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Polymorphism in Exon-40 of FASN Gene in Lesser known Buffalo breeds of India

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ABSTRACT

Present study was aimed to discover the single nucleotide variation present in exon 40 region of the Fatty Acid Synthase (FASN*)* gene. FASN plays a central role in *de novo* lipogenesis in mammals and variation in this enzyme may affect economic traits including fatty acid composition of buffalo milk. A total of 80 DNA samples in two riverine buffalo breeds namely, Gojri and Chhattisgarhi were screened using Restriction Fragment Length Polymorphism (RFLP). FASN gene was found to be polymorphic with an adenine to guanine transition. Three types of genotypes, viz. AA, AG and GG were observed in the studied breeds. Allele A was found to be more frequent than G allele. All the genotypes showed almost the same frequency across the breeds, indicating that there is absence of selection for FASN gene in lesser known buffalo breeds of India. The study will augment the information available and can be applied in future studies to determine the role of bovine FASN gene as a candidate gene marker for a milk-fat content.

Keywords: Buffalo, FASN gene, PCR-RFLP, Milk fat synthesis, Gojri, Chhattisgarhi.

Buffalo contributes about 57% of the total milk produced in India. Buffalo milk is commercially more viable in Indian market than cow milk, for the manufacture of fat-based milk products, such as butter, ghee and milk powders because of its lower water content and higher fat content (Vohra and Chakravarty, 2011). Higher fat in milk makes buffalo a preferred dairy animal. Genetic or marker based selection followed with appropriate breeding methods for higher milk fat can facilitate genetic selection in different buffalo breed improvement programmes in India.

Bovine fatty acid synthase gene (FASN) was mapped to chromosome 19 (BTA19) at q22 band (Roy *et al*., 2001). Several QTLs linked to fat content in milk have been described within the aforementioned chromosome (Taylor *et al*., 1998; Biochard *et al*., 2003). FASN is a complex homodimeric enzyme that catalyses the formation of fatty acids of 16 carbon atoms in length from acetyl-CoA and malonyl-CoA in the presence of NADPH (Chakravarty *et al*., 2004). This synthesis involves a conserved set of chemical reactions for the cyclic step elongation of activated precursors by two carbon units (Smith, 1994). Mammalian FASN is not only an essential metabolic enzyme in fatty acid synthesis during the adult stage, but it also plays an important role during embryonic development (Chirala *et al*., 2003). This cytosolic enzyme catalyses the formation of palmitate from acetyl-coenzyme A and malonyl- coenzyme A in the presence of NADPH. Hence, because FASN plays a central role in *de novo* lipogenesis in mammals (Wakil *et al*., 1983), it is a candidate gene for fat content in milk animals. The studies on the bovine *FASN* gene structure have revealed occurrence of several single nucleotide polymorphisms (SNPs) linked to the fat content and fatty acids composition in milk (Roy *et al*., 2006). Morris *et al*. (2007) identified a QTL on the bovine chromosome 19 (BTA19) containing fatty acid synthase gene and identified FASN gene as a potential candidate gene for some milk production quality traits. However, scanty literature is available regarding the status of genetic variations in FASN gene in river buffaloes. Therefore, it is imperative to explore the genetic variations among the different buffalo populations in our country.

MATERIALS AND METHODS

Population studied and sample size

Gojri buffalo in Punjab and Himachal Pradesh and Chhattisgarhi buffalo from Chhattisgarh. These germplasm have their own socio-economic utility and are generally better adapted to their regions, overall buffalo milk is well known for its higher fat content compared to cow milk. Random blood samples (approximately 8 to 10 mL) were collected from 80 genetically unrelated buffaloes representative of the Gojri (n = 40) and Chhattisgarhi (n = 40) breeds. The blood samples of Gojri and Chhattisgarhi buffaloes were collected from their respective breeding tracts.

DNA isolation and Primers used

Genomic DNA was isolated from aseptically collected venous blood using the standard phenol/chloroform method with minor modifications (Sambrook and Russel, 2001). Quality check and quantification were done by nanodrop spectrophotometer and electrophoresis on 0.8% agarose gel. DNA concentration was determined and samples were diluted 10-40 times (approx. 50-80 ng/µl) with MiliQ water. Forward and reverse primers (P_F 5'-CTCGCACACCTTCGTGATG-3' and P_R 5'-CACGTTGCCGTGGTAGGTAG-3') with T_M of 57.5 °C and 57.4 °C respectively, were designed using Primer 3 software to amplify exon-40 region of FASN gene from published NCBI (National Centre of Bioinformatics, USA) sequences.

PCR amplification and genotyping conditions

DNA amplification of the exon 40 of FASN gene are achieved by PCR. The optimization of PCR was done to get the best possible amplification of the product, PCR was carried out in 25 µl reaction volume consisting of 200***µM*** of each dNTP, 5pM of each primer, 1.5mM $MgCl_2$ and 1.0U Taq polymerase (Invitrogen, CA). Amplification was performed using MASTERCYCLER EP (Eppendorf, Germany) with an initial denaturation at 95˚C for 4 min followed by 30 cycles of 94˚C for 60 sec, annealing temperature 61*℃* for 60 sec and 72˚C for 60 sec, with a final extension for 10 min at 72 °C. All the buffaloes were screened for the presence of FASN gene polymorphism using PCR-RFLP technique. Genotyping was carried out using *Mlucl* restriction enzyme at 37 °C for 6 to 8 hrs. Genotyping was evaluated by running a small aliquot of PCR-RFLP product on 2.5% agarose gel. Genotype and allele frequencies were calculated by gene computing method (Falconer and Mackay, 1996).

RESULTS AND DISCUSSION

A 473 bp fragment of exon 40 of FASN gene was successfully amplified in all samples of Gojri and Chhattisgarhi buffalo. RFLP test using *Mlucl* restriction enzyme indicated that exon-40 region of FASN gene is highly polymorphic in all the three breeds of buffalo, with the presence of three genotypes namely, AA, AG & GG, with A and G alleles (Table). Three types of genotypes, viz. AA, AG and GG were having almost same allele frequency in all three populations studied. GG was rare and AG being most common genotype. Heterozygous AG was found to be most frequent with highest genotype frequency of 0.60 and GG allele was found to be rare with least frequency of 0.025 in Gojri buffalo were as in Chhattisgarhi buffalo Heterozygous AG was found to be most frequent with 0.56 and 0.55 respectively. Homozygous GG was found to be rare genotype in Chhattisgarhi buffalo as 0.125.

Our results were similar to that (0.31) obtained by Morris *et al*. (2007) for Holstein- Friesian cattle where they reported frequency of AA genotype 0.31. Higher FASN (AA) frequencies were also reported by researchers in the following breeds: Holstein-Friesian 0.53 (Schennink *et al*., 2009) and Angus 0.62 (Zhang *et al*., 2008). Significantly lower frequencies were obtained in Jersey breed 0.13 (Morris *et al*., 2007) and Korean breed 0.15 (Oh *et al*., 2011) which is indicative of extensive genetic variability of this fat related marker in different breeds however, within Gojri and Chhattisgarhi breeds of buffalo there seems to be similar trend. Schennink *et al*. (2009) and Zhang *et al*. (2008) reported similar frequencies (0.50 and 0.51, respectively), whereas that showed by Oh *et al*. (2011) was 0.25. A very high FASN (GG) frequency was observed in Korean cattle 0.73 (Oh *et al*., 2011). Zhang *et al*. (2008) have observed more frequent occurrence of the FASN (AA) homozygote 0.36, compared to the FASN (GG) homozygote 0.13. The activity of the KR domain is essential for the FASN protein (Vázquez *et al*., 2008). The T1950A and W1955R may influence the KR domain, resulting in the alteration of the

FASN activity. T2264A was reported to have significant effects on economic traits (Morris *et al*., 2007; Schennink *et al*., 2009; Zhang *et al*., 2008). Moreover, Abe *et al*. (2009) reported that T1950A was completely linked with W1955R in Japanese Black cattle. In recent years, genetic studies have focused on the manipulation of unsaturated fatty acid composition of livestock products which have healthier effects on human metabolism (Taniguchi *et al.,* 2004; Mele *et al.,* 2007; Moioli *et al.,* 2007; Kgwatalala *et al.,* 2009). The mammalian FASN gene is widely regulated at both the transcriptional and post-transcriptional levels. One of the main transcription factors involved in this regulation is SP1 (Schweizer *et al*., 2002). In addition, it has also been described that FASN gene expression is regulated post transcriptionally by mediating mRNA stability (Semenkovich *et al*., 1993). With regards to RNA transcription via 5'-UTR-mediated regulation, Sp1 within 5'-UTRs has been shown to modulate the transcription rate of genes (Muredda *et al*., 2003; Lee *et al*., 2005).

CONCLUSION

It can be concluded that genetic variation is present in the coding region of FASN gene with adenine to guanine transition in lesser known buffalo populations (Gojri and Chhattisgarhi buffalo) is being reported first time. Results suggest that exon 40 region of FASN gene in Gojri and Chhattisgarhi buffaloes is highly polymorphic and sufficient genetic variability is present in this fat related gene in contrast to other well-known candidate gene for fat percentage (DGAT) in buffaloes. This A to G transition can be used further as SNP markers, which could be helpful to breeders for future association studies, selecting superior germplasm and conservation strategies. A detailed analysis of other SNPs localized within this gene, could possibly allow for indicating quantitative trait loci for milk fat, which can be used in marker assisted selection of river buffalo.

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Table 1. Genotype and allele frequencies in different breeds of river buffalo

Breed / Population	Genotype frequencies	Allele frequency
Gojri buffalo	AA = 0.375	A = 0.67
(n=40)	AG = 0.600	G = 0.33
	GG = 0.025	
Chhattisgarhi buffalo	AA = 0.325	A = 0.60
(n=40)	AG = 0.550	G = 0.40
	GG = 0.125	

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