormone on plasma leptin in periparturient dairy cows. American Journal of Physiology, Regulatory, Integrative and Comparative Physiology, 285, 1107-1115.

Liefers, S. C., Pas, M. F. W., Veerkamp, R. F., Van der Lende, T., (2002) Associations between leptin gene polymorphisms and production, live weight, energy balance, feed intake, and fertility in Holstein heifers. Journal of Dairy Science, 85, 227-238.

Liefers, S. C., Veerkamp, R. F., Te Pas, M. F., Chilliard, Y., Van der Lende, T., (2005) Genetics and physiology of leptin in periparturient dairy cows. Domestical Animal Endocrinology, 29 (1), 227-238.

Lindersoon, M., Andersoon-Eklund, L., De Koning, D. J., (1998) Mapping of Serum Amylase-1 and Quantitative Trait Loci for Milk production Trait to Cattle Chromosome 4. Journal of Dairy Science, 81, 1454-1461.

Madeja, Z., Adamowicz, T., Chmurzynska, A., Jankowski, T., Melonek, J., Switonski, M., Strabel, T., (2004) Short Communication: Effect of Leptin Gene Polymorphisms on Breeding Value for Milk Production Traits. Journal of Dairy Science, 87, 3925-3927.

Miller, S. A. – Dykes, D. D. – Polesky, F. H., (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Research, 16, 1215.

Moody, D. E, 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. Animal Genetics, 26, 45-47.

Moravčíková, N. , Trakovická, A. , Hazuchová, E. , Bujko, J. , Kasarda, R., (2012) Associations between polymorphisms in the leptin gene and milk production traits in Pinzgau and Slovak Spotted cattle. Acta agriculturae Slovenica, Supplement 3, 259-263.

Nkrumah, J. D., Li, C., Yu, J., Hansen, C., Keisler, D. H., Moore, S. S., (2005) Polymorphisms in the bovine leptin promoter associated with serum leptin

concentration, growth, feed intake, feeding behavior, and measures of carcass merit. Journal of Animal Science, 83, 20-28.

Oprządek, J., Fliskowski, K., Zwierzchowski, L., Dymnicki, E., (2003) Polymorphisms at loci of leptin (LEP), Pit1 and STAT5A and their association with growth, feed conversation and carcass quality in Black-and- White bulls. Animal Science Papers and Reports, 21, 135-145.

Ozdemir, M., (2012) Determination of Pit-1/Hinf1 polymorphism in Holstein and native Ear cattle raised as genetic resource in Turkey. Journal of Animal & Plant Sciences, 22, 25-28.

Passos, D. T., Hepp, D., Moraes, J. C. F., Weimer, T. A., (2007) Effect of polymorphisms linked to LEP gene on its expression on adipose tissue in beef cattle. Journal of Animal and Breeding Genetics, 124, 157-162.

Radovick, S., Nations, M., DU, Y., Berg, L. A., Weintraub, D. B., Wondisford, F. E., (1992) A mutation in the POU-homeodomain of Pit-1 responsibile for combined pituitary hormone deficiency. Science, 21, 1115-1118.

Renaville, R., Gengler, N., Vrech, E., Prandi, A., Massart, S., Corradini, C., Bertozzi, C., Mortiaux, F., Burny, A., Portetelle, D., (1997) Pit-1 gene polymorphism, milk yield, and conformation traits for Italian Holstein Friesian bulls. Journal of Dairy Science, 80, 3431-3438.

Rosenfeld, M. G., (1991) POU-domain transcription factors: Powerful development regulators. Genes development, 5, 897-907.

Tuggle, C. K., Trenkle, A., (1996) Control of growth hormone synthesis. Domestic Animal Endocrinology, 13, 11-33.

Viorica, C., (2006) RFLP/HinfI polymorphism between exon 5 and exon 6 of the Pit-1 gene in Romanian Simmental cattle. USAMV Journal, 63, 219-222.

Wayne, J., Kuenzel, W. J., Fraley, G. S., (1995) Neuropeptid Y: It is in the Neural Regulation of Reproductive Function and Food Intake in Mammalian species. Poultry Avian Biology Review, 6 (3), 185-209.

Wiliams, G. L., Amstalden, M., Garcia, M. R., Stanko, R. L., Nizielski, S. E., Morrison, C. D., Keisler, D. H., (2002) Leptin and its role in the central regulation of reproduction in cattle. Domestical Animal Endocrinology, 23, 339-349.

Woollard, J., Schmitz, C. B., Freeman, A. E., Tuggle, C. K., (1994) Rapid communication: HinfI polymorphism at the bovine Pit-1 locus. Journal of Animal Science, 72, 3267.

Yang, D., Zhu, F., Tang, L., He, G., Chen, H., (2010) Relationship of Pit-1 polymorphisms with growth traits in Chinese Cattle. 2010 International Conference on Biology, Environment and Chemistry (Proceding of the work of the International Scientific Conference), Singapore, 2010

Zhao, Q., Davis, M. E., Hines, H. C., (2004) Associations of polymorphisms in the Pit-1 gene with gowth and carcass traits in Angus beef cattle. Journal of Animal Science, 82 (8), 2229-2233.