Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2019; 7(3): 414-416

Received: 25-03-2019
Accepted: 27-04-2019

VR Patodkar
Department of Veterinary Physiology, KNP College of Veterinary Science, Shirwal, Maharashtra, India

PV Mehere
Department of Veterinary Physiology, KNP College of Veterinary Science, Shirwal, Maharashtra, India

SN Jadhav
Department of Veterinary Biochemistry, KNP College of Veterinary Science, Shirwal, Maharashtra, India

Correspondence
VR Patodkar
Department of Veterinary Physiology, KNP College of Veterinary Science, Shirwal, Maharashtra, India

Leptin hormone diversity in different species- explained with polymorphism study in Pandharpuri buffalo

VR Patodkar, PV Mehere and SN Jadhav

Abstract
Buffaloes constitute 35% percent of the total bovine population in India and contribute in the 53 % of total milk production. Current technologies enable scientists to improve the accuracy and efficacy of traditional selection by applying the genetic markers. Leptin gene is one of the markers which have been established for milk traits in cattle. But our recent research in buffalo shows that there may be other markers or the sites which may define the milk traits in animals.
This study was aimed to reveal PCR-RFLP pattern of the leptin (exon 2) locus in the Pandharpuri breed of buffaloes. Fragments of leptin 331 bp and OLR1-288bp were amplified by PCR, and subsequently RFLP study was carried out to identify genotypes of the animals with HphI restriction enzyme and HaeIII restriction enzyme to know the relationship of polymorphism in Exon 2 of leptin gene with milk traits but no such relationship has been found. The monomorphic pattern of leptin gene was observed in the present study for exon 2 in Pandharpuri Buffaloes.

Keywords: Hormones, leptin, polymorphism, buffalo

Introduction
Buffaloes constitute 35% percent of the total bovine population in India, but they contribute more than 53 % to the total milk production. That's why Indian buffaloes have a major contribution to make India one of the higher milk producing countries in the world. The Pandharpuri is one of the best milch breeds of buffalo in India and is found in the Western Maharashtra. Current technologies enable scientists to improve the accuracy and efficiency of traditional selection by applying genetic markers. This can be done through analysis of candidate genes. The candidate gene approach consists of the study of different genes potentially involved in a physiological process (e.g., milk protein synthesis, milk fat synthesis) and identification of the allele responsible for a desired phenotype. The leptin gene is considered as a potential QTL, influencing milk performance and reproduction traits in cattle (Buchanan *et al*., 2002) [1]. Leptin is a hormone which is mainly produced in adipose tissue and secreted into the blood stream as a 16 kDa protein. It plays a key role in the regulation of food intake, energy expenditure, fertility and immune functions (Fruhbeck and Gomez, 2001) [4]. Leptin binds to a receptor mainly localized on neuropeptide Y- neurons, which results in reduction of feed intake and increase of energy expenditure. This study was aimed to reveal PCR-RFLP pattern of the leptin (exon 2) locus in the Pandharpuri breed of buffaloes. Fragments of leptin 331 bp and OLR1-288 bp were amplified by PCR, and subsequently RFLP study was carried out to identify genotypes of the animals with HphI restriction enzyme and Hae III restriction enzyme to know its relationship with Fat, SNF, Protein in the milk.

2. Materials and Methods
2.1 Buffalo population, sampling and DNA extraction
To analyze the status of leptin polymorphism, blood samples were collected randomly from 20 unrelated buffaloes of the Pandharpuri breed which were under the lactating phase. DNA was extracted using standard protocol by the salting out method extraction procedure (Sambrook *et al*., 1989) [9].

2.2 Molecular genotyping

The primer pair reported by Hageman *et al*., (2000) [5] (F: 5'GGGAAGGGCAGAAAGATAG3' R: 5'TGGCAGACTGTTGAGGATC3') was used to amplify the region corresponding to leptin exons 2 and the RFLP site the HphI site was targeted. PCR was carried out in a final reaction volume of 25 µl. Amplification cycling conditions for leptin involved initial denaturation 94 °C for 10 minutes, followed by 35 cycles at 94 °C for 1 minute, 53 °C for 45 seconds and 72 °C for 1 minute with a final extension at 72 °C for 10 minutes. For the PCR-RFLP analysis, 20 µl of each PCR amplified product was digested with 10 units of the Hph1 (Method is as per Deshpande *et al*. 2014) [7] and Hae III(GGTGA(N)8 t) in a 50 µl total reaction and incubated in a water bath at 37 °C for 4 h. The digestion products were separated by electrophoresis on a 2% agarose gel in 0.5% TBE buffer.

2.3 Analysis of Milk components

Milk components like fat, protein, SNF from buffalo milk was analyzed using the milk auto analyzer**.**

3. Results and Discussion

Fat, protein, SNF and lactose has been collected and analyzed from 20 lactating Pandharpuri buffaloes as depicted in table 1. Fig 2 shows HPHI digestion of leptin gene. It has been observed that Exon 2 of leptin gene which is typically undergoes restriction digestion using the HpHI in cross breed cattle did not show us the same result in Pandharpuri buffaloes. Deshpande *et al*.,2014[3] reported that HphI is 5'-G G T G A(N)8 ^-3' site was found to be mutated to 5'-G T T G A (N)8 -3' in the Mehsana buffalo. Our results are in accordance with Deshpande *et al*., (2014) [3]. The results obtained for Pandharpuri buffaloes suggest species difference with respect to HphI polymorphism in the leptin gene (Fig 1.). Further with Hae III restriction enzyme we got two bands 275 bp and 56 bp (Fig 2). This genotype was referred as AA type. No different band pattern was observed than that of AA genotype. Hence, the frequency of AA genotype was found to be 1.00 in this study. Thus, the presently observed frequency of 'A' allele is predominant in Pandharpuri buffaloes. The monomorphic pattern of leptin gene was observed in the present study for exon 2. As the results reveal monomorphic pattern of leptin gene at leptin locus, it concludes that monomorphism at this locus is a specific characteristic feature of Pandharpuri buffalo.

The results of the present study are not in accordance with previous reports in cattle (Dandapat *et al*., 2010 and Schmutz, 2001) [2, 10]. Buchanan *et al*., (2003) reported C to T transition in exon 2 of leptin gene that encodes an Arg25Cys substitution (position four of the secreted peptide) associated with body fat deposition in beef cattle.It has been reported that this same genetic variant was also present in six dairy breeds, viz. Holstein, Ayrshire, Brown Swiss, Canadiene, Guernsey and Jersey, and compared lactation performance data using a mixed model (Singh *et al*., 2014) [12]. Animals homozygous for the T allele produced more milk (1.5 kg/d vs. CC animals) and had higher somatic cell count linear scores, without significantly affecting milk fat or protein percent over the entire lactation [13]. Several single nucleotide polymorphisms (SNPs) in the LEP gene have been identified by previous authors (Dandapat *et al*., 2010, Oztabak *et al*., 2010 and references therein) [2, 8]. A SNP resulting from a C>T substitution, causes amino acid change from alanine to valine (A59V) has been observed in LEP exon 3 in various cattle breeds (Silva *et al*., 2014) [11].

As we could not find any polymorphism in exon 2 in leptin gene, association analysis of polymorphism with milk and milk associated traits is null. The results obtained for buffaloes suggest a species difference with respect to HpHI polymorphism in leptin gene in exon 2 (Fig 1) (Deshpande *et al*., 2014) [3] but there may not be any breed difference.

4. Conclusion

In conclusion allele A seems to be frequent in the Shirwal/Satara region in Pandharpuri buffalo in exon 2. Exon 2 did not show any relationship with production traits being monomorphic. Therefore, exon 2 could not be useful in marker assisted selection to improve the reproduction and milk production performance in Pandharpuri buffaloes like in dairy cattle. Targeting other locus for learning the relation between production traits and polymorphism in buffaloes will shed light while selecting the animal for milk trait.

The result obtained from this study may be due to evolutionary changes in the region of exon 2 even though the cattle and buffaloes are from same family Bovidae (Moaeen-ud-Din and Bilal, 2015) [6].

5. Acknowledgment

The authors are grateful to Associate Dean Dr. A. S. Ranade for letting us use the Central instrumentation facility for research

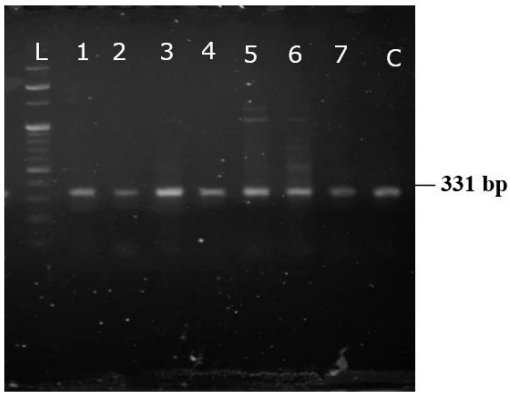

Fig 1: Leptin gene (Exon-2) RE Digest by HpH1 enzyme. Control – Non Lactating buffalo. Obtained the single band suggest the site of HpH1 enzyme may be muted in buffaloes

Re Digestion of Leptin (Exon 2 with Hae III Enzyme)

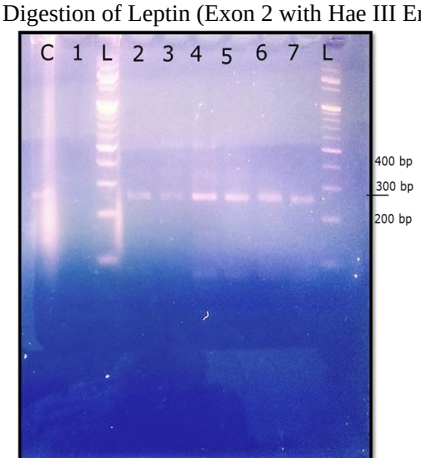

Fig 2: Leptin gene (Exon- 2) Re digest by HaeIII enzyme. Control-Non Lactaing Buffalo Obtained The two bands suggest the only AA genotype is present in the samples Collcted so far

Table 1: Analysis of milk traits from Pandharpuri buffalo

Buffalo (No) in Lactation	Fat %	SNF %	Density	Protein %	Lactose (mg/dl)
1	10.7	8.2	26.2	3.85	3.6
2	5.0	8.9	34.0	4.09	3.7
3	7.2	8.8	31.7	4.08	4.8
4	6.2	9.0	33.3	4.16	3.4
5	6.0	9.0	33.5	4.16	2.4
6	6.7	8.9	35.5	4.06	4.7
7	7.2	8.4	30.1	3.88	4.0
8	7.5	8.3	32.1	4.30	2.4
9	6.2	8.1	31.1	4.1	3.2
10	6.1	8.2	34.2	3.4	3.5
11	6.8	8.1	33.9	3.5	4.2
12	6.3	8.2	34.3	3.7	4.0
13	6.8	8.1	35.1	3.9	4.1
14	7.1	8.3	33.1	3.8	4.5
15	6.1	8.3	33.2	4.12	3.1
16	6.1	8.9	32.4	4.12	2.3
17	6.5	8.7	32.1	4.12	4.4
18	6.4	9.0	33.5	4.16	4.2
19	7.5	8.4	30.1	3.88	4.0
20	6.2	8.8	33.3	4.02	3.7

6. References

1. Gupta MP, Sharma K, Khan BU, Kapoor BC, Bajpai PK, Kumar A *et al*. Tissue specific esterase isozyme variation in Clarias batrachus and C. gariepinus. Global Journal of Pharmacology. 2009; 3(1):1-5.
2. Buchanan FC, Fitzsimmons CJ, Van Kessel AG, Thue TD. Hot topic: an association between a leptin single nucleotide polymorphism and milk and protein yield. Journal of Dairy Science. 2003; 86:3164-6.
3. Dandapat A, Kumar D, Ghosh AK, Umapathi V. A note on a DNA polymorphism study of leptin gene in Sahiwal and crossbred cattle using PCR-RFLP technique. Livestock Research for Rural Development, 2010, 22(1)
4. Despande M, Rank DN, Vataliya PH, Joshi CG. Study of leptin gene polymorphism in Mehsana buffalo (Bubalus bubalis). Buffalo Bulletin. 2014; 33(1):115-119.
5. Frühbeck G, Gómez-Ambrosi J. Modulation of the leptin-induced white adipose tissue lipolysis by nitric oxide. Cell Signal. 2001; 13(11):827-33.
6. Haegeman AA, Zeveren V, Peelman LJ. New mutation in exon 2 of the bovine leptin gene. Animal. Genetics. 2000; 31:79-79.
7. Moaeen-ud-Din M, Bilal G. Sequence diversity and molecular evolutionary rates between buffalo and cattle. Journal of Animal Breeding and Genetics. 2015; 132(1):74-84.
8. Kulig H, Kmieć M. Association between leptin gene polymorphisms and growth traits in Limousin cattle. Genetika. 2009; 45:838-841.
9. Oztabak K, Toker NY, Cemal UN, Akis I, Mengi A, Karadag O *et al*. Leptin gene polymorphisms in native Turkish cattle breeds. Kafkas Üniversitesi Veteriner Fakültesi Dergisi. 2010; 16(6):921- 924.
10. Sambrook J, Russel DW. Molecular Cloning: A laboratory Manual. Cold Spring Harbour. Tab. Press, New York. 2001; 6(11):4-6.
11. Schmutz. Association of a missense mutation in the bovine leptin gene with carcass fat content and leptin mRNA levels. Genetic Selection of Evolution. 2002; l34:105-116.
12. Silva DBS, Crispim BA, Silva LE, Oliveira JA, Siqueira F, Seno LO *et al*. Genetic variations in the leptin gene associated with growth and carcass traits in Nellore cattle. Genetics and Molecular Research. 2014; 13(2):3002-3012.
13. Singh U, Kumar S, Deb R, Mann S, Sharma A. Genotypic profiling of coding region of leptin gene and their association studies on reproductive and milk production traits in Sahiwal and Frieswal cattle of India. African Journal of Biotechnology. 2014; 12(42):6140-6146.
14. Yoon DH, Cho BH, Park BL, Choi YH, Cheong HS, Lee HK *et al*., Highly polymorphic bovine leptin gene. Asian Australian Journal of Animal Science. 2005; 18(11):1548-1551.