DGAT1 GENE POLYMORPHISMS AND ITS ASSOCIATION WITH MILK PRODUCTION TRAITS IN MEHSANA BUFFALO (*BUBALUS BUBALIS*)

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ABSTRACT

India is mega diversity center for buffalo breeds among that Mehsana is one of the best milk breeds of buffalo. Diacylglycerol acyltransferase 1 (DGAT1) is a microsomal enzyme that catalyzes the final step of triglyceride synthesis. The DGAT1 gene is a strong functional candidate for determining milk fat content. In this work, we used different techniques viz., Polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP), Allele specific PCR (AS PCR), Single extension assay (SNaP Shot) and direct sequencing to screen the nineteen reported single nucleotide polymorphisms (SNPs) in DGAT1 gene in 130 unrelated Mehsana buffalo (Bubalus bubalis) reared under field progeny testing programme operated by Dudhsagar Research and Development Association (DURDA) in the Gujarat. SNP g.8259G>A was significantly associated with first lactation yield. However, no SNP was associated with milk fat content in Mehsana buffalo. The result presented here is preliminary and require further investigation on large population.

Keywords: DGAT1, PCR-RFLP, Allele specific PCR, Single extension assay, SNP

INTRODUCTION

There are 177.25 million buffalo in the world distributed 96.4% in Asia, 2.8% in Africa mainly in Egypt, 0.2% in Europe mainly in Italy and 0.6% in America mainly in Brazil. India has 56%, Pakistan 14% and China 13% of world buffalo population constituting nearly 98% of water buffalo in Asia (FAO, 2010). India's diversity in buffaloes is multifarious. There are 13 recognized breeds (Bhadawari, Jaffarabadi, Marathwadi, Mehsana, Murrah, Nagpuri, Nili Ravi, Pandharpuri, Surti, Toda, Banni, Chilika, Kalahandi) of water buffalo (*Bubalus bubalis*) spread out largely in Northern and Western India (http://www.nbagr.res.in/regbuf.html). Mehsana is one of the best milk breeds of buffalo in India (Gupta, 1997) and inhabits the northern part of the Gujarat state (Figure 1) and derives its name from the town "Mehsana" in the North Gujarat State. The average milk yield is between 1200 to 1500 kg per lactation (Pundir *et*

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al., 2000).

Many candidate genes with different functions in metabolism have been proposed as affecting milk yield and composition in dairy cattle, such as Diacylglycerol acyltransferase (*DGAT1*) (Grisart *et al*., 2002), Leptin receptor (*LEPR*) (Silva *et al*., 2002), Butyrophilin (*BTN1A1*) (Ogg *et al*., 2004), *Pit1* (de Mattos *et al.,* 2004), Growth hormone (*GH*) (Muhaghegh *et al.,* 2006), Osteopontin (Leonard *et al*., 2006), Leptin (Javanmard *et al*., 2005), Prolactin (Alipanah *et al*., 2007), αs1-casein (Chianese *et al*., 2009), Kappa-Casein (Riaz *et al*., 2008), Alpha-Lactalbumin (Ramesha *et al*., 2008), etc.

Diacylglycerol acyltransferase (*DGAT1*) is important in lipogenesis in many tissues, including mammary gland (Cases *et al*., 1998). *DGAT1* gene is located in the terminal portion of the centromere referring to chromosome 14 in bovines which is a locus with a quantitative profile (QTL) (Furbass *et al*. 2006). Grisart *et al.* (2001) sequenced *DGAT1* gene of *Bos taurus* and reported two base substitutions at nucleotide position 10,433 and 10,434 in exon 8 leading to amino acid substitution from lysine to alanine (K232A) where Lysine variant (K allele) was associated with high fat yield and Alanine variant (A allele) with high milk yield. Later on, Winter *et al*. (2002) and Roos *et al.* (2007) demonstrated similar results for Lysine variant (K allele) and Alanine variant (A allele) in *Bos taurus* cattle. However, Tantia *et al*. (2006) found fixed allele K at *DGAT1* gene in Indian cattle (*Bos indicus)* as well as buffaloes (*Bubalus bubalis)*. Later on, Mishra *et al.* (2007) also

Figure 1. Breeding tract of Mehsana buffaloes.

confirmed absence of K232A substitution in Indian buffalo breeds. Recently, Ozdil and Jihan (2012) also supported existence of only lysine variant (K allele) in all of the indigenous Anatolian buffalo populations.

Mishra *et al.* (2007) although found fixed K allele in DGAT1 gene of *Bubalus bubalis,* reported 19 single nucleotide polymorphisms (SNPs) (g. 3057A>T, g.3627 C>T, g.3674G>A, g.3741G>C, g.3815C>A, g.1606T>C, g.2141G>T, g.2217C>T, g.2394C>T, g.1179A>T, g.1195C>T, g.1784G>A, g.1875G>A, g.3096G>T, g.5545T>C, g.6067C>T, g.8087C>T, g.8259G>A, g.8426C>T) in buffalo DGAT1. Functional significance of these 19 SNPs has not been investigated in buffalo, particularly their role in genetic variability in milk yield and milk fat %. Although intronic, these SNPs might affect the function of genes for instance, like an enzyme such as endothelial nitric oxide synthase (Buraczynska *et al*., 2004) and the cytochrome P450 isoenzyme CYP1A2 (Sachse *et al*., 1999). Hence, these reported SNPs in DGAT1 gene were screened in Mehsana buffalo for possible association with milk production traits. We also developed rapid and precise SNP genotyping techniques that allow the identification of SNPs in buffalo DGAT1 gene.

MATERIALS AND METHODS

A total of 130 unrelated Mehsana buffalo under field progeny testing programme operated by Dudhsagar Research and Development Association (DURDA) in the Gujarat state were used for the study. In India, selection is based on only two traits, milk yield and milk fat percentage in dairy animals. First lactation milk yield (MY) was derived by totaling ten monthly test day records while milk fat percentage (MF%) was derived by averaging ten monthly test day records. Blood samples were collected from Mehsana buffloes from their breeding tracts (Fig 1). Genomic DNA was isolated by the phenol-chloroform extraction method (Sambrook and Russel, 2001). The quality and purity of the isolated DNA were determined by agarose gel electrophoresis and spectrophotometry.

Genomic regions of DGAT1 gene encompassing 19 SNPs were amplified using flanking primers as described by Mishra *et al.* (2007). PCR reaction was prepared in 25 µl volume containing 90 ng of genomic DNA, 10 pmol of each primer, 12.5 µl 2X PCR master mix (including 4 mM $MgCl_2$, 1.6 mM dNTPs and 0.05 U of Taq DNA polymerase) and amplification was performed in Veriti thermal cycler (Applied Biosystem). PCR amplification protocol was standardized using cyclic condition consisting of initial denaturation at 94 °C for 5 min followed by 30 amplification cycles of denaturation at 94 °C for 45 s, annealing temperature 54°C to 66°C (Table 1) for 45 s and extension at 72 °C for 45 s, with a final extension at 72 °C for 10 min. PCR amplicons were resolved on 2% agarose gel to confirm desired size and purified by GenElute agarose spin columns (Sigma). Details of primer sequences, PCR product size, annealing temperatures and different techniques used in study are presented in Table 1.

Different genotyping methods were attempted for rapid screening of DGAT1 SNPs. Out of 19 SNPs, three (g.3057A>T and g.3627C>T in intron 1 and g.8426C>T in 3' UTR) fall within the recognition sequences of the specific restriction enzymes (Table 1). Hence, these SNPs were screened by PCR-RFLP. The PCR products were digested with *NmuCI (5'-↑GTSAC- 3'), SmaI (5'-CCC↑GGG- 3') and EheI (5'-GGC↑GCC- 3')* endonuclease respectively. Digested PCR products were separated by electrophoresis through 2% - 2.5% agarose gel. Genotyping was performed based on RFLP patterns.

Table 1. Primer Sequences, Product size, SNPs covered, approaches for screening, and region amplified from DGAT1 gene.

Sr. No	Primer sequences	T_a	Product size	SNP position	Approaches	Region
1	F: TTggCAggTTgTAgCATgAg R: gCAAggCCTCCAgTTTTgTA	60°C	503 bp	g. 3057A>T	PCR RFLP RE - *Tsp45I*	Intron 1
2	F: ggCCTCTCCCCTTACAAAAC R: CACACACCAATTCAggATgC	56°C	661 bp	g.3627 C>T* g.3674G>A	PCR-RFLP, RE- *SmaI*	
				g.3741G>C g.3815C>A*	Direct sequencing	
3	F: TggATTTggggTCACTTT R: gTCCCTCTACCAgCCTTCC.	54°C	1146 bp	g.1606T>C g.2141G>T* g.2217C>T g.2394C>T	Direct sequencing	
4	F: gAAggAgTTgggggAgTAAg R: TgAggAggAAgAggAACAgg	60°C	891 bp	g.1179A>T* g.1195C>T	SNaPShot	
5	Wt: F1: ggCAgggCTgggCATTg**g** Mt:F2 : ggCAgggCTgggCATTg**A** R: TCAAAgggCAgCAggCATgg	66°C	734 bp	g.1784G>A	AS- PCR	
6	Wt_F1: TgTTCCTCTTCCTCCTCAg Mt_F2: TgTTCCTCTTCCTCCTCAA *Wt*_ F1: TgTTCCTCTTCCTCCTC**T**g *Mt* _F2: TgTTCCTCTTCCTCCTC**T**A R: CTCATTACATTTggCACCTC	60°C	490 bp	g.1875G>A	AS- PCR	
7	F: TTggCAggTTgTAgCATgAg R: gCAAggCCTCCAgTTTTgTA	60°C	505 bp	g.3096G>T*	SNaPShot	Intron 2
8	F: gCGTgAgTAggggTggAg R: gCAACTAAggACAgAAgCAA	60°C	791 bp	g.5545T>C g.6067C>T	SNaPShot	Intron 3
9	F: CTCCCCCgCAgACACTTC R:GCACAgCACTTTATTgACACATTC	54°C	748 bp	g.8087C>T g.8259G>A	Direct sequencing	Intron-15, 16
10	F: gATAgTgggCCgCTTCTTC R: TgCACAgCACTTTATTgACACA	60°C	413 bp	g.8426C>T	PCR/ RFLP RE - *SfoI*	3' UTR

F=forward, R=reverse, Wt=wild type, Mt=mutant type, *Mt* and *wt* indicates primers with penultimate mismatch at 3' end and bold letters indicate penultimate mismatch, * indicates transversion, T_a= annealing temperature, UTR= Untranslated region, RE=restriction enzyme, AS = Allele specific

Table 2. Primers used in single nucleotide extension assay (SNaPshot assay).

Sr. No.	Primer Name	Targeted SNP	Sequence (5' to 3')	Primer length
1	*DGAT*-SNaP1179	g.1179A>T	$(A)_{40}$TCCAgAgCCAgAggTTATCA	60
2	*DGAT*-SNaP1195	g.1195C>T	$(A)_{35}$AAATCCTTgCTgACTgACCT	55
3	*DGAT*-SNaP3096	g.3096G>T	$(A)_{30}$TCCATCCTgggCAgCACACg	50
4	*DGAT*-SNaP5545	g.5545T>C	$(A)_{27}$GTAgTggTgggAAAgAgC	45
5	*DGAT*-SNaP6067	g.6067C>T	$(A)_{22}$TgggTgAgggTCTgTgTg	40

Table 3. Allelic frequency of ten polymorphic SNPs of *DGAT1* gene in Mehsana buffalo breed and comparison with previously reported frequency by Mishra *et al*., 2007.

Region	SNP	Base Pair (from/to)	Allelic frequency in present study	Allelic frequency reported in other Indian buffaloes by Mishra *et al*., 2007
Intron 1	2217	C → T	0.09	0.14
	3057	A→ T	0.03	0.21
	3627	C → T	0.09	0.20
	3674	G → A	0.71	0.16
	3741	G → C	0.27	0.11
	3815	C → A	0.47	0.16
Intron 2	5545	T → C	0.40	0.20
Intron 3	6067	C → T	0.43	0.06
Intron 16	8259	G → A	0.47	0.41
3' UTR	8426	C → T	0.65	0.13

Allele specific (AS) PCR assay was developed for screening of other two SNPs (g.1784G>A and g.1875G>A) in intron 1 using either wild type (*wt*) primer (F1) or mutant (*mt*) primer (F2) along with a common reverse primer (Table 1) in otherwise identical 25 µL PCR reaction mix.

Single nucleotide extension assay (SNaPshot assay) was used for screening SNPs (g.1179A>T and g.1195C>T) in intron 1, (g.3096G>T) in intron 2 and (g.5545T>C and g.6067C>T) in intron 3. This assay is based on single base extension of an oligonucleotide by DNA polymerase (Sokolvo, 1990). For this assay SNP templates (amplicon covering SNPs) were generated from genomic DNA and used as templates for primers (Table 2). These primers hybridize to the SNP templates just adjacent to the mutation site. Length of these primers varies in size due to addition of poly A tail at 5' end which helps to resolve them based on size difference during the capillary electrophoresis. Multiplex reaction of SNP templates including primers (Table 2), DNA polymerase and fluorescence-labelled dideoxynucleotide (ddNTPs) terminators was subjected to single nucleotide primer extension under thermal cycling condition of annealing at 50^0C and extension at 60^0C for 25 cycles. The obtained SNaPshot PCR products were treated with Calf Intestinal Alkaline Phosphatase (to functionally deactivate unincorporated ddNTPs and primers) and subjected to capillary electrophoresis on ABI PRISM 310 Genetic Analyzer along with LIZ™ 500 size standard. Each of the used ddNTPs was assigned to a different fluorescent dye so that different fluorescence signals generated correspond to different alleles.

Remaining SNPs (g.3741G>C, g.3815C>A, g.1606T>C, g.2141G>T, g.2217C>T, g.2394C>T in intron 1, g.8087C>T in intron 15, g.8259G>A in intron 16 and g.8426C>T in 3' UTR) were genotyped by direct sequencing.

The allele and genotype frequencies were estimated by direct counting. The relationships between *DGAT1* SNP genotypes and milk yield traits in 130 Mehsana buffaloes were evaluated using general linear model (GLM) procedure of SAS software version 9.2. Following linearity model was applied to ascertain the association of *DGAT1* SNP genotypes with milk yield traits in Mehsana buffalo.

$$Y_{ijk} = \mu + S_i + G_j + e_{ijk},$$

Where,

Y_{ijk} = observation of the milk yield traits of the progeny of i^{th} sire, j^{th} genotype

S_i = sire families

G_j = fixed effects of genotypes of each SNP

e_{ijk} = random residual effect

Herd, year, season effects were not considered, as buffalo belonged to same milk shade area (following common management practices) born in the same year and season having same parity (1^{st} lactation).

RESULTS

Genotyping of SNPs g.3057A>T and g.3627C>T in intron 1 and g.8426C>T in 3' UTR was performed based on RFLP patterns as AA (337bp and 166bp) and AT (503bp, 337bp, and 166bp) or TT (503 bp) for SNP g.3057A>T, likewise CC (397bp and 264bp), CT (661bp, 397bp, and 264bp) and TT (661bp) for SNP g.3627C>T and similarly, CC (291bp and 122bp), CT (413bp, 291bp and 122bp) and TT (413bp) for SNP g.8426C>T.

Genotyping of SNPs g.1784G>A and g.1875G>A was carried out according to the amplification of *wt* and/or *mt* allele product after AS-PCR. Allelic discrimination showed that all the samples showed GG genotype for both SNPs on agorse gel indicated that 'G' allele was fixed in the Mehsana buffalo.

SNaPshot assay showed that at SNP positions g.1179A>T, g.1195C>T and g.3096G>T "A", "C" and "G" alleles were fixed respectively in the Mehsana buffalo population whereas, at SNP positions g.5545T>C and g.6067C>T there was higher frequency of "C" and "T" alleles respectively.

All the nineteen SNPs were successfully screened in Mehsana buffalo and the minor allele frequency were estimated (Table 3) which revealed that nine SNPs (g.1179A>T g.1195C>T, g.1606T>C, g.1784G>A, g.1875G>A, g.2141G>T, g.2394C>T, g.3096G>T and g.8087C>T) were monomorphic whereas, ten SNPs (g.2217C>T, g. 3057A>T, g.3627 C>T, g.3674G>A, g.3741G>C, g.3815C>A, g.5545T>C, g.6067C>T, g.8259G>A and g.8426C>T) were polymorphic with varying frequencies in Mehsana buffalo.

Different SNP genotypes of DGAT1 were compared for milk production traits *viz.,* first lactation standard (305 days) milk yield (MY) and milk fat % (MF) to find any association between them. There was no significant difference in milk yield among various SNP genotypes except for SNP g.8259G>A. AA and GA genotypes (2169.40^{a} ± 183.385 kg, 2363.60^{a} ± 41.50 kg respectively) had significantly (P=0.01) higher milk yield than GG genotypes (1577.23^{b} ± 126.28 kg). None of the other SNP had any significant influence on milk yield or milk fat percentage.

DISCUSSIONS

There was intense interest in DGAT1 K to A mutation since its first report (Grisart *et al.*, 2001) of association with milk fat% and milk yield in dairy breeds throughout the Europe, which unanimously revealed high frequency of K allele in European cattle (Cardoso *et al*., 2011). On the other hand, a low frequency of the K allele is found in populations of the zebu breeds (Winter *et al.*, 2002). In Indian Holstein bulls the results indicated higher allelic frequency of K allele (0.59) compared to A allele (0.41) for DGAT1 (Patel *et al*., 2009). However, these two variants are not found in Indian zebu cattle and buffalo. In absence of these mutants we screened other 19 SNPs present in this gene in Indian buffalo to explore possible association with milk production traits. Our findings showed that there was no significant association of these SNP genotypes with milk production traits *viz.,* lactation yield and milk fat percentage except SNP g.8259G>A which showed AA and GA genotypes had significantly higher milk yield than GG genotypes. Earlier, Yuan *et al.* (2007) reported seven polymorphic positions in the complete genomic region of buffalo DGAT1 and detected SNP (C/T) at position 11785 in exon 17 which creates a substitution change for the amino acid sequence, resulting in an Ala residue (GCG) transition to a Val residue (GTG) in position 484 of buffalo DGAT1 protein. However, its effect on functional traits was not studied.

In conclusion, SNP genotyping techniques were developed in this study are for screening of DGAT1 gene polymorphisms in buffaloes. The reported intronic SNPs except SNP g.8259G>A in DGAT1 gene show non significant difference either in milk yield or in milk fat percentage. Since, the study was conducted on animals with

markedly different milk yield and milk fat content which indicates a need for large number of animals to ascertain the association between these SNPs and milk fat content. Surprisingly, 50% SNPs were found to be monomorphic (fixed for either of allele), particularly, SNPs with very low minor allele frequency in other Indian buffalo breeds were observed to be fixed in Mehsana buffalo.

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REFERENCES

Alipanah, M., L. Kalashnkova and G. Rodionov. 2007. Association of prolactin gene variants with milk production traits in Russian Red Pied cattle. *Iran J. Biotech.,* **5**: 158-161.

Buraczynska, M., P. Ksiazek., W. Zaluska., T. Nowicka and A. Ksiazek. 2004. Endothelial nitric oxide synthase gene intron 4 polymorphism in patients with end-stage renal disease. *Neph. Dial. Transp*., **19**: 2302-2306.

Cardoso, S.R., L.B. Queiroz, V.A. Goulart, G.B. Mourao, E. Benedetti and L.R. Goulart. 2011. Productive performance of the dairy cattle Girolando breed mediated by the fat-related genes DGAT1 and LEP and their polymorphisms. *Res. Vet. Sci*., **91**: 107-112.

Cases, S., S.J. Smith, Y.W. Zheng, H.M. Myers, S.R. Lear, E. Sande, S. Novak, C. Collins, C.B. Welch and A.J. Lusis. 1998. Identification of a gene encoding an acyl CoA:diacylglycerol acyltransferase, a key enzyme in triacylglycerol synthesis. *Proceedings of the National Academy of Sciences of the United States of America*, **95**: 13018-13023.

Chianese, L., M. Quarto, F. Pizzolongo, M.G. Calabrese, S. Caira, R. Mauriello, S. De Pascale and F. Addeo. 2009. Occurrence of genetic polymorphism at the αs1-casein locus in Mediterranean water buffalo milk. *Int. Dairy J*., **19**: 181-189.

de Mattos, K.K., S.N. Del Lama, M.L. Martinez and A.F. Freitas. 2004. Association of bGH and Pit-1 gene variants with milk production traits in dairy Gyr bulls. *Pesq. Agropec. Bras. Brasilia*., **39**: 147-150.

FAO. 2010. 2008. *Production Yearbook*.

Grisart, B., W. Coppieters, F. Farnir, L. Karim, C. Ford, P. Berzi, N. Cam bisano, M. Mni, S. Reid, P. Simon, R. Spelman, M. Georges and R. Snell. 2001. Positional candidate cloning of a QTL in dairy cattle: Identification of a missense mutation in the bovine DGAT1 gene with major effect on milk yield and composition. *Genome Res.,* **12**: 222-231.

Gupta, P.R. 1997. Operation flood. *In* Gupta, P.R. (ed.) *Dairy India Year Book,* 5th ed. New Delhi, India. Available on http://www.nbagr.res.in/regbuf.html.

Javanmard, A., N. Asadzadeh, M.H. Banabazi and J. Tavakolian. 2005. The allele and genotype frequencies of bovine pituitary specific transcription factor and leptin genes in Iranian cattle and buffalo populations using PCR-RFLP. *Iranian J. Biotech.,* **3**: 2-5.

Leonard, S., H. Khatib, V. Schutzkus, Y.M. Chang and C. Maltecca. 2006. Effects of

the Osteopontin Gene Variants on Milk Production Traits in Dairy Cattle. *J. Dairy Sci*., **88**: 4083-4086.

Mishra, B., M.S. Tantia, S.T. Bharani Kumar and R.K. Vijh. 2007. Characterization of the DGAT1 gene in the Indian buffalo (*Bubalus bubalis*). *Genetics and Molecular Biology.* **30**(4): 1097-1100.

Muhaghegh, M.H., S.L. Goswami and S. De. 2006. Single strand conformation polymorphism (SSCP) in 3' region of growth hormone gene in five breeds of Indian buffalo. *Anim. Sci. Rep*., **24**: 159-162.

Ogg, S.L., A.K. Weldon., L. Dobbie, A.J.H. Smith and I.H. Mather. 2004. Expression of butyrophilin (BTN1A1) in lactating mammary gland is essential for the regulated secretion of milk-lipid droplets. *Proc Natl Acad Sci*., **101**: 10084-10089.

Ozdil, F. and F. Ilhan. 2012. DGAT1-exon8 polymorphism in Anatolian buffalo. *Livestock Science.* **149**(1): 83-87.

Patel, R.K., J.B. Chauhan, K.J. Soni and K.M. Singh. 2009. Genotype and allele frequencies of DGAT1 gene in Indian Holstein Bulls. *Current Trends in Biotechnology and Pharmacy*, **3**: 4.

Pundir R.K., G. Sahana, N.K. Navani., P.K. Jain, D.V. Singh, S. Kumar and A.S. Dave. 2000. Characterization of Mehsana Buffaloes in India. *AGRI.*, **28**: 53-62.

Ramesha, K.P., H. Khosravinia, S. Gowda and M.R.S. Rao. 2008. Alpha-Lactalbumin Gene Polymorphism: A preliminary study on two breeds of the river Buffalo *(Bubalus bubalis). Asia. Pac. J. of Mol. Bio*., **16**: 47-52.

Riaz, M.N., N.A. Malik., F. Nasreen and J.A. Qureshi. 2008. Molecular marker assisted study of kappa-casein gene in Nili-ravi (buffalo) breed of Pakistan. *Pak. Vet. J*., **28**: 103-106.

Roos, A.P.W.D., C. Schrooten, E. Mullaart., M. P. L. Calus and R. F. Veerkamp. 2007. Breeding value estimation for fat percentage using dense markers on *Bos taurus* autosome 14. *J. Dairy Sci*., **90**: 4821-4829.

Sachse, C., J. Brockmoller, S. Bauer and I. Roots. 1999. Functional significance of a C _ A polymorphism in intron 1 of the cytochrome P450 *CYP1A2*gene tested with caffeine. *Brit. J. Clin. Pharmaco.,* **47**: 445-449.

Sambrook, J. and D.W. Russel. 2001. *Molecular Cloning: A Laboratory Manual,* 3rd ed. Cold Spring Harbor Laboratory Press, New York, USA. 616p.

Silva, L.F.P., M.J. Vandehaar, M.S. Weber Nielsen and G.W. Smith. 2002. Evidence for a local effect of leptin in bovine mammary gland. *J. Dairy. Sci*., **85**: 3277-3286.

Sokolvo, B.P. 1990. Primer extension technique for the detection of single nucleotides in genomic DNA. *Nucleic Acid Research*, **18**: 3671.

Tantia, M.S., R.K. Vijh, B.P. Mishra, B. Mishra, S.T. Bharani Kumar and M. Sodhi. 2006. DGAT1 and ABCG2 polymorphism in Indian cattle (*Bos indicus*) and buffalo (*Bubalus bubalis*) breeds. *BMC Vet. Res.,* **2**(32): 1-5.

Winter, A., W. Kramer, F.A.O. Werner, S. Kollers, S. Kata, G. Durstewitz, J. Buitkamp, J.E. Womack., G. Thaller and R. Fries. 2002. Association of a lysine-232/alanine polymorphism in a bovine gene encoding acyl CoA: diacylglycerol acyltransferase (DGAT1) with variation at a quantitative trait locus for milk fat content. *Proc. Natl.*

Acad. Sci. USA., **99**(14): 9300-9305.

Yuan J., J. Zhou., X. Deng, X. Hu and N. Li. 2007. Molecular cloning and single nucleotide polymorphism detection of buffalo DGAT1 gene. *Biochem Genet*., **45**: 611-621.