International Journal of Biotech Trends and Technology
ISSN: 2249 – 0183 /doi:10.14445/22490183/IJBTT-V11I1P604

Volume 11 Issue 1, 20-25, Jan-Mar 2021

FASN Gene And Its Role In Bovine Milk Production

D.S.Kale[#], Jaya Singh, Y.B. Sathe, and D.V.Patil

Department of Animal Genetics and Breeding, Nagpur Veterinary College, Maharashtra Animal and Fishery Sciences University (MAFSU), Seminary Hills, Nagpur-440006, Maharashtra, India.

Abstract:
FASN (Fatty Acid Synthase) is regarded as a candidate gene for milk production traits in cattle which is a multidomain enzyme complex and functions for the synthesis of palmitic acid through denovo lipogenesis. The current emphasis world over is to identify the genetic mechanisms behind the milk composition and milk production traits like fat synthesis for identification of genetic signatures behind economic traits and their use in the selection of quality animals of breeds. The high concentration of dietary monounsaturated and polyunsaturated fatty acids are found to be associated with low cholesterol and low-density lipoproteins level, which aids in decreasing the risk of several cardiovascular diseases. In view of this, the studies related to the identification of genetic variation within FASN candidate gene reported to be exhibiting significant SNPs related to milk composition and production traits need to be reviewed for understanding the identified significant genetic variants, effective techniques, its impact on its possible use as DNA marker for fat milk traits in selection and breeding decisions of cattle breed improvement.

Keywords*: FASN, DNA Markers, Milk traits, and Bovine*

I. INTRODUCTION

For the last 40 years, improving the fat content of milk has been one of the prime focuses in animal science. Similarly, the nutritional quality of milk fatty acids profile has received considerable attention for improvement. Till date, one of the main objectives has always been being to increase the concentration of polyunsaturated fatty acids in milk. Cattle and buffaloes are considered the most valuable and significant species for dairy production. Currently, large numbers of animal breeds are being exploited for value-added products in the dairy industry. The different region around the world has priority in adopting prevalent breed of their area for sustainable milk production. Comparative studies have been reported about several aspects of physio-chemical property, nutritional and medical value of goat and sheep kinds of milk [1]. Unsaturated fatty acids are beneficial for human health, and fatty acid composition should be considered an economically important trait [2]. In the current review article, our focus is on genetic mechanisms behind milk composition and production traits with special reference to FASN gene polymorphism studies around the world. Milk fat is regarded as the most important content contributing to the nutritional value of milk [3]. The most significant fatty acids present in bovine milk are palmitic acid, myristic acid, and stearic acid. One of the targets of breeding cattle is not only increasing the amount of milk fat and protein

But also it is quality. These studies show that consumption of dietary monounsaturated fatty acids and polyunsaturated fatty acids significantly reduces blood cholesterol and low-density lipoprotein level and helps in decreasing the risk of cardiovascular diseases [4], [5]. The adverse of cholesterol and low-density lipid on human health and it has a correlation with cardiovascular effects [6]. For these reasons, it is important to review genetic research in dairy cattle for improving the quality of milk. Till now, several genes, including ABCG2, PPARGC1A, ACSS2, DGAT1, FASN, and STAT5A, were explored and found to be coordinately involved in fat synthesis in dairy cattle [7]. Our current review article is particularly focused on the Fatty acid synthase (FASN) gene in bovines. In order to identify gene sequences dedicated to the biosynthesis of milk fatty acids, the genome-wide linkage between milk fatty acids and significant DNA sequences was performed [8], [9]. FASN is an important candidate gene affecting milk composition because of its role in denovo lipogenesis in mammals. Molecular genetics solely focuses on the identification of DNA polymorphism which influences the trait of interest in animal breeding [10]. Recent developments in molecular genetics have developed several molecular approaches and made it possible to perform genome-wide association studies using PCR-RFLP and single nucleotide polymorphism (SNP) markers to detect QTL related to milk production traits [11]. Considering the benefits of genomic selection, it is essential to identify SNPs in the candidate genes responsible for milk traits [12]. Maintaining the balance between molecular genetic techniques and conventional animal breeding techniques is crucial.

II. FASN GENE STRUCTURE AND FATTY ACID SYNTHASE COMPLEX

FASN gene was first identified and studied in rats, humans, goose, chickens, and cattle [13], [14], [15]. The bovine FASN gene was first localized on chromosome 19q22 by using fluorescence *In-situ* hybridization and somatic cell hybrid analysis [16]. FASN gene in bovine is 19,770 bp long and consists of 42 exons and 41 introns (GenBank Acc. No. AF285607). There are two main: Thioesterase domain (TE) and β-ketoacylreductase domain (KR) altogether give rise to functionally different long-chain fatty acids with variable chain length, along with upstream Acyl carrier protein domain, assist in chain

length termination [17]. FASN gene expression is controlled by alternate splicing at exon 8-10 region [18]. The KR and TE domain are very close to each other [19]. The TE domain of the FASN gene is considered as a candidate gene in determining the fatty acid composition in bovine and other animals [20]. Determination of the FASN gene at genomic and transcription level with the identification of the sequence and organization of protein and its expression in cattle is depicted in Fig.1. [21]. It was concluded that the bovine FASN gene is highly conserved and similar to humans and rats. The 5' and 3' RACE and RT-PCR products using cloning and sequence analysis revealed a complete bovine FASN cDNA sequence of 7,542bp coding sequence (GenBank Accession No. AY343889) and encoded a protein of 2,513 amino acids with molecular weight 274kD [22].

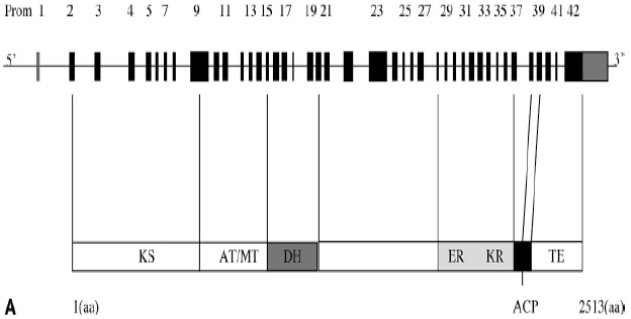

Fig.1 Complete Bovine FASN gene structure (Roy et al., 2005).

Fatty acid synthase is a multienzyme complex comprising the main catalytic domain. The enzymes are not associated with β- oxidation system but are located in the cytosol [20]. Identification of key component of fatty acid synthase complex: the Malonyl transferase, β-ketoacylsynthase, β-ketoacylreductase, dehydrase, and Enoylreductase. Investigation of fatty acid synthase enzymatic activity revealed the main function of fatty acid synthase complex is to catalyze the synthesis of palmitate from acetyl- CoA and malonyl-CoA in the presence of NADPH [21].

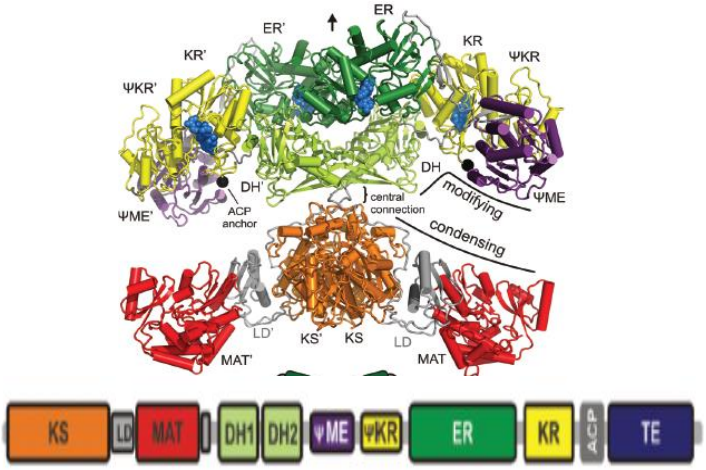

Fig.2 Structural overview of mammalian fatty acid synthase complex and the linear organization in mammalian fatty acid synthase complex gene.

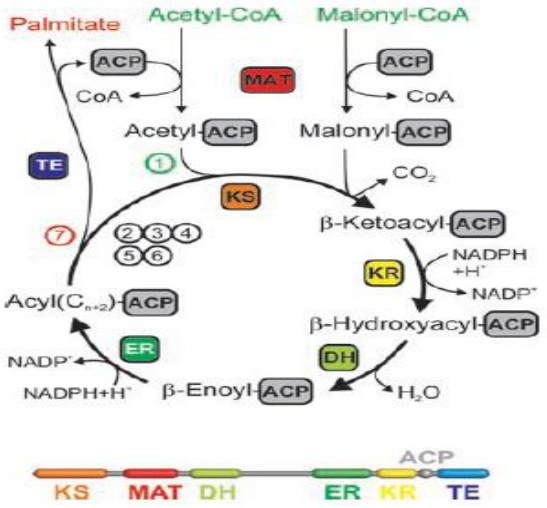

Fig. 3 Catalytic cycle and the catalytic domain of FASN multienzyme complex.

III. FASN GENE POLYMORPHISM STUDIES USING MOLECULAR TECHNIQUES

Till now, several studies have been carried out to determine the possible association between FASN gene polymorphisms and milk production traits. After the identification of the FASN gene sequence and fatty acid synthase complex structure, the researcher now started focusing on the association of polymorphism in the FASN gene and its correlation with milk fatty acid composition. By isolating genomic DNA samples and PCR amplification with the different sets of primer designed from the bovine genomic DNA sequence and direct sequencing for single nucleotide polymorphism, approximately 200 blood samples of Holstein–Friesian animals identified two SNPs. These SNPs include a G>C substitution in the untranslated exon 1 (g.763G>C), altering a potential Sp1 transcription factor-binding site, and an A>G substitution in exon 34 (g.16009A>G), which determines a non-conservative substitution of threonine by alanine. From this, it has been first reported that FASN polymorphism is related to the fat content of the milk [18].

Two SNPs were identified in the bovine FASN gene from two different Jersey and Limousin cattle populations. To confirm SNPs, genomic DNA was subjected for restriction digestion by using *XhoI* and *EcoRI*, proceeded for ligation into plasmid pBluescript KS, and transformed into chemically competent E.coli strains XL-1 Blue. The extracted plasmids from this transformed E.coli culture were further subjected to sequencing, which gives 1.8kb of the bovine FASN gene located on bovine chromosome 19q22. Two SNPS in particular, SNP4 and SNP5, showed large effects, although only SNP6 encodes an amino acid substitution. From this experiment, they concluded that bovine chromosome 19 and FASN could be considered as candidate gene and a total of five SNPs (g.17250–17251AT in-del, g.16907T>C, g.15531C>A, g.15603G>A, and g.17924A>.G) were found to be associated with the fatty acid percentage in milk [22].

FASN is an enzyme that catalyzes the de novo synthesis of fatty acids in cells. This investigation reported

the identification of a single nucleotide polymorphism (SNP), g.763G>C, in the bovine *FASN* 5 flanking region, which was significantly associated with milk fat content in dairy cattle. The g.763G>C SNP was part of a GC-rich region that may constitute a *cis*-element for members of the Sp transcription factor family. Thus the SNP could alter the transcription factor binding ability of the *FASN* promoter and consequently affect the promoter activity of the gene. Therefore, elucidating the underlying molecular mechanism that could explain the association of the SNP with milk fat content was targeted. [23].

Variations in single nucleotide polymorphism (SNP) in thioesterase domain of the fatty acid synthase (g.17924 A>G Threonine>Alanine) and in diacylglycerol acyltransferase-1 (g.10433/10434 GC/AA Alanine>Lysine) genes would explain variations in milk fatty acid composition among Holstein dairy cattle. About 200 cows participated in the study. Milk samples were collected monthly throughout the first ten months of lactation and analyzed for milk fatty acid composition by gas chromatography. Blood samples were used to obtain a DNA sample for each animal. Milk from cows with g.17924GG genotype had lower atherogenic index [AI; (12:0 + 4(14:0) + 16:0)/(MUFA + PUFA)] compared with milk from cows of g.17924AG genotype (P=0.007). Likewise, milk from cows with p.232AA genotype had lower AI compared with that from cows with p.232KK genotype (P<0.016). The decrease in AI for cows with g.17924GG and p.232AA genotypes was achieved by the decrease in the concentration of palmitic acid (P=0.06 and P<0.0001, respectively) and by the increase in the concentration of mono- and poly-unsaturated fatty acids in milk for both genotypes. The results of this study indicate the potential of using earlier mentioned SNPs as DNA markers to select breeding animals that produce progeny with a healthier milk fatty acid composition [24].

Effect of polymorphism was estimated in FASN gene on milk production traits and detailed milk-fat composition. Milk-fat composition phenotypes were available for 1905 Dutch Holstein–Friesian cows. The presence of each SNP in the Dutch Holstein–Friesian population was evaluated by direct sequencing of the PCR product surrounding the SNP in 22 proven Dutch Holstein–Friesian bulls for FASNg.16024G>A and FASNg.17924A>G SNP. FASNg.17924A>G was proved to have a significant effect (P < 0.05) on milk-fat percentage. Moreover, FASNg.16024G>A with higher G allele frequency is found to be responsible for non-synonymous mutation. It was also confirmed that both SNP in FASN had an effect on C14:0 whereas FASNg.16024G>A also affect the C18:2cis9,12 index [25].

The polymorphism of FASN gene exon 2, exon 4, exon 26, exon 34 in the Chinese Holstein population was detected using PCR -SSCP. Results indicated that mutation point was only found in exon 34 (g.16009AG). Holstein cows in southern China have A (point mutation G) and B (point mutation A) alleles. The frequencies of alleles were 0.8 802/0.1 198, respectively, with genotypic frequencies AA 0.7 604 and AB 0.2 396. GLM model was applied to analyze the impact of FASN gene genotype on milk protein percentage, milk fat percentage, milk yield, and somatic cell score (SCS). It was also showed that the two genotypes had a significant difference (P 0.05) on milk fat and SCS. This study provided original information for elucidating the regulatory mechanism of milk fat [26].

Allele frequencies of 10 representative polymorphisms for milk traits were investigated for a total of 240 animals from *Bos taurus* and *Bos indicus* breeds, including two Japanese groups (Japanese Black and Japanese Brown), two East Asian groups (Korean and Mongolian), three European groups (Holstein, Angus, and Hereford) and a *Bos indicus* group in South Asia (Myanmar, Laos, and Cambodia). The Japanese Black revealed unique genetic construction in *GH*, *FASN*, and *SREBP-1*, and the other Asian populations show intermediate frequencies between European and Japanese populations. The *Bos indicus* group showed low favorable allele frequencies in most of the genes. The study showed the variability and distribution of 10 genes affecting economic traits among world representative cattle breeds. The genetic information would contribute to elucidating the genetic background for worldwide cattle breeds and the possibility of improvement using the markers [27].

Polymorphism study using cDNA sample along with the primers specially designed for FASN gene investigation. The amplification of target gene genotyping of 198 Holstein cattle was done by PCR-RFLP, leads to the identification of five novel SNPs. For the PCR-RFLP, restriction enzymes used were as follows: *RsaI*, *MslI*, *HhaI*, *NciI*, *MscI*. Total 13 SNPs were identified, out of which five SNPs (4168bpC/T, 5566bpA/C, 5848bpA/G, 5863bpT/C, and 6790bpA/G) were non-synonymous mutations, found to be causing amino acid substitution where 4168bpC/T leads to tyrosine from histidine (H1390Y), 5566bpA/C from isoleucine to leucine (I1856L), 5848bpA/G from threonine to alanine (T1950A), 5863bpT/C from tryptophan to arginine (W1955R) and 6790bpA/G from threonine to alanine (T2264A). In addition, H1390Y and I1856L are similar, and H1390Y was the novel SNP identified for the first time. From their investigation, they also reported that T1950A and W1955R are present in the KR domain of the FASN gene, which in turn causes altered FASN activity [28].

Identification and correlation of g.17924A>G TE domain of FASN gene (within exon 39-42) mutation with milk fatty acid composition was performed in 50 Chilean Black Friesian cows. DNA extraction was done from a blood sample using AxyPrep Miniprep kit, and the presence of SNP was detected by PCR- RFLP by *MscI* restriction endonuclease. The generated data were further analyzed using a general linear model; for the SNP g.17924A>G, the genotypic frequencies were AA: 9.6%, AG: 42.8, and GG: 47.6%, and the allele frequencies were A=0.31 and G=0.69. This study indicated that the FASN genotype could be used as a genetic tool to improve the nutritional quality of milk in cattle [29].

Correlation between the polymorphism of FASN gene and milk production trait was analyzed in 109 Polish Holstein- Friesian cows. Genomic DNA isolation s carried

out from blood, and genetic analysis of FASN was done by PCR-RFLP method using *AciI* restriction enzyme. The major allele for the analyzed locus was $FASN_{g.17924G}$ (f=0.63), and there was a significant correlation between the $FASN_{g.17924A>G}$ polymorphism and selected production traits, i.e., milk yield ($p \leq 0.05$), fat yield ($p \leq 0.05$) and protein yield ($p \leq 0.05$) [30].

Identification of candidate genomic regions for fatty acid composition by genome-wide association study with 50 K single nucleotide polymorphism (SNP) array in Japanese Black cattle. A total of 461 individuals and 40 657 SNPs were used in this study. They applied genome-wide rapid association using mixed model and regression (GRAMMAR) and genomic control approaches to estimate the associations between genotypes and fatty acid composition. In addition, two SNPs in *fatty acid synthase* (*FASN*) (T1952A) and *stearoyl-CoA desaturase* (*SCD*) (V293A) genes were also genotyped. Association analysis revealed that 30 significant SNPs for several fatty acids (C14:0, C14:1, C16:1, and C18:1) were located in the BTA19 *FASN* gene located within this region, but the *FASN* mutation had no significant effect on any traits. They also detected one significant SNP for C18:1 on BTA23 and two SNPs for C16:0 on BTA25. This study demonstrated novel candidate regions in BTA19, 23, and 25 for fatty acid composition [31].

Searching for promoter variability in Fatty Acid Synthase (FASN), Stearoyl-CoADesaturase (SCD), Perilipin (PLIN), Glycerol-3 phosphate acyltransferase mitochondrial (GPAM), and Melanocortin-4receptor (MC4R), 42 polymorphisms were found. Forty-one of them were reported for the first time in *B. taurus* and *B. indicus* species. Higher nucleotide diversity was found in *B. indicus* for FASN, SCD, PLIN, and MC4R, whereas GPAM was the only more polymorphic promoter in B. Taurus. The lower diversity in the *B. Taurus* breeds may reflect their population history with a stronger selection pressure for some economic and productive traits. Alternatively, the ancestors of the indicine breed may have had high genetic diversity. This research underlines the significance of local breeds for preserving genetic diversity for sustainable agriculture. [32].

The effect of polymorphisms of the FASN gene on milk yield and milk composition of Chinese Holstein cattle has been analyzed to improve the breeding of Chinese Holstein cattle. Around 464 improved descendants from Holstein cows and frozen semen of Holstein bull were selected in this study. Milk yield and milk composition information were collected based on DHI production performance measurement. DNA was extracted from the experimental group, and genotypes were detected using PCR-SSCP and DNA sequencing. The least-squares method was used to analyze the correlation between polymorphisms and milk traits. There were two alleles and two genotypes in the locus of *FASN* gene exon 34. The allelic frequencies of A and B were 0.813 9 and 0.186 1, respectively, and the population was not Hardy-Weinberg equilibrium at this locus ($P<0.01$). Heterozygosity (*He*) and the polymorphism information content (*PIC*) were 0.372 3 and 0.257 0, respectively. Comparing with the sequence of Bos Taurus (Accession #: AF285607), mutations A→G and C→T occurred at the 16 024 bp and 16 039 bp of allele B, respectively. 16 024 bp A→G mutation resulted in the transformation of Threonine into Alanine, and 16 039 bp C→T resulted in the transformation of Arginine into Tryptophan. Allele A was the same as that of AF285607. The Least Squares method showed that the fat milk yield for 305 d with genotype AA was significantly higher than that with genotype AB ($P<0.05$), indicating that allele A was the dominant gene of high milk fat yield. *FASN* gene exon 34 mutations can be used as molecular genetic markers for high milk-fat in marker-assisted selection (MAS) for Chinese Holstein cattle breeding [33].

Investigation of polymorphisms in FASN resulted in, six SNPs which were found and then genotyped in 752 Chinese Holstein cows. It was found that g.17924A>G was non-synonymous, g.13965 C>T, g.16907 T>C and g.18663T>C were synonymous mutations, and two other two SNPs, g.8948 C>T (ss491228481) and g.14439T>C (rs133498277), were in intronic sequences of the gene. All such identified SNPs were found to be associated with milk yield and composition traits ($P = 0.0441$ to <0.0001). Significant additive and allele substitution effects were observed for three yield traits at all six loci as well ($P < 0.05$ to <0.01). Complete linkage disequilibrium among the five SNPs, with the exception of g.8948 C>T, was observed [34].

Correlation between the polymorphism of FASN gene and milk production trait was analyzed in 109 Polish Holstein- Friesian cows. Genomic DNA isolation s carried out from blood, and genetic analysis of FASN was done by PCR-RFLP method using *AciI* restriction enzyme. The major allele for the analyzed locus was $FASN_{g.17924G}$ (f=0.63), and there was a significant correlation between the $FASN_{g.17924A>G}$ polymorphism and selected production traits, i.e., milk yield ($p \leq 0.05$), fat yield ($p \leq 0.05$) and protein yield ($p \leq 0.05$) [35].

Variants of TE and KR domains of the FASN gene has been discovered to be linked with fatty acid composition and evaluate its potential as a genetic marker. The aim of this study was to carry out genotyping by using primers for amplification of regions including g.18663T>C, g.1924A>G and g.18440G>A polymorphisms in the TE domain and g.16039T>C polymorphism in the KR domain. Amplicons of the TE domain were digested with *MscI* and *Hpy188III* restriction enzyme; for KR domains, amplicons digestion HhaI and NciI enzymes were employed. Polymorphism g.18440G>A in the TE domain led to the substitution of glutamic acid into lysine and determined the effect of amino acid changes in the KR domain on the function of TE domain has an important role in fatty acid biosynthesis. Also, the g.18663T>C is found to be a silent mutation [50]. The unique genetic property, along with recent advances in biotechnological tools and advantages of the molecular marker, makes the genetic research more amenable, and these molecular markers greatly help in the improvement of livestock [36]. Discovery of the single nucleotide variation present in exon 40 region of the Fatty Acid

Synthase (FASN*)* gene was done. 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 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 the G allele. All the genotypes showed almost the same frequency across the breeds, indicating that there is the absence of selection for the 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 the bovine FASN gene as a candidate gene marker for a milk-fat content [37].

FASN gene is associated with the milk production traits through a comparative analysis of phenotypic data of candidate gene with its genotype. For FASN, a total of 30 pairs of primers were designed to amplify all exons, and purified PCR products were sequenced subsequently, followed by genotyping done by using MALDI/TOF mass spectroscopy for 346 Chinese Holstein cows. As a result, six SNPs were found out of which one was non-synonymous and two were synonymous and suggested that the FASN gene mainly affects the medium and long-chain saturated fatty acids [38].

Genetic variability in the thioesterase (TE) domain of the fatty acid synthase gene of Murrah Buffalo was studied. Terminal exon 38-42, including the TE domain, were explored for nucleotide variation using PCR-RFLP. An important novel SNP at g.18433A>G in the exon-40 region of the *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 the non-synonymous transition in the *FASN* gene. The study will augment the information available and can be applied in future studies to determine the role of the buffalo *FASN* gene as a candidate gene marker for a milk-fat content [39].

The effect of polymorphism of fatty acid synthase gene on eight traits related to milk production and its composition in 162 Murrah buffaloes was evaluated. The trait study included 305 days milk yield, lactation fat average (LFA), lactation solid not fat average (LSA), 305 days solid not fat yield, lactation total solid average (LTSA), 305 days total solid yield, and peak yield. Restriction fragment length polymorphism was used to identify the SNP in a 472bp amplified product of exon 40 in the FASN gene. It was found to be polymorphic with guanine to adenine transition, and three genotypes, namely AA, AG, and GG, were observed. Allele A was found to be more frequent than the G allele, and exon 40 is associated with LFA, LTSA, and peak yield [40].

A total of 105 cows (72 Simmental and 33 crossbred Holstein) were genotyped using the PCR-RFLP method, and their fatty acid profiles were analyzed. The Holstein × Simmental cows with the diplotype AR/AR were also characterized by a significantly lower content of C16:0 and saturated fatty acids, but higher C18:1n9, monounsaturated fatty acid, and monounsaturated fatty acid/saturated fatty acid content compared to the same diplotype of the Simmental cattle. These results indicated that with accurate breeding plans, crossbreeding Holstein cows with Simmental bulls could be directed towards a more desirable fatty acid composition of milk and dairy products [41].

The benefits of genomic selection were investigated and found to be very important to identify the SNPs in the candidate gene responsible for milk traits. The aim of this study was to determine different polymorphisms that may be found in different generations, and their effects on milk can also be different, and to investigate their associations with milk traits. The study used 100 samples, and genotyping for polymorphism was carried out by isolating DNA from the blood sample and PCR-RFLP mapping by using *MscI*. Results were found with two SNPs in the FASN gene (GG,342bp/355bp and AG 167bp/188bp). This investigation also reported that the AG genotype of FASN results in a higher protein percentage than the GG genotype. However, no association of the SNP in FASN with milk traits was found [12].

Evaluation of the influence of polymorphic loci and other factors on milk performance and the technological properties of milk was performed on Simmental and Holstein cows. *FASN* gene alleles A and G genotyped through PCR-RFLP technique. For the *FASN* gene, the protein content was slightly but significantly higher in GG homozygous cows, and the A allele was associated with higher milk, protein, and fat yields than the G allele [42].

It is shown that milk infrared (IR) spectroscopy can be used to predict detailed milk fat composition. In addition, polymorphisms with substantial effects on milk fat composition have been identified. Investigation of the combined use of milk IR spectroscopy and genotypes of dairy cows can be employed on the accuracy of predicting milk fat composition. This study demonstrated the potential of combining milk infrared (IR) spectra with genotypic information to predict milk fat composition for genomic breeding value [43].

IV. CONCLUSION

FASN gene is one of the potential candidate genes responsible for milk quality and its fatty acid composition. DNA polymorphism studies within the FASN gene using various molecular markers can be used for the improvement of milk properties of dairy cattle. A detailed study of the unexplored FASN locus of the gene can further lead to the identification of various novel and economically beneficial SNPs, which will aid in selection and breeding decisions for the genetic improvement of dairy cattle.

REFERENCES

[1] Jandal, J.M., Comparative Aspects of Goat and Sheep Milk. Small Ruminant Research, 22(2)(1996) 177–85.

[2] Koletzko, B., et al., Growth, Development and Differentiation: A Functional Food Science Approach. British Journal of Nutrition,. 80(S1)(1998) S5–45.

[3] Lock A.L., and D.E Bauman ., Modifying Milk Fat Composition of Dairy Cows to Enhance Fatty Acids Beneficial to Human Health. Lipids, 39(12)(2004) 1197–1206.

[4] Mele, M., et al., Stearoyl-Coenzyme a Desaturase Gene Polymorphism and Milk Fatty Acid Composition in Italian Holsteins. Journal of Dairy Science, 90(9)(2007) 4458–65.

[5] Kgwatalala, P.M., et al., Influence of Stearoyl-Coenzyme A Desaturase 1 Genotype and Stage of Lactation on Fatty Acid Composition of Canadian Jersey Cows. Journal of Dairy Science, 92(3)(2009) 1220–28.

[6] Krauss, R.M, Lipoprotein Subfractions, and Cardiovascular Disease Risk. Current Opinion in Lipidology, 2010. 21(4)(2010) 305–11.

[7] Bouwman, C.A., et al., Genome-Wide Association of Milk Fatty Acids in Dutch Dairy Cattle. BMC Genetics,. 12(43)(2011) 1-12.

[8] Roy, R., et al., Association of Polymorphisms in the Bovine FASN Gene with Milk-Fat Content. Animal Genetics, 37(3)(2006) 215–18.

[9] Schennink, A., et al., Effect of Polymorphisms in the FASN, OLR1, PPARGC1A, PRL and STAT5A Genes on Bovine Milk-Fat Composition. Animal Genetics, 40(6)(2009) 909–16.

[10] Bionaz, M. and J.J. Loor, Gene Networks Driving Bovine Milk Fat Synthesis during the Lactation Cycle. BMC Genomics, 9(366)(2008) 1–21.

[11] Bouwman, C.A., et al., Genomic Regions Associated with Bovine Milk Fatty Acids in Both Summer and Winter Milk Samples. BMC Genetics, 13(93)(2012) 2-13.

[12] Rahayu, A.P., et al., Association of Single Nucleotide Polymorphisms in the Fatty Acid Synthase, LOC514211, and Fat Mass and Obesity-Associated Genes with Milk Traits in Indonesian-Holstein Dairy Cattle. Veterinary World, 12(7)(2019) 1160–66.

[13] Amy, M.C., et al., Molecular Cloning of the Mammalian Fatty Acid Synthase Gene and Identification of the Promoter Region. Biochemical Journal, 271(3)(1990) 675–79.

[14] Jayakumar, A., et al., Isolation and Chromosomal Mapping of Genomic Clones Encoding the Human Fatty acid Synthase Gene. Genomics, 23(2)(1994) 420-424.

[15] Chirala, S.S., et al., Animal Fatty Acid Synthase: Functional Mapping and Cloning and Expression of the Domain I Constituent Activities. Proceedings of the National Academy of Sciences of the United States of America,. 94(11)(1997) 5588–93.

[16] Roy, R., et al., Assignment of the Fatty Acid Synthase (FASN) Gene to Bovine Chromosome 19 (19q22) by in Situ Hybridization and Confirmation by Somatic Cell Hybrid Mapping. Cytogenetics and Cell Genetics,. 93(2001)(1–2) 141–42.

[17] Chakravarty, B., et al., Human Fatty Acid Synthase: Structure and Substrate Selectivity of the Thioesterase Domain. Proceedings of the National Academy of Sciences of the United States of America, 101(44)(2004) 15567–72.

[18] Roy, R., et al., Genomic Structure and Alternative Transcript of Bovine Fatty Acid Synthase Gene (FASN): Comparative Analysis of the FASNgene between Monogastric and Ruminant Species. Cytogenetic and Genome Research., 111(1)(2005) 65–73.

[19] Maier, T., S. Jenni and B. Nenad, Architecture of Mammalian Fatty Acid Synthase at 4.5 å Resolution. Science, 311(5765)(2006). 1258–62.

[20] Gibson, D.M., E.B. Titchener, and S.J. Wakil, Studies on the Mechanism of Fatty Acid Synthesis V. Bicarbonate Requirement for the Synthesis of Long-Chain Fatty Acids. BBA - Biochimica et Biophysica Acta, 30(2)(1958) 376–83

[21] Alberts, W.A.., et al., Acyl-Carrier Protein. II. Intermediary Reactions of Fatty acid Synthesis. Biochemistry,. 3(10)(1964) 1563-72.

[22] Morris, C.A., et al., Fatty Acid Synthase Effects on Bovine Adipose Fat and Milk Fat. Mammalian Genome,. 18(1)(2007) 64–74.

[23] Ordovás, L. et al., The g.763G>C SNP of the Bovine FASN Gene Affects Its Promoter Activity via Sp-Mediated Regulation: Implications for the Bovine Lactating Mammary Gland. Physiological Genomics,. 34(2)(2008) 144–48.

[24] Nafikov, R., et al., Effects of A17924G Genotypes Associated with Thioesterase Domain of Fatty Acid Synthase and K232A Genotypes of Diacylglycerol Acyltransferase-1 on Milk Fatty Acid Composition in Holstein Dairy Cows. Animal Industry Report, 2009. 655(1). 56., ASL R2433.

[25] Schennik, A., et al., Effect of Polymorphism in the FASN, OLR1, PPARGC1A, PRL and STAT5 genes on bovine milk-fat composition. Animal Genetics,. 40(6)(2009) 909-916.

[26] Wu, X., et al., Genetic diversity analysis of FASN gene and its correlation with milk performance in Southern Chinese Holstein. Chinese Journal of Animal Science,. 1(1)(2010) 1-4.

[27] Kaneda, M., et al., Allele frequnecies of gene polymorphism related to economic traits in Bos indicus and Bos taurus cattle breeds. Animal Science Journal, 82(6)(2011) 717-721.

[28] Matsumoto, H., et al., Identification of SNPs in the FASN Gene and Their Effect on Fatty Acid Milk Composition in Holstein Cattle. Livestock Science, 144(3)(2012) 281–84.

[29] Inostroza, B Karla., Erick S Scheuermann and Nestor A Sepulveda, Stearoyl CoA desaturase and Fatty acid synthase gene polymorphism and milk fatty acid composition in Chilean Black Frieian cows. Rev Colomb Cienc Pecu., 26(4)(2013) 263-269.

[30] Ciecierska, D., et al., The influence of Fatty Acid Synthase Polymorphism on milk production traits in Polish Holstein-Friesian Cattle. The Journal of Animal and Plant Sciences, 23(2)(2013) 376-379.

[31] Ishii, A., et al., Genome-wide association study for fatty acid composition in Japanese Black cattle. Animal Science Journal, 84(10)(2013) 675-82.

[32] Sanz, A., et al., 5' Cis-regulatory polymorphisms in candidate genes in Bos taurus and Bos indicus. Livestock Science, 157(1)(2013) 88–92.

[33] YanNan, M., et al., Correlation between PCR-SSCP polymorphism of part FASN gene sequence and milk traits in Chinese Holstein cattle. Journal of Northwest A & F university-Natural Science Edition,. 41(12)(2013)1-6.

[34] Alim, M.A., et al., Effect of FASN gene on milk yield and milk composition in the Chinese Holstein dairy population. Animal Genetics, 45(1)(2013) 111-3.

[35] Fontanesi, L., et al., A candidate gene association study for nine economically important traits in Italian Holstein Cattle. Animal Genetics, 45(4)(2014) 576-580.

[36] Oztabak, K., et al., FASN gene polymorphism in Indigenous Cattle breeds of Turkey. Folia Biologica (Kraków),. 62(1)(2014) 29-35.

[37] Vohra, V., et al., Polymorphism in Exon 40 of FASN Gene in Lesser-known Buffalo Breeds of India. Journal of Animal Research,. 5(2)(2015) 325-328.

[38] Li, C., et al., Genetic Effects of FASN, PPARGC1A, ABCG2 and IGF1 Revealing the Association with Milk Fatty Acids in a Chinese Holstein Cattle Population Based on a Post Genome-Wide Association Study. BMC Genetics,. 17(1)(2016) 110.

[39] Kumar, M., et al., SNP identification in thioesterase domain of fatty acid synthase gene in Murrah Buffalo. The Journal of Animal and Plant Sciences, 26(6)(2016) 1828-1832.

[40] Kumar, M., et al., Influence of FASN gene polymorphism on milk production and its composition traits in Murrah buffaloes. Indian Journal of Animal Research,. 51(4)(2017) 640-643.

[41] Mauric, M., et al., Effects of Different Variants of the FASN Gene on Production Performance and Milk Fatty Acid Composition in Holstein × Simmental Dairy Cows. Veterinarni Medicina,. 64(03)(2019) 101–108.

[42] Citek, J., et al., Gene polymorphisms influencing on yield, composition and technological properties of milk from Czech Simmental and Holstein cows. Asian- Australasian Journal of Animal Sciences, 34(1)(2020) 2-11.

[43] Wang, Q., and Bovenhuis H, Combined use of milk infrared spectra and genotypes can improve prediction of milk fat composition. Journal of Dairy Science,.103(3)(2020) 2514-2522.