BENEDITO RODRIGUES DA SILVA NETO
(ORGANIZADOR)

A MEDICINA VOLTADA À PROMOÇÃO DA SAÚDE E DO BEM-ESTAR

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CIÊNCIAS BIOLÓGICAS E DA SAÚDE

A medicina voltada à promoção da saúde e do bem-estar

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Dados Internacionais de Catalogação na Publicação (CIP)

M489 A medicina voltada à promoção da saúde e do bem-estar / Organizador Benedito Rodrigues da Silva Neto. – Ponta Grossa - PR: Atena, 2023.

Formato: PDF
Requisitos de sistema: Adobe Acrobat Reader
Modo de acesso: World Wide Web
Inclui bibliografia
ISBN 978-65-258-1004-1
DOI: https://doi.org/10.22533/at.ed.041231502

1. Medicina. 2. Saúde. I. Silva Neto, Benedito Rodrigues da (Organizador). II. Título.

CDD 610

Elaborado por Bibliotecária Janaina Ramos – CRB-8/9166

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APRESENTAÇÃO

Sabemos que classicamente a saúde é definida pela Organização Mundial da Saúde (OMS) como “o bem-estar físico, mental e social, envolvendo algo a mais do que a mera ausência de doença”. Com esse conceito em mente podemos também definir a promoção da saúde como o conjunto de políticas, planos e programas de saúde pública com ações individuais e coletivas voltadas, para evitar que as pessoas se exponham a situações que podem causar doenças. Deste modo entendemos que promover o bem-estar populacional é bem mais que prevenir doenças.

Com este conceito abrangente em mente é que desejamos recomendar a nova obra intitulada “A medicina voltada à promoção da saúde e do bem-estar” apresentada inicialmente em dois volumes.

Se promover a saúde não se limita a melhorar apenas a saúde, mas envolve melhorar a qualidade de vida e o bem-estar, torna-se necessária uma perspectiva multidisciplinar integradas e em redes, utilizando-se das ciências biológicas, ambientais, psicológicas, físicas e médicas. Deste modo almejamos oferecer ao nosso leitor uma produção científica de qualidade fundamentada no fato de que a integridade da saúde da população aprofundando no conhecimento nas diversas técnicas de estudo do campo médico que tragam retorno no bem estar físico, mental e social da população.

Esta obra, portanto, compreende uma comunicação de dados muito bem elaborados e descritos das diversas sub-áreas da saúde.

A obra “A medicina voltada à promoção da saúde e do bem-estar” oferece ao nosso leitor uma teoria bem fundamentada desenvolvida em diversos pesquisadores de maneira concisa e didática. A divulgação científica é fundamental para o desenvolvimento e avanço da pesquisa básica em nosso país, e mais uma vez parabenizamos a estrutura da Atena Editora por oferecer uma plataforma consolidada e confiável para estes pesquisadores divulguem seus resultados.

Desejo à todos um ano de 2023 rico em conhecimento cientifico!

Benedito Rodrigues da Silva Neto

SUMÁRIO

INVESTIGATION OF THE POLYMORPHISM IN THE LEPTIN GENE IN BUFFALO HERDS OF NORTHEASTERN BRAZIL AND ITS ASSOCIATION WITH MILK PRODUCTION

Data de submissão: 09/12/2022 *Data de aceite: 01/02/2023*

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ABSTRACT: Genetic polymorphism LEP-1620 (A / G), located in intron 2 leptin gene, has been associated with characteristics as percentage of fat, protein and other characteristics of milk production in cattle. However, in the literature there are few studies related to this polymorphism to milk production in buffaloes. Thus, the objective of this study was to identify the presence of the LEP-1620 (A / C) polymorphism in the gene which coding for leptin hormone in buffaloes and verify their associations with milk production. Blood samples were collected from 139 buffaloes Murrah from two farms of the Northeast of Brazil being A farm in the state of Pernambuco and F farm in the state of Alagoas. After DNA extraction, the samples were genotyped by PCR-RFLP technique. Genotypes AA, AG, GG were found, GA was associated with higher milk production by buffalo from farm A when compared to the buffalo from farm F. These genotypes were not associated with the variables analyzed: lactation days and average daily production. From these data, we can conclude that further studies on polymorphisms in other regions of this gene should be carried out so that we can better understand the function of leptin and its effects on buffalo milk production.
KEYWORDS: Buffalo, Murrah, blood, SNP.

RESUMO: O polimorfismo genético LEP-1620 (A / G), localizado no íntron 2 do gene leptina, tem sido associado a porcentagem de gordura, proteína e outras características da produção de leite em bovinos. No entanto, na literatura existem poucas publicações relacionadas a esse polimorfismo na produção de leite em búfalos. Assim, o objetivo deste estudo foi identificar a presença do polimorfismo LEP-1620 (A / C) no gene que codifica o hormônio leptina em búfalos e sua associação com a produção de leite. Assim, foram coletadas amostras de sangue de 139 búfalas Murrah de duas fazendas denominadas A e F no Nordeste do Brasil, sendo que a fazenda A fica no estado de Pernambuco e a fazenda F no estado de Alagoas. Após a extração do DNA, as amostras foram genotipadas pela técnica de PCR-RFLP. Dos resultados, os seguintes genótipos foram encontrados: AA, AG e GG, sendo que o genótipo AG foi associado à maior produção de leite de búfalas da fazenda A quando comparado ao de búfalas da fazenda F. Entretanto, esses genótipos não foram associados às variáveis analisadas como, dias de lactação e produção média diária. A partir desses dados podemos concluir que outros estudos sobre polimorfismos em outras regiões desse gene devem ser realizados para que possamos melhor entender a função da leptina e seus efeitos na produção de leite de búfalas.
PALAVRAS-CHAVE: Búfalo, Murrah, sangue, SNP.

1 I INTRODUCTION

Molecular markers associated with the study of molecular genetics, in addition to traditional genetic improvement, are tools that can be used to aid the selection of animals that have a characteristic of economic interest, through the study of polymorphisms in genes that determine this characteristic (GALLEGOS et al., 2006), which has been studied to detect markers that are useful for association studies (MARRERO et al., 2016).Research has also focused on single nucleotide polymorphisms (SNPs) that are common throughout the genome and can predict an important step for studies of association with the characteristics of milk production in cattle and buffaloes (ANAND et al., 2008).In order to identify milk markers associated with milk components in buffaloes (REN et al., 2009; COSENZA et al., 2009), as well as the association of molecular markers with milk production in cattle and buffaloes (LARA, 2011; ZETOUNI et al., 2013). Although buffaloes and cattle have strong genotypic and phenotypic similarities and belong to the subfamily Bovinae (SCHERF, 2000; BONDOC, 2013), few studies with molecular markers have been carried out with buffaloes.

Leptin is a non-glycosylated peptide hormone (16 kDa) product from ob gene, has an initial polypeptide chain of 167 amino acids, of which the first 21 amino acids represent a signal peptide that is discarded before the mature protein is secreted into the circulation, and it is produced almost exclusively in adipose tissue (ZHANG, 1995; TERMAN, 2005).

This protein is involved with the mechanisms that regulate both, energy intake and metabolism, preventing the excessive deposition of body fat, homeostatic regulation of food intake, immune function, energy distribution, milk production and fertility (ARGAWAL et al. 2010; GIBLIN et al., 2010; CLEMPSON et al., 2011; ORRÙ et al., 2012). The genetic

polymorphism LEP-1620 (A / G), located in intron 2 of the leptin gene, was positively associated with the percentage of fat and protein (ZETOUNI et al., 2013). This polymorphism was also correlated with weaning weight in Nellore cattle (SOUZA et al., 2009). Cattle and buffalo have great contributions to the world agricultural economy with their products. According to MAPA (2016), the Brazilian herd of buffalo is estimated at about 1.15 million buffaloes, the North region being the largest producer in the country, with 720 thousand animals, the Northeast region with 135 thousand heads and the Southeast region, with 104 thousand heads.

In relation to milk production, cattle are the most studied animals in genetic research. However, studies that relate LEP-1620 SNP to milk production in buffaloes are rare in the literature. Based on these data, the objective of the present study was to identify the LEP-1620 (A / G) polymorphism in the gene coding for leptin hormone in buffaloes and to predict their association with milk production.

2 | MATERIAL AND METHODS

2.1 Samples

Samples of whole blood were collected through the coccygeal tail vein of 139 buffaloes from two farms located in Northeast Brazil, Pernambuco, Mata Meridional (08º 42 '25 "S 35º 31' 51" W) and Alagoas, Mata (09º 19 '04 " S 35 ° 33'39 "W).

2.2 Biological Material Collection and Sample Processing

Five ml of whole blood (tubes for collection of blood by vacuum) were collected from the Murrah buffaloes, clinically healthy, created in a semi-intensive manner. The blood samples were conditioned in styrofoam and sent to the Laboratory of Applied Molecular Animal Physiology - FAMA - of the Department of Morphology of the Federal Rural University of Pernambuco - UFRPE.

2.3 DNA extraction

DNA extraction from whole blood was performed using the modified method (MANIATIS et al., 1989). In a 1.5 ml eppendorf tube, were added:100 µl whole blood, 100 µl TE (10 mM Tris - 1 mM EDTA pH 8.0) and 100 µl equilibrated phenol pH 8.0 The samples were mixed in a vortex for 1 minute and centrifuged at 14000 rpm for 5min at 4°C. The supernatant was transferred to another 1.5 ml eppendorf tube. After the time, was added 50 µl of phenol-chloroform (1: 1), then vortexed for 1min and centrifuged at 14000rpm for 5min. The supernatant was transferred to another 1.5ml eppendorf tube where 100µl of chloroform was added, mixed for 1min and centrifuged at 14000rpm for 5min. Again, in another 1.5 ml eppendorf tube, was added in this order: 1º-10 µl of 3M ammonia acetate; 2º - 100 µl of the supernatant from the anterior tube (where the DNAs are located); and 3º -

100 µl of isopropanol. After this, the sample was mixed for 1 min in a vortex and incubated during 30 min in the freezer.

After that time, the samples were centrifuged at 14000rpm for 15min, the supernatant was discarded and the pellet washed with 500µl of 70% ethanol, this solution was centrifuged at a rotation of 14000rpm for 5min at 4 ° C. The 70% ethanol was removed and the pellet was allowed to dry at room temperature; The pellet was resuspended in 30 µl of injection water. The extracted DNA was analyzed and quantified in the Spectrophotometer, followed by electrophoresis in a 0.8% agarose gel, stained with blue Green, visualized in ultraviolet light and photographed to verify its quality.

2.4 Spectrophotometer

After extraction of DNA, the samples were evaluated on 1.0% agarose gel, then standardized for concentration using the spectrophotometer (BioMate 3 - Thermo Scientific) and 50 ng / µl L of each sample was used for the reaction of PCR.

2.5 Polymerase Chain Reaction (PCR)

The extracted DNA samples were submitted to the PCR technique for amplification of the regions of interest. The sequences of the primers used were: (LEP 1) 5'-GTC TGG AGG CAA AGG GCA GAG T-3 'and (LEP 2) 5'-CCA CCA CCT CTG TGG AGT AG -3', described by (LIEN et al., 1997). Mix solutions for PCR (MultiGene - Labnet ®) were prepared with a final volume of 20 µL, containing 3 µL of DNA (100 ng), 1 µL of each primer (15 pM), 5 µL qsp water and 10 µL of Go Taq® Green Master Mix (Promega). For amplification of the regions of interest, the following cycling was used: 94 ° C 5 minutes; 94 ° C 15 seconds, 58 ° C 30 seconds and 72 ° C 1 minute (35 cycles); followed by final extension at 72 ° C 4 minutes. After this, the samples were stored at 4 ° C.

PCR products were stained with Blue Green Loauding Dye (LGC Biotechnology), subjected to agarose gel electrophoresis (1%) for 30 minutes at 100 V and subsequently visualized in ultraviolet (UV) light. The expected size of the fragment was 522 bp.

2.6 Restriction fragment length polymorphism-PCR-RFLP

PCR-RFLP was carried out using a mixture containing, 10 µL of the PCR product, 0.5 µL of restriction enzyme *BsaAI,* 0.5 µL of the 10X enzyme buffer and 8.0 µL of water in one volume end of 19 µL. The samples were submitted to 37ºC for 3 hours for the digestion of the fragment, after were stained with Blue Green Loauding Dye (LGC Biotechnology) and subjected to agarose gel electrophoresis (2%) for approximately 90 minutes at 110 V.

The digestion products were submitted to agarose gel electrophoresis (3%) and visualized in ultraviolet light, where three different genotypes were identified: AA (522pb), AG (522, 441 and 81pb) and GG (441 and 81pb).

2.7 Description of study variables

In the present study, the data of milk production of buffaloes of the two farms A (Pernambuco) and F (Alagoas) were analyzed, such as the dairy production of the animals between June 2013 and June 2014, lactation days and daily lactation. Other variables also were observed during the study, such as peak production, lactation numbers, birth weight and weaning weight of the animals. These data (Prodap Professional Program GP) were provided by the owners of the farms.

2.8 Statistical analyzes

The results were expressed as mean percentages, median and standard deviation. The Kolmogorov-Smirnov normality test was used, which showed a parametric distribution for the variables, milk production, lactation days and non-parametric distribution for the other variables studied. Pearson's Chi-square test was used to compare the farms in relation to the investigated genotypes. In relation to the numerical variables (milk production and lactation days), the T-student test was used for parametric variables and Mann-Whitney as non-parametric test.

To test the differences between the genotypes investigated (AA, AG, GG) in the study variables, the ANOVA test was used with Tukey comparisons for parametric data and Kruskal-Wallis for the other variables.

Statistical analyzes were performed in SPSS (Statistical Package for the Social Sciences) software, version 21. A margin of error of 5% was adopted for statistical significance ($p < 0.05$).

3 I RESULTS AND DISCUSSION

PCR technique was used to amplify the 139 samples studied and a 522 bp fragment was observed (Figure 1).

After amplification, the products obtained were digested with restriction enzyme BsaAI (Thermo Scientific).Three bands patterns were observed: AA genotype showing a single band of 522 bp, AG genotype with fragments of 522, 441 and 81 bp and GG showing two fragments of 441 and 81 bp (Figure 2). The result obtained is in agreement with the one found by LIEN et al. (1997).

Genotype frequencies were calculated for the animals from farms F, A and Total Group (Table 1). A higher percentage of the AG genotype was observed in all situations, 53.8% in farm F, 45.2% in farm A and 50.4% in the Total Group.

These data are in agreement with those found by AZARI et al., (2012) in the three populations studied for polymorphism of the leptin LEP 1620 gene in Holstein cows, native bovine (Mazandarani) and buffalo where was observed the highest frequency for the genotype heterozygous AG. Data also found by ZETOUNI et al., (2013) for a population of

buffaloes from Jabuticabal, São Paulo-Brazil. However, in the present study, no significant difference between the farms in relation to the genotypic distribution of animals was verified ($p > 0.05$).

When the associations between the genotypes and the variables of the study were analyzed, such as milk production, lactation days and average daily production, independent of the farm, it was verified that the average milk production was higher among the animals that presented the GG genotype and lower in those with AG genotype. However, these data do not agree with the data found by ZETOUNI et al., (2013) in which it was observed that the AA genotype showed a higher average milk yield. When analyzing the average daily production variable, it was observed that the genotype AA had the highest average. However, there was no significant difference between the genotypes for the three variables ($p > 0.05$), according to the results presented (Table 2).

These differences between studies can be justified for different reasons, such as the different frequencies of genotypes among the populations studied, or even due to the interaction between genotype and environment.

In the present study, the leptin gene and its receptor in Holstein cows showed a tendency ($p < 0.10$) of the LEP-963 T allele (C / T) to association with the decrease in milk production. Lep-1238 (C / G) and LEP-963 (C / T) SNPs with milk production in cows were not found by LIEFERS et al. (2005). ZETOUNI et al. (2013) and also, did not find this association in their studies with buffaloes.

The comparison between farms by type of genotype for the variables, milk production and lactation days (Table 3), showed that the median dairy production was higher in the farm animals A than in the F for each one of the genotypes studied, while in the variable days of lactation, the means were higher on farm F than on “A”.

The significant difference ($p < 0.05$) occurred only in relation to the milk production variable, when the data were analyzed by the multiple comparisons test for the AG genotype. Therefore, even the F farm having animals with averages of lactation days a little higher than the A farm, this one has a higher milk yield in a lower average days of lactation. This can be justified by the way in which the animals are managed on farm A.

Even the F farm containing animals with higher average days of lactation than farm A, and it showed a higher milk yield in a lower mean of days of lactation. This can be justified by the way in which the animals are managed on farm A.

According to (ARKER et al., 2010; BARKER et al., 2010; BERTENSHAW et al., 2009), the management of animals influence the milk production. They concluded that stress in aversive management causes a decrease in milk production and that the application of new technologies is necessary for animal welfare, guaranteeing the quality in production.

Farms A and F presented data in common, however, farm F presented other variables, such as peak production, weight at birth and weight at weaning (Table 4).

In this case, no significant difference was found between these variables and the

studied genotypes. SOUZA et al, 2013 studying Nellore cattle, found association between the LEP-1620 SNP (A/G) and birth weight. The differences found between the studies can be justified by the difference between the species.

4 I CONCLUSION

In conclusion, we can say that in this study on the leptin polymorphism as a marker for the association of milk production in buffaloes, only the AG genotype presented statistical differences for milk production at farm A when compared to farm F. This is probably due the adoption of new technologies prioritizing animal welfare with an improvement in management quality, which may result in better milk production of the buffaloes of farm A. We can still suggest that future studies should be conducted on polymorphisms in other regions of the leptin hormone gene so that we can better understand the function of this protein and its effects on buffalo milk production.

ACKNOWLEDGEMENTS

To CAPES and CNPq, for granting the research and financial support to carry out this study.

VERBAL REPORT

The concepts and statements contained in this article are of responsibility of the respective authors.

ETHICS COMMITTEE

This study was previously approved by the Committee on Ethics in the Use of Animals - CEUA (nº 123/2014) of the Federal Rural University of Pernambuco (UFRPE). The same is also in accordance with the rules in force in Brazil, especially Law 11794/2008.

REFERENCES

ALASHAWKANY, A.R.; SHAHROUDI, F.E.; NASSIRY, M.R.; MOUSSAVI, A.H.; HEYDARPOUR, M.; SADEGHI, B. Association of SNP in the Exon II of Leptin gene with Milk and Reproduction Traits in Holstein Iranian Cows. **Biotechnology**, v. 7, p. 347-350, 2008.

ANAND, V.K.; SHARMA, V.; KUMAR, A.; VERMA, V.K.; RANA, V.P.; KHIRBAT, R. AND SANGWAN, M. L. PCR-RFLP based leptin gene polymorphism and its association with mastitis in Murrah buffalo. **Journal of Cell and Tissue Research,** v. 15, n. 2, p. 5125-5132, 2015.

ARCHER, S.; GREEN, M.; HUXLEY, J. Association between Milk Yield and Serial Locomotion Score Assessments in UK Dairy Cows. **Journal of Dairy Science,** v. 93, p. 4045-4053, 2010.

ARGAWAL P.; ROUT, P.K.; SINGH, S.K.; Leptin: A biomolecule for enhancing livestock productivity**.** **Indian Journal Biotechnology**, v. 8, p. 169-176, 2009.

AZARI, M. A.; HASANI, S.; HEIDARI, M.; YOUSEFI, S. Genetic polymorphism of leptin gene using pcr-rflp method in three different populations. **Slovak Journal of Animal Science**, v. 45, n. 2, p. 39-42, 2012.

BARKER, Z. E.; LEACH, K. A.; WHA, H. R.; BELL, N. J.; MAIN, D. C. J. Assessment of lameness prevalence and associated risk factors in dairy herds in England and Wales. **Journal of Dairy Science,** v. 93, p. 932–941, 2010.

BERTENSHAW, C.; ROWLINSON, P. Exploring Stock Managers' Perceptions of the Human–Animal Relationship on Dairy Farms and an Association with Milk Production. **Anthrozoös**, v. 22, p. 59-69, 2009.

BONDOC, ORVILLE. Genetic Diversity and Relationship of Domestic Buffalo and Cattle Breeds and Crossbreeds (Subfamily Bovinae) in the Philippines Based on the Cytochrome C Oxidase I (COI) Gene Sequence. **The Philippine Agricultural Scientist**. v. 96, n. 1, p. 93-102, 2013.

CLEMPSON, A.M.E.;, POLLOTT, G.E.; BRICKELL, J.S.; BOURNE, N.E, MUNCE N.; WATHES D.C.; Evidence that leptin genotype is associated with fertility, growth, and milk production in Holstein cows. **Journal Dairy Science**, v.94, p.3618– 3628, 2011

COSENZA, G.; PAUCIULLO, A.; FELIGINI, M.; COLETTA, A.; COLIMORO, L.; DI BERARDINO, D.; RAMUNNO, R. A point mutation in the splice donor site of intron 7 in the alphas2-casein encoding gene of the Mediterranean River buffalo results in an allele-specific exon skipping. **Animal Genetics**, v. 40, n. 5, p. 791, 2009.

DA-XI REN; SHU-YING MIAO; YOU-LIANG CHEN; CAI-XIA ZOU; XIAN-WEI LIANG; JIAN-XIN LIU. Genotyping of the kcasein and β-lactoglobulin genes in Chinese Holstein, Jersey and water buffalo by PCR-RFLP. **Journal of Genetics**, v. 90, n. 1, 2011.

DEB, R.; SINGH, U.; KUMAR, S.; SINGH, R.; SENGAR, G.; SHARMA, A. Genetic polymorphism and association of kappa-casein gene with milk production traits among Frieswal (HF × Sahiwal) cross breed of Indian origin. **Iranian Journal of Veterinary Research,** v. 15, n. 4, p. 406-408, 2014.

GALLEGOS, M, P.; TOCA, J. A.; RODRIGUEZ, P.; PINZÓN, C. E.; REVELES, F; A.; QUINTERO, J.S. Role of leptin gene in cattle production: review. **Journal of Animal and Veterinary Advances**, v. 14, n. 4, p. 81-90, 2015.

GIBLIN, L.; BUTLER, S.T.; KEARNEY, B.M.; WATERS, S.M.; CALLANAN, M.J.; BERRY, D.P. Association of bovine leptin polymorphisms with energy output and energy storage traits in progeny tested Holstein-Friesian dairy cattle sires. **BMC Genetics**, v, 11, p. 73, 2010. https://doi.org/10.1186/1471-2156-11-73

LARA, M, A. C.; PINATTI, E.; FARIA, M. H.; RESENDE, F.D.; PIVETTA, A. J.; GUTMANIS, G.; CAVALCANTE NETO, A. Polimorfismo do gene leptina (snp305) em bovinos e sua implicação na Maciez de carne. **Actas Iberoamericanas de Conservación Animal,**v. 1, p. 195-198, 2011.

LIEFERS, S. C.; VEERKAMP, R. F.; TE PAS, M. F. W.; CHILLIARD, Y.; VAN DER LEN, T. Genetics and physiology of leptin in periparturient dairy cows. **Domestic Animal Endocrinology**, v. 29, p. 227–238, 2005.

LIEN, S.; SUNDVOLD, H.; KLUNGLAND, H.; VAGE, D. I. Two novel polymorphisms in the bovine obesity gene (OBS). **Animal Genetics,** v.28, p.238–246, 1997.

MAPA - MINISTÉRIO DA AGRICULTURA PECUÁRIA E ABASTECIMENTO. Bovinos e Bubalinos. 2016.

MARRERO, N. M.; MASCENA, L. A.; MACEDO, J. L.; VALDÉS, A. M.; GOMES FILHO, M. A.; REINOSA, O. U. Genotyping of the kappa-casein and leptin genes in Cuban water buffalo by PCR-RFLP. **Revista de Salud Animal**. v. 38, n2, p. 71-78, 2016.

ORRÙ, L.; ABENI, F.; CATILLO, G.; GRANDONI, F.; CRISÀ, A.; DE MATTEIS, G.; CARMELA SCATÀ, M.; NAPOLITANO, F.; MOIOLI, B. Leptin gene haplotypes are associated with change in immunological and hematological variables in dairy cow during th e peripartum period. ***Journal of Animal Science,*** v.90, n. 1, p. 16-26, 2012.

SCHERF, B.D. World watch list for domestic animal diversity. **Food and Agriculture Organization of the United Nations (FAO)**, 2000.

SOUZA, F. R. P.; MERCADANTE, M. E. Z.; FONSECA, L. F. S.; FERREIRA, L. M. S.; REGATIER, I. C.; AYRES, D. R.; TONHATI, H.; SILVA, S. L.; RAZOOK, A. G.; ALBUQUERQUE, L. G. Assessment of *DGAT1* and *LEP* gene polymorphisms in three Nelore (*Bos indicus*) lines selected for growth and their relationship with growth and carcass traits. **Journal of Animal Science** v.88, p.435-441, 2013.

TERMAN, A. Effect of the polymorphism of prolactin receptor (PRLR) and leptin (LEP) genes on litter size in Polish pigs. **Journal of Animal Breeding and Genetics,** v. 122, n. 6, p. 400-4, 2005.

ZETOUNI, L.; CAMARGO, G. M. F.; FONSECA, P. D. S.; GIL, F. M. G.; LUGO, N. A. H.; ASPILCUETA-BORQUIS, R. R.; CERVINI, M.; TONHATI H. Effects of a single nucleotide polymorphismin the leptin gene on the productive traits of dairy buffaloes (Bubalus bubalis). **Molecular Biology Reports**. v. 40, p. 5159–5163, 2013.

ZHANG, Y.; PROENCA, R.; MAFFEI, M.; BARONE, M.; LEOPOLD. L.; FRIEDMAN, J.M. Positional cloning of the mouse obese gene and its human homologue. **Nature**, v. 30, n. 374(6521)-479, 1995.

Ladder

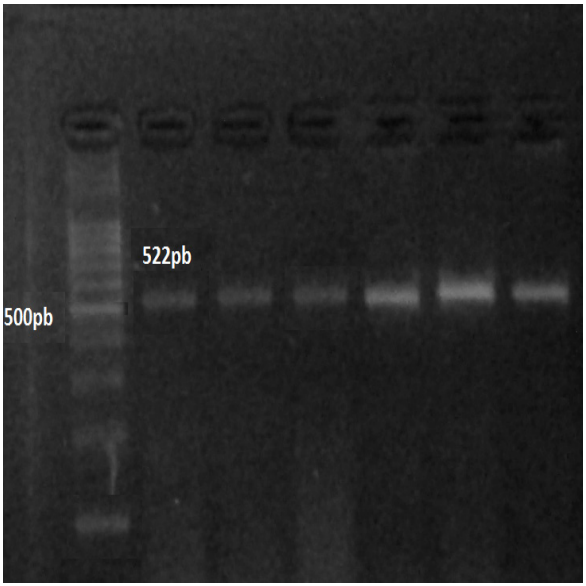

Figure 1.Agarose gel electrophoresis (1.0%) reveals the PCR products of 522bp. Ladder 100pb

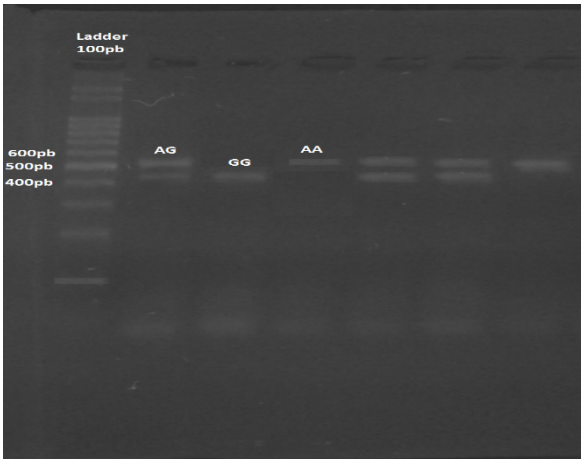

Figure 2. Agarose gel electrophoresis (3%) reveals the PCR products of the restriction enzyme digestion *BsaAI* (Genotypes AA, AG, GG). Lane M: 100pb DNA Ladder

Genotype	Farms F		Farms A		Total Group		P value
	N	%	N	%	N	%	
AA	18	23.1	11	18.0	29	20.9	p (1) = 0.240
AG	42	53.8	28	45.2	70	50.4	p (1) = 0.224
GG	18	23.1	22	36.1	40	28.8	p (1) = 0.068
Total	**78**	**100**	**61**	**100.0**	**139**	**100**	

(1): Pearson's Chi-square Test.

Table1. Genotypic distribution in the sample studied

Variables	Genotypes AA Mean ± DP (Median)	Genotypes AG Mean ± DP (Median)	Genotypes GG Mean ± DP (Median)	P value
Dairy production (l)	1,803.89 ± 628,02 (1,708.90)	1,694.93 ± 546,78 (1,577.40)	1,969.41 ± 594.12 (1,875.00)	p(1)= 0.093
Days lactation	284.41 ± 74.40 (271.00)	276.61 ± 63,05 (269.00)	301.76 ± 70,36 (294.00)	p(1)= 0.214
Average daily production	5.62 ±2.54 (5.47)	5.40 ± 2.65 (5.60)	5.44 ± 2.92 (5.60)	p(2)= 0.786

Table 2. Association of lactation variables by genotypes studied (n = 139)

Variables	Genotype	Farms F Mean ± DP (Median)	Farms A Mean ± DP (Median)	P value
Dairy production	AA	1,624.57 ± 501.91 (1,595.22)	2,090.80± 726.02 (2,096.50)	p(1) = 0.060
	AG	1,434.28 ± 362.45 (1,398.97)	2,229.25± 469.46 (2,190.00)	p(1)<=0.001*
	GG	1,734.90 ± 505.30 (1,803.14)	2,218.56± 593,41 (2,118.00)	p(2)=0.017*
Days of lactation	AA	294,71 ± 79,16 (277.00)	266,90 ± 65.63 (240.50)	p(1) = 0.227
	AG	276.27 ± 70.10 (269.00)	277.30 ± 46.99 (268.50)	p(2) = 0.953
	GG	309.59 ± 72.98 (294.00)	293.44 ± 68.83 (289.50)	p(2) = 0.519

Table 3.Analysis among the variables, amount of lactation and days of lactation between the farms by genotype

Variable	Genotypes			P value
	AA	AG	GG	
	Mean ± DP	Mean ± DP	Mean ± DP	
	(Median)	(Median)	(Median)	
Peak production (L)	1,020.89 ± 419.75	928.05 ± 298.82	987.61 ± 257.73	p(2)=0.873
	(988.50)	(913.00)	(960.00)	
Weight at birth (kg)	78.63 ± 96.91	82.95 ± 122.93	45.22 ± 34.21	p(2)=0.084
	(39.00)	(40.00)	(36.50)	
Weight at weaning (kg)	558.61 ± 54.69	547.64 ± 64.93	559.06 ± 72.89	p(2)=0.382
	(541.00)	(565.00)	(580.00)	

(*): Significant difference to 5%.

(1): F (ANOVA) test with Tukey's comparisons.

(2): Kruskal-Wallis test

Note: If the letters between parentheses are distinct it is verified significant differences between the corresponding genotypes.

Table4. Analysis of the variables, lactation liters, lactation days, number of lactations, peak production and birth weight at the F farm by genotype

ÍNDICE REMISSIVO

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