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SNP IDENTIFICATION IN THIOESTERASE DOMAIN OF FATTY ACID SYNTHASE GENE IN MURRAH BUFFALOES

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ABSTRACT

The aim of present study was to screen for the presence of genetic variability in thioesterase (TE) domain of the fatty acid synthase (*FASN*) gene in Murrah buffalo, an important milch breed of India. TE domain of *FASN* gene is responsible for termination of fatty acid chain during its *de-novo* synthesis which in turn affects the fatty acid composition and quality of milk fat. In this study, terminal exons 38 to 42, including TE domain were explored for the presence of SNP variations using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. An important SNP at g.18433A>G in exon-40 region of *FASN* gene was identified in Murrah buffaloes. Three types of genotypes, viz. AA, AG and GG were observed having 34%, 56% and 10% frequencies, respectively. The allele frequencies of A and G alleles were 0.62 and 0.38, respectively. The identified polymorphism was non-synonymous transition in *FASN* gene. The study will augment the information available and can be applied in future studies to determine the role of buffalo *FASN* gene as a candidate gene marker for a milk-fat content.

Key words: Murrah buffaloes, *FASN*, Genotypes, SNP, Thioesterase domain.

INTRODUCTION

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. Fatty acid synthase *FASN* is a multifunctional enzyme complex that regulates *de novo* biosynthesis of long chain fatty acids. This cytosolic enzyme catalyses palmitic acid synthesis from acetyl coenzyme-A and malonyl coenzyme-A in the presence of NADPH (Wakil *et al*., 1983). The *FASN* gene has been identified in rat, human, goose, chicken and cattle (Kameda and Goodridge 1991; Amy *et al.,* 1992; Jayakumar *et al.,* 1995; Chirala *et al.,* 1997; Roy *et al.,* 2006). Bovine fatty acid synthase gene *(FASN)* was mapped to chromosome 19 (BTA19) at q22 band (Roy *et al*., 2001) and is 19770 bp long and consists of 42 exons and 41 introns (Gen Bank Accession. No. AF285607.2).

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). Based on QTL studies, Morris *et al.* (2007) identified *FASN* gene as a potential candidate gene for some milk production quality traits. Moreover, the fatty acid composition of milk is an important factor affecting human health (Mele *et al.,* 2007; Kgwatalala *et al.,* 2009). The thioesterase (TE) domain within the *FASN* complex regulates the termination of fatty acid synthesis. C14 acyl-ACP and C16acyl-ACP are both substrates of the *FASN* (TE) domain. The hydrolysis rate of C14 acyl-ACP by *FASN* (TE) is slower than that of C16 acyl-ACP (Lin and Smith 1978; Pazirandeh *et al.,* 1991). The TE domain has an important role in determining the product chain length of *FASN*. Therefore, the TE domain of the *FASN* gene can be a candidate gene for variance in fatty acid composition and might help to produce healthier livestock products regarding fatty acid composition (Zhang *et al*., 2008). The TE domain is the part between exons 39 to 41 of the *FASN* gene (Abe *et al*., 2009). SNPs affecting fatty acid composition, g.18663 (g – genomic sequence) T>C and g.17924 A>G, were identified in the TE domain (Zhang *et al*., 2008; Bhuiyan *et al*., 2009). Furthermore a *Bos indicus* associated SNP, g.18440 G>A is also reported in the same region (Bhuiyan *et al*., 2009). However, scanty literature is available regarding genetic variations in TE domain of the *FASN* gene among buffaloes. Keeping this in mind, an attempt was made to explore the polymorphism in *FASN* gene in Murrah buffaloes and to study its partial sequence comparison with other livestock species.

MATERIALS AND METHODS

Population studied and sample size: Random blood samples (approximately 8 to 10 mL) were collected from 196 buffaloes representative of the Murrah breed. Samples of Murrah buffaloes were collected from Livestock Farm of National Dairy Research Institute, Karnal (Haryana).

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. For amplification of *FASN* gene (exon 38, 39, 40, 41 and exon 42) in Murrah buffalo the primers were designed from published cattle (*Bos taurus*) sequences available online in the Gen bank (www.ncbi.nlm.nih.gov) having accession no. AF285607.2 for *FASN* gene (Table 1). Designing of primers were done with help of primer 3.0 software (http://frodo.wi.mit.edu/cgi-in/primer3/primer3_www.cgi.) The PCR-RFLP reaction conditions are shown in Table 2.

Statistical analysis: Genotype and allele frequencies of g. 18433 A>G polymorphisms in the TE domain were calculated by using the gene counting method as suggested by Falconer and Mackay (1996). A chi-square test was also performed to check Hardy-Weinberg equilibrium status in the studied population.

RESULTS AND DISCUSSION

The available genotypes of *FASN* exon 38 to 42 at position g.17980 G>T, g. 18025 C>T, g. 18433 A>G, g. 18628 C>G and g. 19056 C>T polymorphisms in the TE domain were determined by the PCR-RFLP method using the *RsaI*, *PstI*, *MluCl*, *MspI* and *DdeI* restriction enzymes in Murrah buffaloes. The genotype and allele frequencies are shown in Table 3. Digestion of the 228bp PCR product of exon38 with *RsaI* revealed two fragments of 133 and 95bp and show the monomorphic band pattern. Digestion of the 696bp PCR product of exon39 with *PstI* revealed three fragments of 296, 229 and 171bp in all the samples and was found to be monomorphic band pattern. Digestion of the 373bp PCR product of exon41 with *MspI* revealed two fragments of 256 and 117bp and show the monomorphic band pattern, and digestion of the 453bp PCR product of exon42 with *DdeI* revealed three fragments of 135, 121 and 97bp and also show the monomorphic band pattern. Digestion of the 472bp PCR product of exon40 with *MluCl* revealed three fragments of 281, 191bp and uncut 472bp show the polymorphic band pattern and it is novel finding. RFLP test using *MluCl* restriction enzyme indicated that exon40 region of *FASN* gene is polymorphic, with the presence of three genotypes namely, AA, AG & GG, with two alleles in Murrah buffaloes. Frequency of AA, AG and GG genotypes were 34%, 56% and 10%, respectively. Out of the three GG was rare and AG being most common genotype. Allele frequencies of A and G alleles were 0.62 and 0.38, respectively. Moreover, the genotype frequencies of the exon40 polymorphisms in the TE domain was found to be in Hardy-Weinberg equilibrium. Inferring that so far the studied population of Murrah buffaloes followed random mating and has not been subjected to selection with respect to TE domain of *FASN* gene for delete milk fat composition.

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; Schennink *et al.,* 2009; Kgwatalala *et al.,* 2009). Zhang *et al*. (2008) studied the association between g.17924A>G and g.18663 T>A polymorphisms in the *FASN* gene TE domain and beef fatty acid composition in Angus cattle. According to their results, g.17924 GG genotyped cattle had lower myristic acid (C14:0; P<0.0001), palmitic acid (C16:0; P<0.05), total SFA contents and higher oleic acid (C18:1; P<0.001) and total MUFA contents. The substitution from threonine to alanine due to this polymorphism may result in less C14:0 content and higher C:16:0 to C14:0 ratio in animals with the 17924 GG genotype (alanine) than in animals with the 17924 AA genotype (threonine) (Zhang *et al.,* 2008). Bhuiyan *et al.* (2009) reported a significant association of the GG genotype of g.17924 A>G with C16:0 and C18:1 fatty acid content. GG genotype frequency was calculated as 13% in Angus cattle (Zhang *et al.,* 2008) and 73% in Hanwoo cattle (Bhuiyan *et al.,* 2009).

The selected genotypes obtained through RFLP results were sequenced an on sequence comparison (Fig.1) it was revealed that the g.18433 G>A polymorphism in the TE domain leads to non-synonymous transition as observed in the third base of codon in *FASN* gene. The protein translation of exon40 (Fig.2) reveals a change of L (*Bos taurus*) to P Murrah buffaloes. However, both Leucine and Proline are non-polar neutral amino acid. In Murrah this change in amino acid may affect the quality and quantity of milk fat. However, detailed association studies needs to be carried out to confirm such association. On analysis of variations in adjacent sites to g.18433 it is confirmed that buffalo germplasm is quite different from *Bos taurus* at many locations but within buffalo samples it is polymorphic at this particular site (Fig.1). Phylogenetic analysis of sequence characterized for exon40 in Murrah was compared with other reported sequences (NCBI Accession No. AF_285607.2, NW_005395032, NW_005100886.1, XM_012186804.1, NC_006105.3, NW_005785022.1) in different livestock species (Fig.3) which revealed that Murrah is closer to *Bubalus, taurus* cattle and Yak species which lies in an adjacent cluster. Other livestock species forms different clusters. Therefore, it is suggested that A allele of g.18433 G>A is a Murrah associated allele.

It is concluded that genetic variation is present in the coding region of TE domain in *FASN* gene with guanine to adenine transition in well-known Murrah buffalo is being reported first time. Results suggest that

exon40 region of *FASN* gene in Murrah buffaloes is polymorphic and sufficient genetic variability is present in this gene in contrast to other well-known candidate gene for fat percentage like DGAT gene and its alleles are reported fixed in Murrah buffaloes. The genotypic frequencies of the exon40 polymorphisms in the TE domain were found to be in Hardy-Weinberg equilibrium. It infers that there is no selection of animal for *FASN* gene in Murrah herd. This G to A transition can be used further as SNP marker, which could be helpful to breeders for conducting future association studies and detailed analysis of other SNPs localized within this gene, could also possibly allow for indicating quantitative trait loci for milk fat and its metabolism, and can be used in marker assisted selection of river buffaloes.

Table 1. Primer sequences used for amplifying various exon regions of *FASN* gene

Gene / Region	Primer sequence	Product length	A. (ºC)	GC (%)
EXON 38	F- 3'GACCTTGACACGGCTCAACT-5'	228 bp	60	55
	R- 3'GGGCACAGCATGAGGTTTAG-5'			55
EXON 39	F- 3'AGAGCTGACGGACTCCACAC-5'	696 bp	58	60
	R- 3'CTGCATGAAGAAGCACATGG-5'			50
EXON 40	F- 3'CTCGCACACCTTCGTGATG-5'	472 bp	60	58
	R- 3'CACGTTGCCGTGGTAGGTAG-5'			60
EXON41	F- 3'CGCTCACTGTCCTGTCCTAC-5'	373 bp	60	60
	R- 3'GCTGTGAATAATACTAAGGATGGA-5'			37.5
EXON 42	F- 3'GCCTGGGCGCCGACTACAATCTG-5'	453 bp	61	65.2
	R- 3'CCCCCATGGCGACGCAATAAAT-5'			54.5

A. is annealing temperature

Table 2. PCR-RFLP reaction conditions used for digesting the different exons of *FASN* gene.

Region	Exon 38	Exon 39	Exon 40	Exon 41	Exon 42
Template DNA Conc. ng/µl	150	200	150	180	100
Restriction Enzyme	*RsaI*	*PstI*	*MluCl*	*MspI*	*DdeI*
Restriction Site	G|TAC	CTGCA|G	|AATT	C|CGG	C|TNAG
Buffer used	5 µl (10x)	5 µl(10x)	5 µl (10x)	5 µl (10x)	5 µl (10x)
Enzyme Concentration	3 IU	6 IU	4 IU	7 IU	3 IU
Time for digestion	8 to 12 hr. at 37^0C	8 to 12 hr. at 37^0C	8 to 12 hr. at 37^0C	8 to 12 hr. at 37^0C	8 to 12 hr. at 37^0C
Temp. & Time of Denaturation	80^0C for 15 min.	80^0C for 15 min.	65^0C for 20 min.	65^0C for 20 min.	80^0C for 15 min.

Table 3. Genotype and allele frequencies of different exons in *FASN* gene.

Position	Region	Genotype	Genotype frequency	Allele	Allele frequency
g 17980 G>T *(RsaI)*	Exon38	GG	1.00	G	1.00
		GT	0.0	T	0.0
		TT	0.0		
g 18025 C>T *(PstI)*	Exon39	CC	1.00	C	1.00
		CT	0.0	T	0.0
		TT	0.0		
18433 A>G (MluCl)	Exon40	AA	0.34	A	0.62
		AG	0.56	G	0.38
		GG	0.10		
g 18628 C>G *(MspI)*	Exon41	CC	1.00	C	1.00
		CG	0.0	G	0.0
		GG	0.0		
g 19056 C>T *(DdeI)*	Exon42	CC	1.00	C	1.00
		CT	0.0	T	0.0
		TT	0.0		

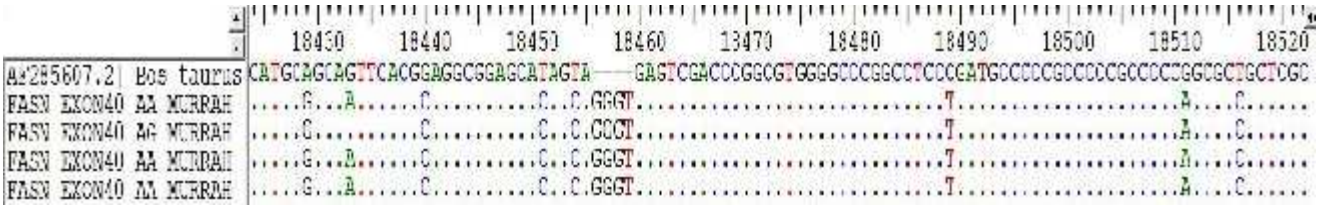

Figure 1. Nucleotide sequence alignment of different genotypes of exon-40 region in *FASN* gene and SNP at g.18433A>G position among different *Bubalus bubalis* (Murrah buffalo) and their comparison with *Bos Taurus* (Accession no. AF285607.2)

>Bos Taurus: Met P P P P P P A **L** L A H C P V L Q V L E A L L P L G D L E A R V A A T V E L I V Q S H A G L D R H A L S F A A R S F Y H K L R A A E

>**Murrah Buffalo:** Met P P P P P P A **P** L A H C P V L Q V L E A L L P L G D L E A R V A A T V E L I V Q S H A G L D R H A L S F A A R S F Y H K L R A A E

Figure 2. Comparison of different protein sequence (single letter symbol of proteins) of exon 40 region of TE domain of *FASN* gene

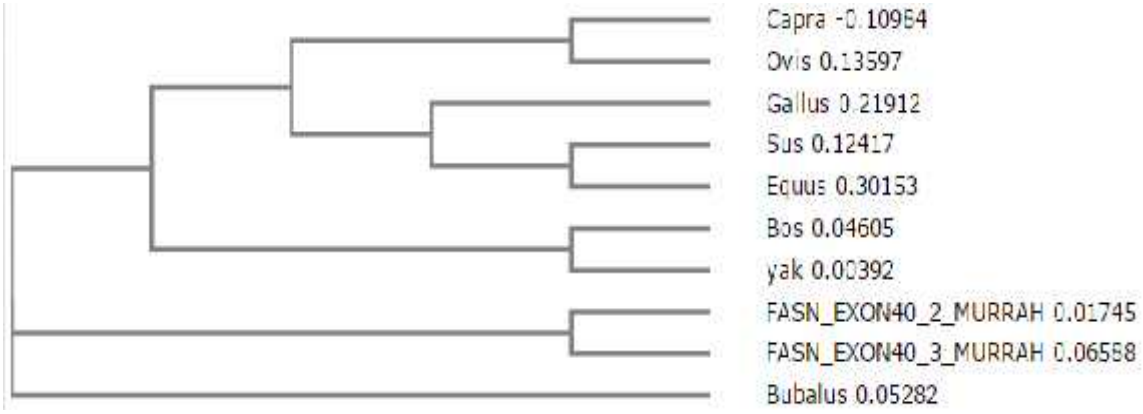

Figure 3. Phylogenetic diagram of TE domain of *FASN* gene based on NJ method using ClustalW2-Phylogeny program.

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REFERENCES

Abe, T., J. Saburi, H. Hasebe, T. Nakagawa, S. Misumi, T. Nade, H. Nakajima, N. Shoji, M. Kobayashi and E. Kobayashi (2009). Novel mutations of the *FASN* gene and their effect on fatty acid composition in Japanese Black beef. Biochem. Genet. 47: 397-411.

Amy, C. M., B. Williams-Ahlf, J. Naggert and S. Smith (1992). Molecular cloning of the mammalian fatty acid synthase gene and identification of the promoter region. Bioch. J., 271: 675–679.

Bhuiyan, M. S. A., S. L. Yu, J. T. Jeon, D. Yoon, Y. M. Cho, E. W. Park, N. K. Kim, K. S. Kim and J. H. Lee (2009). DNA polymorphisms in SREBF1 and *FASN* genes affect fatty acid composition in Korean cattle (Hanwoo). Asian-Aust. J. Anim. Sci. 22: 765-773.

Biochard, D., C. Grohs, F. Bourgeois, F. Cerqueira, R. Faugeras, A. Neau, R. Rupp, Y. Amigues, M. Y. Boscher and H. Leveziel (2003). Detection of genes influencing economic traits in three French dairy cattle breeds. Genet. Selec. Evol. 35: 77-101.

Chirala, S. S., W. Y. Huang, A. Jayakumar, K. Sakai and S. J. Wakil (1997). Animal fatty acid synthase: functional mapping and cloning and expression of the domain I constituent activities. Proc. Natl. Acad. Sci. 94: 5588–5593.

Falconer, D. S. and T. F. C. Mackay (1996). Introduction to Quantitative Genetics. 4th edn. Addison Wesley Longman Ltd. Essex, England.

Jayakumar, A., M. H. Tai, W. Y. Huang, W. Al-Feel, M. Hsu, L. Abu-Elheiga, S. S. Chirala and S. J. Wakil (1995). Human fatty acid synthase: properties and molecular cloning. Proc. Natl. Acad. Sci. 92: 8695–8699.

Kameda, K. and A. G. Goodridge (1991). Isolation and partial characterization of the gene for goose fatty acid synthase. J. Biol. Chem. 266: 419–426.

Kgwatalala, P. M., M. Ibeagha-Awemue, X. Mustafaa and X. Zhao (2009). Influence of stearoyl-coenzyme-A desaturase 1 genotype and stage of lactation on fatty acid composition of Canadian Jersey cows. J. Dairy Sci. 92: 1220-1228.

Lin, C. Y. and S. Smith (1978). Proporties of the thioesterase component obtained by limited trypsinization of fatty acid synthetase multienzyme complex. J. Biol. Chem. 253: 1954-1962.

Mele, M., G. Conte, B. Castiglioni, S. Chessa, N. P, Macciotta, A. Serra, A. Buccioni, G. Pagnacco and P. Secchiari (2007). Stearoyl-coenzyme A desaturase gene polymorphism and milk fatty acid composition in Italian Holsteins. J. Dairy Sci. 90: 4458-4465.

Morris, C. A., N. G. Cullen, B. C. Glass, D. L. Hyndman, T. R. Manley, S. M. Hickey, J. C. McEwan, W. S. Pitchford, C. D. Bottema and M.A. Lee (2007). Fatty acid synthase effects on bovine adipose fat and milk fat. Mamm. Genome. 18: 64–74.

Pazirandeh, M., S. S. Chirala and S. J. Wakil (1991). Site directed mutagenesis studies on the recombinant thioesterase domain of chicken fatty acid synthase expressed in Escherichia coli. J. Biol. Chem. 266: 20946-20952.

Roy, R., M. Gautier, H. Hayes, P. Laurent, R. Osta, P. Zaragoza, A. Eggen and C. Rodellar (2001). Assignment of the fatty acid synthase *(FASN)* gene to bovine chromosome 19 (19q22) by in situ hybridization and confirmation by somatic cell hybrid mapping. *Cytogenet.* Cell Genet. 93: 141-142.

Roy, R., L. Ordovas, P. Zaragoza, A. Romero, C. Moreno, J. Altarriba and C. Rodellar (2006). Association of polymorphisms in the bovine *FASN* gene with milk-fat content. Anim. Genet. 37: 215-218.

Sambrook, J. and D. W. Russel (2001). Molecular Cloning: A laboratory manual. 3rd ed. vol 1. Cold Spring Harbor Lab. press. New York, USA.

Schennink, A., H. Bovenhuis, K. M. Leon-Kloosterziel, J. A. M. Van Arendonk and M. H. P. Visker (2009). Effect of polymorphisms in the *FASN*, OLR1, PPARGC1A, PRL and STAT5A genes on bovine milk-fat composition. Anim. Genet. 40: 909-916.

Taniguchi, M., T. Utsugi, K. Oyama, H. Mannen, M. Kobayashi, Y. Tanabe, A. Ogino and S. Tsuji (2004). Genotype of stearoyl-coenzyme-A desaturase is associated with fatty acid composition in Japanese Black cattle. *Mamm. Genome.*14: 142-148.

Wakil, S. J., J. K. Stoops and V. C. Joshi (1983). Fatty acid synthesis and its regulation. Annual Review of Biochem. 52: 537–579.

Zhang, S., T. J. Knight, J. M. Reecy and D. C. Beitz (2008). DNA polymorphisms in bovine fatty acid synthase are associated with beef fatty acid composition. Anim. Genet. 39: 62-70.