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Polymorphism in fatty acid synthase (FASN) gene in swamp buffalo of Assam and Manipur, India

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Abstract
The present study has been aimed to screen the presence of variation with respect to the exon- 40 region of fatty acid synthase **(**FASN) gene in two native swamp buffalo populations, Luit of Assam and Manipuri eroi of Manipur, India as the FASN gene has been suggested to be associated with fatty acid composition in milk and its quality. Blood samples for DNA extraction were collected from a total of 30 randomly chosen swamp buffaloes each from different districts of Assam and Manipur, India. The detection of SNP variation present in the FASN gene within these two populations has been investigated using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. Three genotypes have been detected in the Luit buffalo population AA (0.37), AG (0.56) and GG (0.07) while the genotype GG was found to be absent in the Manipuri eroi buffalo with AA and AG having frequencies of 0.47 and 0.53, respectively. The A allele was observed to be predominant in both the populations with an overall allele frequency of 0.692. In regards to FASN gene and its allelic frequencies, the two populations do not show much variation between them. There is presence of variability in both the buffalo population in FASN locus which indicates the potential for genetic improvement in milk and milk fat production performance by implying proper selection methods and breeding systems.

Keywords: FASN gene, swamp buffalo, PCR-RFLP, SNP, variability

1. Introduction
Buffalo milk is known to have higher fat than cow milk. In India, the economic importance of buffalo milk is rising gradually with time. The Murrah group and Gujarat group of buffaloes are usually known for their milk production potential in the country. Unlike the buffaloes of most Indian states, the buffaloes of North eastern states of India like Assam and Manipur are swamp type and wild in nature. These animals are mainly reared for land preparation in wet paddy fields by the locals as they are known for their adaptability to the sub-tropical climate of the region. The marginal and poor farmers usually prefer these multipurpose animals for various uses like milk, meat and drought power under zero input system.

The higher fat and lower water content makes buffalo a highly desired dairy animal as compared to other livestock [1]. The bovine Fatty acid synthase (FASN) gene has been mapped to the chromosome 19 (BTA19) where several QTLs have been identified [2] including QTL for adipose fat composition in meat, milk fat and milk production [3] in bovines. The TE domain which is a part lying between the exon 39 to 41 of the FASN gene regulates the termination of fatty acid chain during its synthesis and thus plays a role in the quality of fat and its composition present in the milk [4]. Due to this role of FASN gene in milk fat determination it also becomes crucial in human nutrition and determining the metabolism aspects of the milk produced. Therefore, the FASN gene can be considered as a chief candidate gene for fatty acid composition and its variation from animal-to-animal. Study regarding its variation within a population might prove to be helpful in producing healthier animal products [5]. However, scanty information is available regarding the FASN gene polymorphism within the swamp buffaloes of India which are native to states like Assam and Manipur.

Therefore, the main aim of this study has been dedicated to the detection of polymorphism in FASN gene in swamp buffalo population of Assam (Luit) and Manipur (Manipuri eroi) and observation of the genetic variablility as well as heterozygosity present within and between these two populations and possibility of making genetic improvement with the help of the information obtained in the present study.

2. Materials and Methods

2.1 Ethical approval

The protocols followed abide by the standard rule of animal ethics and has been approved by the Institutional Animal Ethics Committee (IAEC) of India with the approval reference number CVSC/CAU/IAEC/19-20/P-20.

2.2 Animals and Blood samples

Random blood samples (approximately 5 mL) were collected for the present study from a total of 30 swamp buffaloes each from the states of Assam and Manipur. The animals from fields of Nagaon, Morigaon, Hojai, Sivsagar, Cachar and Jorhat districts of Assam and Imphal east, Imphal west and Senapati districts of Manipur were randomly chosen irrespective of their sex and age. Blood samples have been collected aseptically from the jugular vein in EDTA coated vials and then kept immediately in ice after collection and after bringing to the laboratory were stored at -20˚C till further use.

2.3 Genomic DNA isolation

Genomic DNA was extracted from the collected samples using commercially available GeneJET Genomic DNA Purification Mini Kit (K0782, Thermo Fisher Scientific) following the protocols provided with the kit. Quantity and quality checking of the extracted DNA from the samples were done using a Nanodrop Spectrophotometer (Thermo Scientific, USA) and electrophoresis on 0.75% agarose gel. DNAs having optical density ratio (OD_{260}/OD_{280}) of 1.7 to 1.9 were subjected to further analysis for molecular and quantitative data.

2.4 Polymerase Chain Reaction and Restriction Fregment Length Polymorphism

The PCR amplification was carried out in a 25 µL reaction mixture of 10X PCR buffer, 200 µM of each dNTPs, 2mM of $Mgcl_2$, 2U Taq DNA polymerase, 5 pM of each forward and reverse primers and 60 ng of extracted genomic DNA. The exon 40 of FASN gene was amplified using forward primer 5'-ctcgcacaccttcgtgatg -3' and reverse primer 5'-cacgttgccgtggtaggtag -3' [6]. PCR amplification of the desired region was done using the following cycles : Initial Denaturation at 95 ºC for 5 min, followed by 30 cycles of denaturation at 95 ºC for 45 sec, primer annealing at 61ºC for 60 sec, elongation at 72 ºC for 45 sec and final extension at 72ºC for 5 min. The amplified DNA of 472 bp was subjected to RE digestion using *Mluc*I restriction endonuclease enzyme by incubating at 37ºC for 8 hours. The digested products were separated for visualisation of the bands using 2.5% agarose gel in 0.5 X TAE containing 1.0 µM ethidium bromide and observed under UV trans-illuminator after which photographs were taken using Gel Doc system for further analysis. The primers and restriction enzyme used for PCR-RFLP analysis are given in Table 1.

Table 1: Gene, PCR amplicon size, primer sequence, annealing temperature for PCR and restriction endonuclease (RE) enzyme used for RFLP analysis:

Gene		Primer sequence (5´ - 3´)	T^A (°C)	Product size (bp)	RE	Incubation
FASN	F	CTCGCACACCTTCGTGATG	61	472	*Mluc*I	37 ºC for 8 hours
	R	CACGTTGCCGTGGTAGGTAG				

2.5 Statistical Analysis

The allele frequencies, genotypic frequencies, Hardy-Weinberg Equilibrium (HWE), observed and expected heterozygosity along with F-statistics were calculated using the POPGENE 32 software [7] for population genetics analysis.

3. Results and Conclusion

The PCR-RFLP analysis of FASN gene was done by restriction digestion of a 472 bp size amplicon which revealed the presence of polymorphism, yielding three genotypes AA (472 bp), AG (472, 281, 191 bp) and GG (281, 191 bp) showing 1, 3 and 2 bands respectively for each genotypes (Figure 1). The genotype AG was predominantly found in both the swamp buffalo populations of Assam (0.56) and Manipur (0.53). The GG genotype was completely absent in the Manipur buffalo population and was also very rarely distributed in the buffaloes of Assam with a frequency of 0.07. The calculated Chi-square value revealed that both the populations under the present study were confronting to the Hardy-Weinberg equilibrium (Table 2).

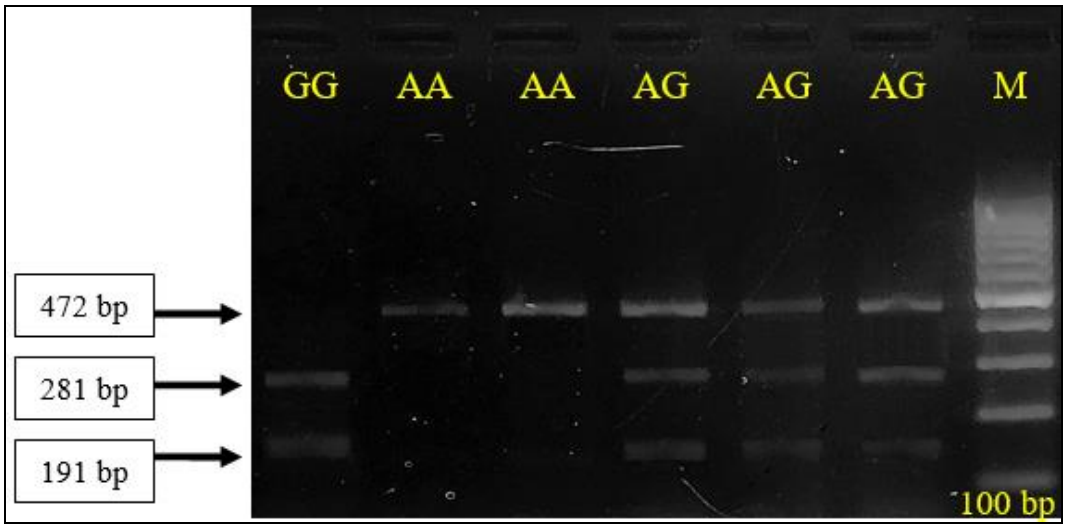

Fig 1: Genotypes of FASN gene digested with RE *Mluc*l in 2.5% agarose gel

Table 2: Genotypic frequency distribution in Swamp buffaloes of Assam (Luit) and Manipur (Manipuri eroi)

Genotypes	Assam (Luit) (n=30)	Manipur (Manipuri eroi) (n=30)
AA	0.37 (11)	0.47 (14)
AG	0.56 (17)	0.53 (16)
GG	0.07 (2)	0.00 (0)
χ^2 value	1.58 ^{NS}	3.68 ^{NS}
Observed Heterozygosity	0.57	0.53
Expected Heterozygosity	0.46	0.40

n = Number of animals; NS = Not significant; the figures in parenthesis are the number of animals

In Luit buffalo population the observed and expected homozygosity was found to be 0.43 and 0.53 respectively for the FASN locus while the observed and expected heterozygosity was calculated to be 0.57 and 0.46 respectively. Whereas, in Manipur eroi buffalo population, for FASN gene, the observed and expected homozygosity was calculated to be 0.47 and 0.60 while the observed and expected heterozygosity values were found to be 0.53 and 0.40 respectively.

The A and G allele frequency of FASN gene were found to be 0.65 and 0.35 respectively for Luit buffalo of Assam and a frequency of 0.74 and 0.26 was observed for the respective alleles in Manipuri eroi. The allele A was predominantly found in both the populations and showed an overall frequency of 0.692 (Table 3). The predominant allele A was observed with higher frequency in the Manipur buffalo population (0.74).

Similar to present findings, higher A allele frequency (0.62) in Murrah buffalo population was reported. The heterozygote AG genotype was found to be most frequent in the population showing a genotypic frequency of 0.56 [6]. In Gojri and Chattisgarhi breeds of India the A allele was more frequently found than G allele [1]. On the contrary, a higher frequency of the G allele was reported in Polish Holstein-Friesian Cattle population [8] where the G allele showed a frequency of 0.63. The AG genotype was most frequently found and had a frequency of 0.52 followed by GG (0.37) and AA (0.11) genotypes.

In various previous studies, the FASN locus was also regarded to be associated with production traits like Lactation Fat Average (LFA), Lactation Total Solid Average (LTSA) and Peak yield of milk in buffaloes where the A allele was found to be showing better results as compared to the G variants [6]. The AA genotype was also mentioned to be linked with an increased fat content and decreased Mono unsaturated fatty acid (MUFA) content hence making it less valuable for human nutrition unlike the GG genotype which has a low milk fat yield but a high MUFA proportion [8].

The influence of variation within the FASN locus was observed in the milk fat amount of Dutch Holstein-Friesian Cattle [9]. The difference between the various FASN genotypes was found to be statistically significant with respect to saturated fatty acid (SFA) and Mono unsaturated fatty acid (MUFA) content. The AA genotype animals were observed to be highest fat content and increased myrystic acid content while the GG genotype animals were characterised with low fat content but a higher MUFA content. Moreover, in Korean cattle [10] an increased MUFA/SFA ratio was observed for the GG genotype individuals along with an increased oleic acid and decreased palmitic acid content.

Table 3: Allele frequency of FASN locus in Swamp buffalo of Assam and Manipur

Locus	Allele	Allelic frequency		Overall frequency
		Assam	Manipur	
FASN	A	0.65	0.74	0.692
	G	0.35	0.26	0.308

The present study can be concluded by stating that genetic variation for the FASN locus is present within the Assam and Manipuri swamp buffaloes. Sufficient amount of heterozygosity and genetic variability is present among both the populations. The genotypic frequencies for the exon 40 polymorphisms in the TE domain of FASN gene were found to be conforming to the Hardy-Weinberg equilibrium for both the studied population indicating the absence of selection regarding fat composition in milk production. Thus, this information can be further utilized for association study and can be subsequently used for marker assisted selection in Luit and Manipuri eroi buffaloes of Assam and Manipur respectively.

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