



Leptin gene polymorphism in association with lactation milk yield in Murrah buffaloes

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In India, buffalo contributes 56.65% of the total milk production, which is about 61.6 million tonnes (Anonymous 2012). Association studies of major candidate genes for milk production, viz. leptin gene will facilitate marker assisted selection in Buffalo Improvement Programme in India. Leptin, a 16-kDa protein (Gong *et al.* 1996), performs important roles in regulation of milk production besides feed intake, energy balance, fertility, immune functions and reproduction (Block *et al.* 2001, Buchanan *et al.* 2003). It is one of the most important bio-molecule as a marker for identifying high performing individuals (Matteis *et al.* 2012). Leptin protein binds to a receptor resulting in a reduction of feed intake and an increase of energy expenditure (Magni *et al.* 2000). The coding region for the leptin gene is contained within exons-2 and 3. Leptin gene has been mapped to chromosome 4 in bovine (Stone *et al.* 1996) and chromosome 8 (BBU 8q32) in buffaloes (Vallinato *et al.* 2004). Leptin genotype TT plays an important role in increasing milk production in Holstein cows (Buchanan *et al.* 2003, Chebel *et al.* 2008, Matteis *et al.* 2012), Karan Fries (Vohra *et al.* 2011), and CC genotype of leptin gene in Jersey cows (Kulig *et al.* 2009). However, little reports were available on polymorphism and association studies of leptin gene with production traits in Murrah buffaloes. Hence, the present study was conducted to evaluate the association of polymorphism in leptin gene with 305 days or less milk yield in Murrah buffaloes.

The present study was conducted on 154 lactating Murrah buffaloes maintained at the Institute Livestock Farm. All the buffaloes were screened for the presence of leptin gene polymorphism.

DNA isolation and polymorphism detection: Genomic DNA was isolated from aseptically collected venous blood using the standard phenol/chloroform method with minor

modifications (Sambrook and Russel 2001). Forward and reverse primers (P_F 5'-GTGCCACGTGTGG TTTCTTC-3' and P_R 5'-CCTCCCTACCGTGTGTGAGA-3') with T_M of 61.57°C and 61.12°C, respectively, were designed using Primer 3 software to amplify exon-2 region of leptin gene from published NCBI (National Centre of Bioinformatics, USA) sequences. The optimization of PCR was done to get the best possible amplification of the product, at an annealing temperature of 58°C for 1 min. The genotyping was carried out using PCR-RFLP (polymerase chain reaction- restriction fragment length polymorphism) technique with AclI restriction enzyme at 37°C for 6 to 8 h. Genotype and allele frequencies were calculated and compared by gene computing method (Falconer and Mackay 1996). The PCR products of different genotype were sequenced using the automated dye terminator cycle sequencing method with Ampli Taq DNA polymerase in ABI PRISM 377 DNA sequencer.

Phenotypic information, statistical and genotype analysis: The first lactation production information of 154 Murrah buffaloes was collected from Dairy Cattle Breeding division of National Dairy Research Institute. The data of 305 days or less milk yield were adjusted for non-genetic factors, viz. season of calving, period of calving and age group at first calving using fixed linear models (Harvey 1990). Following model was considered:

$$Y_{ijkl} = \mu + S_i + P_j + AG_k + e_{ijkl}$$

where, Y_{ijkl} , observation of the i^{th} buffalo calved in k^{th} age group, j^{th} period and i^{th} season; μ , overall mean of the trait; S_i , fixed effect of i^{th} season of calving (winter, summer, rainy and autumn); P_j , fixed effect of j^{th} period of calving (1 to 8); AG_k , fixed effect of k^{th} age group of first calving (< 37, 37–51 and >51 months); and e_{ijkl} , random error is NID (0, σ^2_e). The effects of non-genetic factors, viz. season and period of calving and age group of first calving on milk yield were adjusted through analysis of variance.

To study the effect of leptin genotypes on adjusted 305 days or less milk yield in Murrah buffaloes, fixed linear model with SNP genotypes as fixed effects was used. The model considered was: $Y_{ij} = \mu + G_i + e_{ij}$ where, Y_{ij} , 305 days or less milk yield of j^{th} buffalo for i^{th}

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genotype; μ , overall mean; G_i , effect of i^{th} genotype ($i = 1-3$) and e_{ij} , random error is NID ($0, \sigma_e^2$). The differences of least square means of 305 days or less milk yield between subclasses of leptin genotype were tested for significance using Duncan's Multiple Range Test (DMRT) as modified by Kramer (1957).

Identification of genotypes: PCR amplification generated a 210 bp for exon-2 of leptin gene in Murrah buffaloes. Datta *et al.* (2013) also reported similar results in Murrah buffaloes. RFLP of leptin gene done with *Acil* restriction enzyme revealed 2 products of 130 bp and 80 bp. It indicated the presence of 3 types of genotypes TT, TC and CC for leptin gene in Murrah buffaloes of NDRI herd. Frequency of CC, TC and TT genotypes were 0.26, 0.73 and 0.01% respectively.

Sequencing results of amplified PCR product revealed the 2 (T and C) alleles for the exon-2 region of leptin gene in Murrah buffaloes. T and C alleles of leptin gene of Murrah buffaloes were compared (Clustal W software) with *Bos taurus* which showed variation at 1180th position. Translated products, showed difference in amino acid sequence of leptin protein. Amino acid arginine of the C allele was replaced with cysteine in T allele of Murrah buffaloes. This difference was due to presence of transition mutation (C to T) in the first base of 25th codon. Datta *et al.* (2013) reported absence of polymorphism for leptin gene in Murrah buffaloes. However, many researchers reported polymorphism of leptin gene in cattle. Vohra *et al.* (2011) reported genotype frequency of 0.13%, 0.52% and 0.35% for TT, TC and CC genotypes in Karan Fries cattle. Buchanan *et al.* (2003) reported a single nucleotide polymorphism in the exon-2 region of the leptin gene in Holstein Friesian cattle. Chebel *et al.* (2008) reported the frequency of TT, TC and CC genotypes as 17.2, 48.2 and 34.6% in Holstein cows. Dandapat *et al.* (2010) observed polymorphism using *HphI* -PCR-RFLP in Holstein crossbred (HF $\times$ Sahiwal) cattle exhibiting AA, AV and VV genotypes with their respective frequency of 0.57, 0.36 and 0.07 and gene frequency as 0.75 and 0.25 for the A and V alleles, respectively.

Association of leptin genotypes with lactation milk yield: Overall least-squares mean of 305 days or less milk yield was estimated as 2078.20 ± 31.21 kg. The period of calving ($P < 0.01$) had significant effect on 305 days milk yield in Murrah buffaloes. The result was in conformity with the findings of many workers (Patil *et al.* 2013, Kumar *et al.* 2014, Jamuna *et al.* 2014). Adjusted 305 days or less milk yield records were further analyzed to assess the effect of different leptin genotypes as fixed effect. The least-squares mean of 305 days or less milk yield for TT, TC and CC genotypes of leptin were 1,358 kg, 2,012.72 kg and 1,888.24 kg, respectively. The mean difference of TT, TC, and CC genotypes differ significantly with 305 days milk yield in Murrah buffaloes. The buffaloes with TC genotype produced more milk yield than TT (654.72 kg) and CC (124.48 kg) genotypes. Vohra *et al.* (2011) reported that Karan Fries cattle with TT genotype had significantly higher

305 days milk yield as compared to cattle with CC genotype. Buchanan *et al.* (2003), reported that Holstein Friesian cattle homozygous for T allele produced more milk (1.5 kg/day) vs. homozygous for C allele cows. Most of workers (Buchanan *et al.* 2003, Vohra *et al.* 2011 and Matteis *et al.* 2012) reported that leptin genotype TT is highly associated with increasing milk production in Holstein cows and its cross breeds. However, overview of results revealed that C allele can be use a marker for higher milk production in marker assisted selection of Murrah buffaloes under Buffalo Improvement programme.

SUMMARY

PCR amplification of exon-2 of leptin gene yielded an amplified product of 210 bp in Murrah buffaloes. RFLP of leptin gene done with *Acil* restriction enzyme revealed 3 types of genotypes CC, TC and TT in Murrah buffaloes of NDRI herd with a frequency of 0.26, 0.73 and 0.013% respectively. Results suggested that CC genotype of leptin gene in Murrah buffaloes is significantly associated with 305 days milk yield. Overall study suggested the scope of considering genetic information of leptin gene in breeding and management strategies for the improvement of Murrah buffaloes of NDRI herd.

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