JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

ASSESSMENT OF CHAROLAIS BULLS POPULATION STRUCTURE BASED ON SNPs ANALYSES

Nina Moravčíková, Tomáš Minarovič, Anna Trakovická*

Address(es): Ing. Nina Moravčíková, PhD.,
[1]Slovak University of Agriculture in Nitra, Faculty of Agrobiology and Food Resources, Department of Animal Genetics and Breeding Biology, Tr. A. Hlinku 2, 94976 Nitra, Slovak Republic, phone number: +421 37 641 4824.

*Corresponding author: nina.moravcikova1@gmail.com

ARTICLE INFO

Received 16. 10. 2013
Revised 12. 11. 2013
Accepted 8. 1. 2014
Published 1. 2. 2014

Regular article

OPEN ACCESS

ABSTRACT

The aim of this study was identification of SNPs in leptin (*LEP*), leptin receptor (*LEPR*), growth hormone (*GH*) and specific pituitary transcription factor (*Pit-1*) genes in order to analyze genetic structure of Charolais bulls' population. The total numbers of genomic DNA samples were taken from 52 breeding bulls and analyzed by PCR-RFLP method. After digestion with restriction enzymes were detected in bulls' population alleles with frequency: LEP/*Sau*3AI A 0.83 and B 0.17 (±0.037); LEPR/*Bse*GI C 0.95 and T 0.05 (±0.021), GH/*Alu*I L 0.62 and V 0.38 (±0.048) and Pit1/HinfI A 0.40 and B 0.60 (±0.048). Based on the observed vs. expected genotypes frequencies population across loci were in Hardy-Weinberg equilibrium (P>0.05), only in case of *Pit-1* locus was detected disequilibrium. Predominant were in analyzed breeding bulls *LEP/Sau3AI*^{AA} (0.69), *LEPR/T945M*^{CC} (0.90), *GH/AluI*^{LL} (0.43) and *Pit-1/HinfI*^{AB} (0.65) genotypes. The observed heterozygosity of SNPs was also transferred to the low (*LEP/Sau3AI*/0.248 and *LEPR/T945M*/0.088) or median polymorphic information content (*GH/AluI*/0.366 and *Pit-1/HinfI*/0.370). Within genetic variability estimating negative (*LEPR/T945M* and *Pit-1/HinfI*) and positive values (*LEP/Sau3AI* and *GH/AluI*) of fixation indexes F_{IS} indicating slight heterozygote excess or deficiency based on analyzed genetic marker were observed.

Keywords: cattle, GH, genetic diversity, LEP, LEPR, Pit-1, polymorphism

INTRODUCTION

Farm animal genetic diversity is required to meet current production needs in various environments, to allow sustained genetic improvement, and to facilitate rapid adaptation to changing breeding objectives. Key questions in management of farm animal genetic diversity involve the distribution of potentially useful quantitative trait locus alleles among livestock breeds and populations. A greater understanding of the potential that these mechanisms have for supporting long-term genetic improvement and genetic relationship among livestock populations are priorities for managing farm animal genetic diversity (**Notter, 1999**). Recent developments in molecular biology and statistics have opened the possibility of identifying and using genomic variation and major genes for the genetic improvement of livestock. During the last 20 years, use of molecular markers has been playing an important part for investigating genetic diversity and structure in livestock animals. In cattle, the analyses of population structure and diversity have provided insight into their origin and evolution (**Lin *et al.*, 2010**). In recent years, single nucleotide polymorphism (SNP) has attracted attention as an alternative marker. Recently, a large number of SNP markers have become available in cattle (**Tassell *et al.*, 2008**), and it is possible to perform genome-wide population genetic analyses. Although biallelic SNP is less informative than multiallelic microsatellites, it can be powerful when many SNP loci are applied for genetic analyses (**Lin *et al.*, 2010**).
In this study were as genetic markers used 4 SNPs – *LEP/Sau3AI*, *LEPR/7945M*, *GH/AluI* and *Pit-1/HinfI*, significantly associated with production traits in cattle. These SNPs affecting phenotypes of different traits in livestock and can be used also as genetic marker for enhancing the productivity of cattle and are potential candidates for marker assisted selection in breeding strategies. Leptin (*LEP*) is a polypeptide hormone synthesized predominantly in the adipose tissue and affects a number of processes in the body. It is involved in maintaining the energy equilibrium by controlling food intake and energy expenditure, as well as in regulating reproductive functions and immune response (**Zhang *et al.*, 1994**). The leptin gene is located on bovine chromosome 4q32. Its DNA sequence has more than 15,000 base pairs and contains three exons, which are separated by two introns (**Stone *et al.*, 1996**). The SNP *LEP/Sau3AI* is situated in the second intron and results in amino acid change at position 2059 of the protein chain (cytosine, C to thymine, T). Effects of leptin are exerted trough six receptors isoforms, but only its longest form (*LEPR-b*) is fully functional and responsible for most hormone physiological functions of leptin (**Tartaglia, 1997**). The *LEPR* gene is located on bovine chromosome 3. The leptin receptor gene consists of 20 exons divided over 1.75 Mb. Inside the *LEPR* gene, **Liefers *et al.* (2004)** described a missense mutation *T945M*. It is a cytosine to thymine base substitution at position 115 in exon 20, which results in a substitution of the amino acid (threonine, T to methionine, M) at residue 945 of leptin. Bovine growth hormone (*GH*) a single chain polypeptide produced in the anterior pituitary gland is necessary for tissue growth, fat metabolism and homeorhesis, thus, it has an important role in reproduction, lactation and normal body growth (**Svennersten-Sjaunja & Olsson, 2005**). The *GH* gene with approximately 1800 bp length, five exons and four introns is a part of multiple gene family that contains prolactin and placental lactogenes and assigned with chromosome region 19q26 in bovine genome. **Lucy *et al.* (1993)** reported a polymorphic site for *Alu*I restriction endonuclease, localized in the exon 5 in bovine *GH* gene and characterized by the substitution of cytosine for guanine at position 2,141 caused an amino acid change from leucine to valine at residue 127. *Pit-1* is the cellular specific transcription factor for activating expression of growth hormone, prolactin and thyrotropin β-subunit genes in anterior pituitary gland but also is a regulatory factor in differentiation and proliferation of cells of pituitary gland (**Tuggle and Trenkle, 1996**). The Pit-1 gene located on bovine chromosome 1 (**Moody *et al.*, 1995**) consists of 6 exons. In the 6 exon of bovine *Pit-1* gene the restriction fragment length polymorphism using restriction enzyme *HinfI* detected **Moody *et al.* (1995)**.
The objective of our study was assessment of genetic diversity and population structure of Charolais breeding bulls population based on analyses of SNPs associated with cattle production traits.

MATERIAL AND METHODS

DNA samples collection

Biological samples were collected from total of 52 Charolais breeding bulls. Genomic DNA for analyses was extracted from whole blood samples with extraction method according to **Miller *et al.* (1988)**. DNA concentrations were estimated by spectrophotometer measuring the optical density at wave length of 260 nm.

Analyses of SNPs and genotyping

Genotyping of *LEP*, *LEPR*, *GH* and *Pit-1* genes was performed by PCR-RFLP method. In table 1 are presented sequence of the primers, size and region of the amplified fragments in PCR and observed genotypes with size of the digestion patterns. A 422 bp and 260 bp fragments covering sequences containing polymorphic sites involving A and B allelic types of *LEP* and *Pit-1* genes were amplified by multiplex PCR using primers and procedure according **Liefers *et al.* (2002)** and **Ozdemir (2012)**. After amplification genotyping of *LEP* and *Pit-1* allelic variants was carried out by digestion of PCR products with *Sau*3AI and *Hin*fI restriction endonucleses, respectively. To analyze loci of *LEPR* and *GH* genes, a 197 bp and 428 fragments covering the sequences containing the mutation sites were amplified according to the protocols proposed by **Almeida *et al.* (2008)** and **Balogh *et al.* (2009)**, respectively. The PCR products were digested with *Bse*GI and *Alu*I restriction endonucleases to differentiate alleles C/T for *LEPR* and L/V for *GH* genes. Results from PCR amplifications and digestion of PCR products were analyzed by horizontal electrophoresis in 3% agarose gels in 0.5 x TBE (130 V for 40 min) stained with GelRed (Biotium) prior to visualization under UV light.

Table1 Sequence of the primers, size and region of the amplified fragments in PCR and observed genotypes with size of the digestion patterns

Gene	Primer sequences	Size (bp)	Amplified region	Genotype/Digestion patterns (bp)	Reference
LEP	F 5´ -TGG AGT GGC TTG TTA TTT TCT TCT- 3´ R 5´-GTC CCC GCT TCT GGC TAC CTA ACT- 3´	422	intron	AA 390, 32 AB 390, 303, 88, 32 BB 303, 88, 32	**Liefers *et al.* (2002)**
Pit-1	F 5´ -ACT CGC TAT TAC ACA ATA GGA GAG CCT- 3´ R 5´-TCC TGC CAA CTC CTC ACC TCC C - 3´	260	exon	AA260 AB260,190,70 BB190,70	**Ozdemir (2012)**
LEPR	F 5´-ACTACAGATGCTCTACTTTGG-3´ R 5´-TGCTCCTCCTCAGTTT-3´	197	exon	CC 130, 67 CT 130, 93, 67, 37 TT 93, 67, 37	**Almeida *et al.* (2008)**
GH	F: 5´-CGGACCGTGTCTATGAGAAGCTGAAG-3´ R: 5´-GTTCTTGAGCAGCGCGTCGTCA-3´	428	exon	LL 265, 96, 51, 16 LV 265, 147, 96, 51, 16 VV 265, 147, 16	**Balogh *et al.* (2009)**

Statistical analyses

Frequency of alleles and genotypes of analyzed markers for the entire population were estimated by direct counting and the differences of the observed and expected frequencies of genotypes were tested using Chi-square (χ^2) analysis in order to verify if the population was in Hardy-Weinberg equilibrium. Differences between the distributions of genotypic frequencies were also tested by F_{IS} fixation index. This Wright's fixation index was used further as a measure of heterozygote excess or deficiency. Together with heterozygosity and the estimation of Hardy-Weinberg equilibrium, these indexes allowed further comprehension of the reproductive structure of analyzed populations. Genetic indices of populations, including observed and expected genes heterozygosity (H_e), homozygosity (H_o), effective allele numbers (N_e) and fixation index (F_{IS}) were performed by Popgene32 software version 1.3 (**Yeh *et al.*, 2000**). Moreover, polymorphism information content (PIC) was calculated according to **Botstein *et al.* (1980)**.

RESULTS AND DISCUSSION

In table 2 are presented frequencies of individual alleles and genotypes in the analyzed populations of Charolais bulls. The highest frequencies were observed for *LEP/Sau3AI*^{AA}, *LEPR/T945M*^{CC}, *GH/AluI*^{LL} homozygotes and *Pit-1/HinfI*^{AB} heterozygotes, lower for heterozygous *LEP/Sau3AI*^{AB}, *LEPR/T945M*^{CT}, *GH/AluI*^{LV} and *Pit-1/HinfI*^{BB} genotypes, and lowest for the homozygous *LEP/Sau3AI*^{BB}, *GH/AluI*^{VV} and *Pit-1/HinfI*^{AA} genotypes. The *LEPR/T945M*^{TT} homozygous genotype was not detected in analyzed group of breeding bulls. Populations of Charolais bulls were in Hardy-Weinberg equilibrium (P>0.05). Only in one case was observed Hardy – Weinberg disequilibrium (P<0.05) caused by the differences between the observed and expected frequencies of *Pit-1/HinfI* genotypes.

Table 2 Genetic structure of analyzed population of Charolais bulls

Genotype frequency			Allele frequency		χ^2 test
LEP/Sau3AI^{AA}	*LEP/Sau3AI*^{AB}	*LEP/Sau3AI*^{BB}	*LEP/Sau3AI*^{A}	*LEP/Sau3AI*^{B}	
0.69	0.27	0.04	0.827±0.037	0.173±0.037	0.256^{-}
LEPR/T945M^{CC}	*LEPR/T945M*^{CT}	*LEPR/T945M*^{TT}	*LEPR/T945M*^{C}	*LEPR/T945M*^{T}	
0.90	*0.10*	-	*0.951±0.021*	*0.049±0.021*	0.393^{-}
GH/AluI^{LL}	*GH/AluI*^{LV}	*GH/AluI*^{VV}	*GH/AluI*^{L}	*GH/AluI*^{V}	
0.43	0.38	0.19	0.615±0.048	0.385±0.048	2.027^{-}
Pit-1/HinfI^{AA}	*Pit-1/HinfI*^{AB}	*Pit-1/HinfI*^{BB}	*Pit-1/HinfI*^{A}	*Pit-1/HinfI*^{B}	
0.08	0.65	0.27	0.404±0.048	0.596±0.048	6,315^{+}

$^{-}$P > 0.05, $^{+}$P < 0.05

The observed heterozygosity, effective allele numbers, polymorphism information content and fixation index of SNPs *LEP/Sau3AI*, *LEPR/T945M*, *GH/AluI* and *Pit-1/HinfI* in analyzed population are presented in Table 3.The loci that presented both alleles at frequency of around 0.50 had higher heterozygosities (*GH* and *Pit-1*), while loci showing one allele at a much higher frequency than other showed lower values (*LEP* and *LEPR*). According to the classification of polymorphic information content bulls population belonged to median or low polymorphism level. Provided that the in biallelic system both alleles result in the genotypes creation is threshold value of N_e 2,000 comparison of loci N_e showed higher effective allele numbers in loci *GH* and *Pit-1*. Observed Wright's fixation indexes F_{IS} had negative values for SNPs *LEPR/T945M* and *Pit-1/HinfI*. These negative values represent slight excess of heterozygote compared with Hardy-Weinberg equilibrium expectations. Values observed for investigated genetic markers suggest (values close to zero) a condition of equilibrium in the population as confirmed by the results of $\chi 2$ test used to verify Hardy-Weinberg equilibrium.

Table 3 Genetic diversity parameters evaluated in population of Charolais bulls

Genetic marker	H_o	H_e	N_e	PIC	F_{IS}
LEP/Sau3AI	0.711	0.289	1.401	0.248	0.069
LEPR/T945M	0.908	0.092	1.101	0.088	-0.041
GH/AluI	0.522	0.478	1.899	0.366	0.197
Pit-1/HinfI	0.514	0.486	1.929	0.370	-0.349

H_o – observed homozygosity, H_e – observed heterozygosity, N_e – effective allele numbers; PIC – polymorphism information content

In comparison with our results reported **Passos *et al.* (2007)** in population of Brangus Ibage cattle and **Kulig and Kmieć (2009)** in Limousin cattle similar high frequency of *LEP/Sau3AI*^{A} allele (0.91 and 0.81). **Othman *et al.* (2011)** detected in several beef cattle breeds total dominance of *LEP/Sau3AI*^{A} allele. In addition to structure of Limousin calves population **Kulig and Kmieć (2009)** revealed, that the *LEP* genotypes affected significantly average daily gain between 3 and 210 days. Significance levels for mean live weight and live weight at week 15 of different *LEP/Sau3AI* genotype were presented in study **Liefers *et al.* (2002)**. These authors reported that the genotype AB tended to show the

higher body weight in comparison with genotype AA. The *LEP* gene has been also associated with higher leptin mRNA levels in adipose tissue and increased fat deposition in mature beef cattle (**Kononoff *et al.*, 2005**). The absence of *LEPR/T945M*^{TT} genotype was observed similarly in studies **Almeida *et al.* (2008)** and **Silva *et al.* (2012)**. In contrary **Pinto et al. (2011)** detected in population of Nelore cattle *LEPR/T945M*^{TT} genotype, but also with low frequency. **Almeida *et al.* (2008)** reported high levels of genetic diversity of studied beef cattle population based on averaged heterozygosity (0.71 – 0.81) of analyzed genetic markers. Authors evaluated also postpartum cows production and the effect of *LEPR/T945M* genotype on reproduction and weight gain of postpartum cows was not detected. **Ferraz *et al.* (2009)** and **Silva *et al.* (2012)** detected significant effect of T945M genotypes on weight gain of evaluated animals. Dominance of *GH/AluI*^{LL} genotype was also observed in populations of different beef cttle breeds (**Krasnoporiova *et al.*, 2012**), Canchim cattle (**Silveira *et al.*, 2008**) and Limousin cattle (**Hartatik *et al.*, 2013**). In contrary with our results was detected in several beef cattle breeds dominance of *Pit-1/HinfI*^{BB} genotype (**Carrijo *et al.*, 2008**; **Dybus *et al.*, 2003**). Single nucleotide polymorphisms *GH/AluI* and *Pit-1/HinfI* were also evaluated as markers for beef cattle production. The effect of *GH/AluI* on growth and production performance of cattle was confirmed in study **Tambasco *et al.* (2003)**, **Maj *et al.* (2004)** and **Ruprechter *et al.* (2011)**. The SNP *Pit-1/HinfI* was indicated as available genetic marker for evaluation of beef cattle growth in studies **Carrijo *et al.* (2008)** and **Yang *et al.* (2010)**.

CONCLUSION

Preservation of gene diversity in beef cattle populations is crucial for their genetic resources conservation and long-term survival. The results presented in this paper shows important findings of allelic richness and level of heterozygosity of charolais breeding bulls, which shows that this reserve is a precious source of genetic variation that should be maintained or used in beef cattle improvement. The analysis of SNPs indicated a relatively high variability of the breeding bulls' populations herein investigated, suggesting that the artificial selection applied to the Charolais breed has not reduced their diversity at least in these systems.

Acknowledgments: This work has been supported by the Excellence Centre for Agrobiodiversity Conservation and Benefit project implemented under the Operational Programme Research and Development financed by European Fund for Regional Development (project number 26220120015) and by the Slovak Research and Development Agency under the contract No. APVV-0636-11 and No. LPP-0220-09).

REFERENCES

ALMEIDA, S.E.M, SANTOS, L.B.S., PASSOS, D.T., CORBELLINI, A.O., LOPES, B.M.T., KIRST, C., TERRA, G., NEVES, J.P., GONCALVES, P.B.D., MORAES, J.C.F., AZEVEDO WEIMER, T. 2008. Genetic polymorphsims at the leptin receptor gene in three beef cattle breeds. *Genetics Molecular Biology*, 31(3), 680-685.

BALOGH, O., KOVÁCS, K., KULCSÁR, M., GÁSPÁRDY, A., ZSOLNAI, A., KÁTAI, L., PÉCI, A., FÉSÜS, L., BUTLER, W.R., HUSZENICZA, G. 2009. *Alu*I polymorphism of the bovine growth hormone (GH) gene, resumption of ovarian cyclicity, milk production and loss of body condition at the onset of lactation in dairy cattle. *Theriogenology*, 71(4), 553-559.

BOTSTEIN, D., WHITE, R. L., SKOLNIK, M., DAVIS, R. W. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphism. *American Journal of Human Genetics*, 32 (3), 314-331.

CARRIJO, S.M., DE ALENCAR, M.M., TORAL, F.L.B., CORREIA, L., REGITANO A. 2008. Association of PIT1 genotypes with growth traits in Canchim cattle. *Scientia Agricola*, 65 (2), 116-121.

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 (6), 527-534.

FERRAZ, J.B.S., PINTO, L.F.B., MEIRELLES, F.V., ELER, J.P., DE REZENDE, F.M., OLIVEIRA, E.C.M., ALMEIDA, H.B., WOODWARD, B., NKRUMAH, D. 2009. Association of single nucleotide polymorphisms with carcass traits in Nellore cattle. *Genetics and Molecular Research*, vol. 8 (4), 1360-1366.

HARTATIK, T., VOLKANDARI, S. D., RACHMAN, M. P., SUMADI. 2013. Polymorphism leu/val of Growth Hormone Gene Identifi ed from Limousin Cross Local Cattle in Indonesia. *Procedia Environmental Sciences*, 17, 105-109.

KONONOFF, P.J., DEOBALD, H.M., STEWART, E.L., LAYCOCK, A.D., MARQUESS, F.L. 2005. The effect of a leptin single nucleotide polymorphism on quality grade, yield grade, and carcass weight of beef cattle. *Journal of Animal Science*, 83 (4), 927-932.

KRASNOPIOROVA, N., BALTRĖNAITĖ, L., MICEIKIENĖ, I. 2012. Growth hormone gene polymorphism and its influence on milk traits in cattle bred in Lithuania. *Veterinary Medicine and Zootechnics*, 58 (80), 42-46.

KULIG, H., KMIEĆ, M. 2009. Association between leptin Gene Polymorphisms and growth Traits in Limousin Cattle. *Russian Journal of Genetics*, 45 (6), 738741.

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 (6), 227-238.

LIEFERS, S.C., VEERKAMP, R.W., TE PAS, M.F.W., DELAVAUD, C., CHILLIARD, Y., VAN DER LENDE, T. 2004. A missense mutation in the bovine leptin receptor gene is associated with leptin concentrations during late pregnancy. *Animal Genetics*, 35 (2), 138-141.

LIN, B.Z., SASAZAKI, S., MANNEN, H. 2010. Genetic diversity and structure in *Bos taurus* and *Bos indicus* populations analyzed by SNP markers. *Animal Science Journal*, 81 (3), 281-289.

LUCY, M.C., HAUSER, S.D., EPPARD, P.J., KRIVI, G.G., CLARK, J.H., BAUMAN, D.E., COLIER, R.J. 1993. Variants of somatotropin in cattle: gene frequencies in major dairy breeds and associated milk production. *Domestic Animal Endocrinology*, 10 (4), 325-333.

MAJ, A., OPRZADEK, J., OPRZADEK, A., DYMNICKI, E., ZWIERCHOWSKI, L. 2004. Polymorphism in the 5´-noncoding region of the bovine growth hormone receptor gene and its association with meat production traits in cattle. *Animal Research*, 53 (6), 503-514.

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 (3), 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 (1), 45-47.

NOTTER, D.R. 1999. The importance of Genetic Diversity in Livestick Populations of the Future. *Journal of Animal Science*, 77 (1), 61-69.

OTHMAN, O.E., ZAYED, F.A., EL GAWEAD, A.A. 2011. Genetic polymorphism of two genes associated with carcass trait in Egyptian buffaloes. *Journal of Genetic Engineering and Biotechnology*, 9 (1), 15-20.

OZDEMIR, M. 2012. Determination of Pit-1/Hinf1 polymorphism in Holstein and native Ear cattle raised as genetic resource in Turkey. In *Journal of Animal & Plant Sciences*, vol. 22, 2012, p. 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 Breeding and Genetics*, 124 (3), 157-162.

PINTO, L.F., FERRAZ, J.B., PEDROSA, V.B., ELER, J.P., MEIRELLES, F.V., BONIN, M.N., REZENDE, F.M., CARVALHO, M.E., CUCCO, D.C., SILVA, R.C. 2011. Single nucleotide polymorphisms in CAPN and leptin genes associated with meat color and tenderness in Nellore cattle. *Genetics and molecular research*, 10 (3), 2057-2064.

RUPRECHTER, G., CARRIQUIRY, M., RAMOS, J.M., PEREIRA I., ANA, M. 2011. Metabolic and endocrine profiles and reproductive parameters in dairy cows under grazing conditions: effect of polymorphisms in somatotropic axis genes. *Acta Veterinaria Scandinavica*, vol. 53 (1), 35.

SILVA, R.C., FERRAZ, J.B., MEIRELLES, F.V., ELER, J.P., BALIEIRO, J.C., CUCCO, D.C., MATTOS, E.C., REZENDE, F.M., SILVA, S.L. 2012. Associations of single nucleotide polymorphisms in the bovine leptin gene and leptin receptor genes with growth and ultrasound carcass traits in Nellore cattle. *Genet Mol Res*, 11 (4), 3721-3728.

SILVEIRA, L.G.G., FURLAN, L.R., CURI, R.A., FERRAZ, A.L.J., ALENCAR, M.M., REGITANO, L.C.A., MARTINS, C.L., BENI ARRIGONI, M., SUGUISAWA, L., SILVEIRA, A.C., OLIVEIRA, H.N. 2008. Growth hormone 1 gene (GH1) polymorphisms as possible markers of the production of beef cattle using the Brazilian Canchim breed as a model. In *Genetics and Molecular Biology*, 31 (4), 874-879.

STONE, R.T., KAPPES, S.M., BEATTIE, C.W. 1996. The bovine homology of the obese gene maps to chromosome 4. *Mammalian Genome*, 7 (5), 399-400.

SVENNERSTEN-SJAUNJA, K., OLSSON, K. 2005. Endocrinology of milk production. *Domestic Animal Endocrinology*, 29 (2), 241-258.

TAMBASCO, D. D., PAZ, C. C. P., TAMBASCO-STUDART, M., PEREIRA, A.P., ALENCAR, M.M., FREITAS, A.R., COUTINHO, L.L., PACKER, U., REGITANO, L.C.A. 2003. Candidate genes for growth traits in beef cattle crosses Bos taurus x Bos indicus. *Journal of Animal Breeding and Genetics*, 120 (1), 51-56.

TARTAGLIA, L.A. 1997. The leptin receptor. *Journal of Biological Chemistry*, 7 (272), 6093-6096.

TASSELL, C., SMITH, T., MATUKUMALLI, L., TAYLOR, J., SCHNABEL, R., LAWLEY, C., HAUDENSCHILD, C., MOORE, S., WARREN, W., SONSTEGARD, T. 2008. SNP discovery and allele frequency estimation by deep sequencing of reduced representation libraries. *Nature Methods*, 5 (3), 247-252.

TUGGLE, C. K., TRENKLE, A. 1996. Control of growth hormone synthesis. *Domestic Animal Endocrinology*, 13 (1), 11-33.

YANG, D., ZHU, F., TANG, L., HE, G., CHEN, H. 2010. Relationship of Pit-1 polymorphisms with growth traits in Chinese Cattle. In *2010 International*

Conference on Biology, Environment and Chemistry (Proceding of the work of the International Scientific Conference), Singapore, 2010.
YEH, F.C., YANG, R., BOYLE, T.J., YE, Z., XIYAN, J.M. 2000. PopGene32, Microsoft Windows-based freeware for population genetic analysis, version 1.32. *Mol. Biol. Biotechnol*. Centre, Univ. Alberta, Edmonton, Alberta, Canada.
ZHANG, Y., PROENCA, R., MAFFEY, M., BARONE, M., LEOPPLD, L., FRIEDMAN, J.M. 1994. Positional cloning of the mouse obese gene and its human homologue. *Nature*, 372(6505), 425-432.