Shiraz University

IJVR

ISSN: 1728-1997 (Print)
ISSN: 2252-0589 (Online)

Vol.26 No.1 Ser. No. 90 2025

IRANIAN JOURNAL OF VETERINARY RESEARCH

Original Article

Effects of *FASN* gene polymorphism on milk production traits in Bangladeshi cattle: Insights from mutant protein function

Alim, M. A.[1*]; Hossain, M. M. K.[2]; Jahan, N.[1, 3]; Rubaya, R.[2]; Bhuyan, A. A.[1]; Alam, J.[1]; Ahammad, I.[4]; Siddiki, M. S. R.[5] and Moniruzzaman, M.[6]

[1]Animal Biotechnology Division, National Institute of Biotechnology, Ganakbari, Ashulia, Savar, Dhaka-1349, Bangladesh; [2]Ph.D. in Animal Genetics, Animal Biotechnology Division, National Institute of Biotechnology, Ganakbari, Ashulia, Savar, Dhaka-1349, Bangladesh; [3]Department of Animal Nutrition, Genetics and Breeding, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka 1207, Bangladesh; [4]Bioinformatics Division, National Institute of Biotechnology, Ganakbari, Ashulia, Savar, Dhaka-1349, Bangladesh; [5]Department of Dairy Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh; [6]Molecular Biotechnology Division, National Institute of Biotechnology, Ganakbari, Ashulia, Savar, Dhaka-1349, Bangladesh

[*]***Correspondence:*** *M. A. Alim, Animal Biotechnology Division, National Institute of Biotechnology, Ganakbari, Ashulia, Savar, Dhaka-1349. E-mail: alimmdcau@gmail.com*

10.22099/ijvr.2025.49855.7351

(Received 31 Mar 2024; revised version 12 Jan 2025; accepted 2 Feb 2025)

Abstract

Background: To meet up the demand gap of milk in Bangladesh, short-term, midterm, and long-term goal have been set up by the government through crossing with Bangladeshi local cattle and high-producing foreign cattle like Friesian, Jersey, Sahiwal, etc. **Aims:** The purpose of this study was to identify the single nucleotide polymorphisms (SNPs) in the *FASN* gene, to check the structural and functional impact of mutant proteins on milk production traits that are significantly associated. **Methods:** Four SNPs were identified in exons 26, 36, 38, and 41 of the *FASN* gene using pooled DNA sequencing, but only one SNP g.17924 A>G was a non-synonymous that changed the amino acid threonine to alanine in the FASN protein and the other three SNPs were silent mutations. Structural and functional prediction analysis were done with a series of techniques to detect remote protein homology and predict structures, structural integrity, structure quality, protein stability, protein motion, flexibility, and stability impact, conservation profile and finally molecular dynamics simulations for wild-type and mutant protein expression differences. **Results:** The non-synonymous g.17924 A>G mutation showed a clear difference between wild and mutant proteins, indicating the impact on the observed phenotype. Then, SNP g.17924 A>G was genotyped in 100 milking cows aiming to check the association effects. SNP g.17924 A>G was found to have significant allele substitution effects on milk yield traits. **Conclusion:** Our results suggest that the identified polymorphism affects milk yield traits in the studied population and could be used as genetic marker for cattle selection processes aiming to increase productivity.

Key words: Association, Bangladeshi cattle, Candidate gene, *FASN*, Single nucleotide polymorphism

Introduction

Crossbred cattle have largely replaced native cattle in Bangladesh over recent decades to meet higher milk demand. Crossbreeding began in 1936 with imported Hariana bulls from India to upgrade the local cattle. In 1958, the Directorate of Livestock Services (DLS) launched artificial insemination (AI), a program that was expanded in 1975-76 and is still in operation today. In the 1960s, bulls from Pakistan (Sindhi, Tharparkar, Sahiwal) were imported, followed by Friesian and Jersey bulls from Australia in 1973, to enhance milk production. Bangladesh's cattle now consist of both crossbreeds and pure local breeds (Hossain *et al*., 2002; Hamid *et al*., 2017). Native cattle are small, horned, and used mainly for plowing, but their production is low even under ideal conditions (Hossain *et al*., 2002). The recommended daily milk intake is 250 ml/person, but actual consumption averages only 193 ml/person, failing to meet national recommended demand (DLS, 2021). For over 50 years, Bangladesh has struggled to close this gap because of the lack of effective selective breeding guidelines. Short-term (inseminate the top most cross bred Holstein-Friesian cows producing 10 kg or more milk reared under intensive management system with imported semen of progeny tested bulls), midterm (inseminate cross bred Holstein-Friesian cows yielding 6-10 kg milk reared under semi intensive management system with semen of progeny tested 50% Holstein-Friesian bulls) and long term goal (inseminate native cows reared under low input production system with semen of superior progeny tested/pedigree bulls of local

cattle for milk production also been set up by National Livestock Development Policy, Ministry of Fisheries and Livestock (NLDP, 2007). Considering the demerits of overall crossbreeding, selective breeding within the breeds is also recommended to conserve the cattle genetic resources. In both crossbreeding and selective breeding, animal selection is crucial for genetic improvement. However, identifying high-yielding animals remains a challenge, with conventional, time-consuming selection methods still in use. As a result, sustainable genetic improvement for high production has been limited over the past few decades. Recently, the Bangladesh Livestock Research Institute (BLRI) attempted to identify SNP markers for Bangladeshi cattle from genes previously reported in other cattle. This was the first effort toward marker-assisted selection for milk production traits in Bangladeshi cattle. However, they were unable to link the identified marker to phenotypic data.

Milk, a white opaque fluid rich in lipids, protein, lactose, and calcium, is often considered one of the best dietary sources of nutrition. It also contains a wide variety of micronutrients and other bioactive components produced by the mammary gland. These include vitamins, minerals, oligosaccharides, immunoglobulins, cytokines, antibodies, enzymes, enzyme inhibitors, growth factors, hormones, and antibacterial agents. Each component is crucial for the newborn's health and development, which is why milk and other dairy products are essential for a healthy, balanced diet (Wickramasinghe *et al*., 2012; Pereira, 2014). The milk fat content and composition are key factors that determine the nutritional and technological quality of dairy products (Chilliard *et al*., 2003). Fat content of milk is abundant in saturated fatty acids (SFA) and low in polyunsaturated fatty acids (PUFA). Therefore, fatty acid (FA) composition is valued economically, and enhancing milk FA composition, particularly by raising unsaturated fatty acid, is essential (Mannen, 2011). The fatty acid composition of milk is a heritable trait, with heritability ranging from 0.31 to 0.73. (Inoue *et al*., 2008). Several recent studies have proposed genetic improvements to the nutritional quality of milk, focusing on its fatty acid profile (Abe *et al*., 2009; Conte *et al*., 2010; Matsumoto *et al*., 2012; Mauric *et al*., 2019).

To gain better insight into mammary biology and accelerate the rate of genetic gain in dairy cattle, numerous researchers have focused on identifying the genes and polymorphisms that influence bovine milk production. Fatty acid synthase, a multifunctional enzyme complex that controls the de novo synthesis of long-chain fatty acids, is a potential candidate gene for determining fat content in bovine milk and beef (Roy *et al*., 2006; Schennink *et al*., 2009; Matsumoto *et al*., 2012; Li *et al*., 2016; Mauriae *et al*., 2017). The bovine *FASN* gene, spanning 19,770 bp and consisting of 42 exons and 41 introns, is located on BTA19 (Roy *et al*., 2006; Kale *et al*., 2021). It contains two main domains: the thioesterase (TE) and β-ketoacylreductase (KR) domains, which together produce long-chain fatty acids of varying lengths, while the upstream Acyl carrier protein domain helps terminate chain elongation (Chakravarty *et al*., 2004). The TE domain is thought to play a key role in regulating the fatty acid composition and content in bovine and other animals (Gibson *et al*., 1958).

Till now, several studies have been conducted to explore the possible association between *FASN* gene polymorphisms and milk production traits. Following the identification of the *FASN* gene sequence and the structure of the fatty acid synthase complex, researchers have shifted their focus to investigating the association between polymorphisms *FASN* gene and their correlation with milk fatty acid composition (Kale *et al*., 2021).

The *FASN* gene is found in a linkage region containing QTL for milk fat content and is a key enzyme for fatty acid synthesis, making it a promising candidate for milk production traits such as milk, protein, and fat yields, which are the primary breeding goals in dairy cattle selection. The present study aimed to screen SNPs in the *FASN* gene, analyze the protein's three-dimensional (3D) structure to understand the functional consequences of the polymorphism and evaluate its link to milk production traits in Bangladeshi local and Holstein cross cattle with the goal of potential application in cattle breeding.

Materials and Methods

The complete workflow used in this study is illustrated in Fig. 1

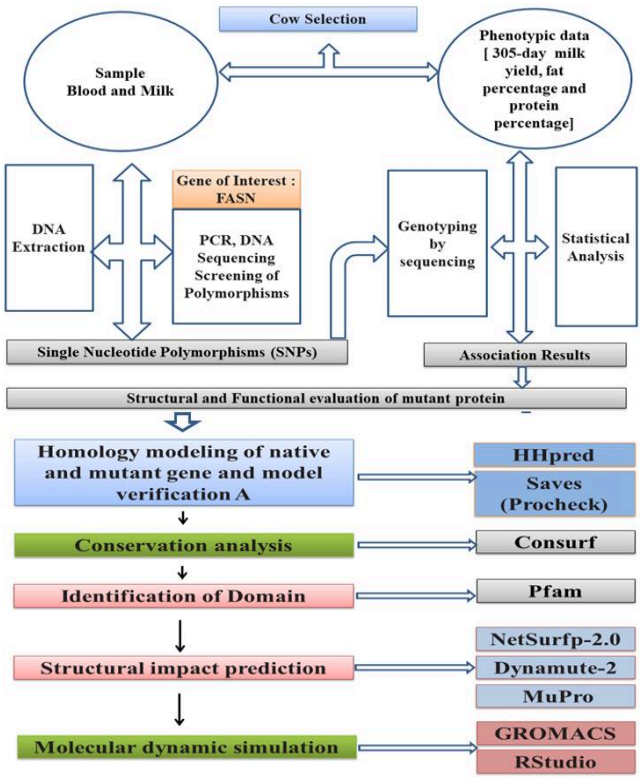

Fig. 1: The complete workflow used in this study

Ethical statement

All procedures involving animals and samples were approved (approval: NIBREC2017-02) by the Ethical Review Committee of the National Institute of Biotechnology (NIB), Bangladesh, where the experiment was conducted.

Experimental animals and phenotypic data collection

The National Institute of Biotechnology (NIB) ethical review committee in Bangladesh gave their clearance to the experiment (approval: NIBREC2017-02). A total of 100 crossbred F1 generation cows (Bangladeshi Local X Holstein) were selected from Central Cattle Breeding and Dairy Farm (CCBDF), Savar, Dhaka, where they were kept under the same management and environment condition. Milk samples were collected from each milking cow once during the whole lactation period, specifically between 90 and 100 days. The milk samples were then promptly sent to the Animal Biotechnology Division of the National Institute of Biotechnology (NIB) for phenotypic data generation (protein and fat percentage) using an auto milk analyzer (Lactoscan, Milk Analyzer, Bulgaria). Milk yield data was collected from CCBDF for each selected milking cow (305 days milk yield).

Screening polymorphisms and genotyping

DNA was extracted from collected blood samples (subset of cows, n=100) using the TIANamp Blood DNA Kit (TIANGEN BIOTECH (BEIJING) Co., Ltd.) according to the manufacturer's guidelines and instructions. Primers were designed and used based on Alim *et al*. (2013) and synthesized by Invitrogen (Invitrogen Life Technologies, China). A DNA pool was prepared with an equal volume and concentration of DNA samples taken from each animal (50 ng/µL/animal). Pool DNA was sequenced for preliminary screening of potential SNPs. After getting potential SNPs, a series of analyses was done to identify the most effective SNP. PCR amplifications were conducted with pooled samples using a programmable thermal cycler (Biometra GmbH, Germany). The 25 µL reaction volume mixture contained 2 µL DNA (50 ng), 1 µL (1 µM) each specific *FASN* gene forward and reverse primers, 8.5 µL molecular grade water and 12.5 µL of Invitrogen's DreamTaq Green PCR Master Mix. The amplification consisted of an initial denaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, annealing at 58°C for 30 s, extension at 72°C for 30 s, and a final extension at 72°C for 7 min. Azure c150 gel imaging workstations were used for gel electrophoresis of PCR the products to confirm amplification. The gel was prepared by 2% agarose. After confirmation, the PCR products were sequenced using the ABI3500 sequencer (Applied Biosystems, USA) at the Molecular Biotechnology Division, NIB. Sequence chromatographs were then checked. If double peak is found in the same place of chromatographs, it is assumed to be polymorphism. After that, all sequence data was analyzed to confirm the detected genetic polymorphisms using BioEdit Sequence Alignment Editor version 7.0.9.0 and ClustalW multiple sequence alignment programs. After structural and functional impact analysis of all identified SNPs, only polymorphism (g.17924 A>G) was genotyped using PCR and sequencing techniques in all selected animals.

Structural and functional impact prediction of *FASN* mutant protein

Identified non-synonymous polymorphisms in the *FASN* gene were examined for their possible effects on protein structure and function, which may affect the milk phenotype. To detect remote protein homology and predict structures, HHpred (https://toolkit.tuebingen.mpg.de/tools/hhpred) was used, while PROCHECK (https://servicesn.mbi.ucla.edu/PROCHECK/) was employed to assess structural integrity. The PDB-formatted three-dimensional model of the protein (Fig. 2) was created by the HHPred server. The PROCHECK server was used to produce a Ramachandran plot to verify the quality of the model. The Ramachandran plot and SAVES web-based server confirmed structure quality. The effect of SNPs on protein stability was predicted by MUpro (https://www.ics.uci.edu/~baldig/mutation.html) using Support Vector Machines and Neural Networks. Protein motion, stability and flexibility impact was analyzed by DynaMut2 (Rodrigues *et al*., 2021). The Consurf server was used for conservation profile analysis (Ashkenazy *et al*., 2016), while domain identification was performed with the Pfam serve (Apweiler *et al*., 2001). To check the impact of the mutations, the ConSurf web-based tool was employed to examine the evolutionary conservation of amino acid residues in the proteins. Molecular dynamics simulations of mutant FASN proteins and wild-type protein were performed using GROMACS for 100 ns (Abraham *et al*., 2015).

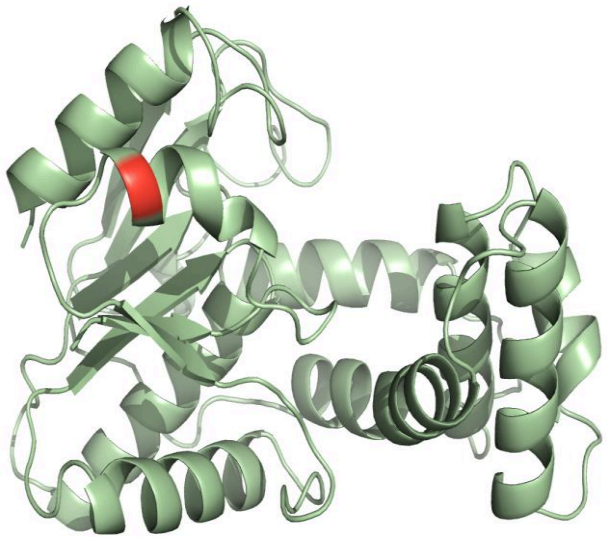

Fig. 2: Three dimensional model of FASN mutant g.17924 G>A (T2264A). The position of the mutated amino acid is marked in red

Root Mean Square Deviation (RMSD) was calculated to assess structural changes in a molecule over time, particularly in proteins. Root Mean Square Fluctuation (RMSF) evaluated the regional flexibility of the protein, as mutations can either decrease or increase flexibility in previously stable regions. These flexible regions often play crucial roles in molecular recognition, binding, and enzymatic activity. A higher RMSF value indicates greater flexibility at a specific amino acid position. In molecular dynamics (MD) simulations, Solvent Accessible Surface Area (SASA) was employed to predict the impact of mutations on the stability of a protein's hydrophobic core. The radius of gyration was used to measure the protein's compactness, where a relatively stable value indicates proper folding, while fluctuations suggest unfolding. Mutations that increase the exposure of hydrophobic regions to the solvent (reflected by an increase in SASA) can destabilize the protein. SASA is a key property for understanding molecular interactions, stability, folding, and binding, as it reveals which regions of the molecule are solvent-exposed, buried, or involved in interactions.

Statistical analysis

MATLAB software (ver. 7.11.0.584) was used to trace pedigree data back one generation, a result total number of animals increased 300 for association analysis. POPGENE software (ver. 1.32) was used to perform the Hardy-Weinberg equilibrium test and to calculate allelic and genotypic frequencies at the loci. SAS software (ver. 9.1.0, SAS Institute Inc., USA) was used to estimate the effects of genotypes on milk production traits. The analysis was performed using the mixed procedure with an animal model (Lynch and Walsh, 1997):

$$Y=\mu+hys+L+G+\alpha+e$$

Where,
Y: Phenotypic value
μ: Average mean
hys: An effect of herd-year-season
L: Fixed lactation effect
G: Refer to fixed effect corresponding to the genotype of polymorphisms
α: Refer to random polygenic component for pedigree relationships
e: A random residual

The Bonferroni correction was applied to adjust the significance threshold for multiple comparisons, reducing the likelihood of false positives in multiple t-tests. This correction was calculated by dividing the significance level of a single test by the number of tests conducted. Since each trait had three genotype levels, three t-tests were performed. Consequently, the Bonferroni-adjusted significance thresholds were set at 0.0167 (0.05/3) and 0.0033 (0.01/3).

Least squares mean values were used for multiple comparisons to estimate the effects of *FASN* polymorphic genotypes on milk production traits, likely by comparing the mean values of different genotypic groups. Falconer and Mackay's equation (1996) was applied to calculate the additive (a), dominance (d), and allele substitution (α1) effects, providing insights into how specific alleles influence the phenotype. These effects were computed using the equations:

$$\text{Additive effect (a):}\ a = \frac{AA - BB}{2}$$

$$\text{Dominance effect (d):}\ d = AB - \left(\frac{AA + BB}{2}\right)$$

$$\text{Allele substitution effect (}\alpha1\text{):}\ \alpha1 = a + d(q - p)$$

Where,
AA and BB: Represent homozygous genotypes
AB: The heterozygous genotype
p and q: The allele frequencies

Results

Screening of single nucleotide polymorphisms and genotypes

Based on the bovine *FASN* sequence available in the GenBank database (accession No.: AF285607.2), the gene consists of 41 exons with a total length of approximately 19,760 base pairs. According to our sequencing result using previously reported primers, four SNPs were identified in this study: g.13965 C>T, g.16907 T>C, g.17924 A>G, and g.18663 T>C, located in exons 26, 36, 38, and 41, respectively. Among these, the g.17924 A>G polymorphism was predicted to alter the FASN protein by substituting threonine (ACC) with alanine (GCC). The remaining three SNPs (g.13965 C>T, g.16907 T>C, and g.18663 T>C) were silent mutations. The allelic and genotypic frequencies are summarized in Table 1. The Chi-square (χ^2) test confirmed that the genotypic frequencies of the loci were in Hardy-Weinberg equilibrium (P>0.05) within the population, suggesting that selection pressure at these sites was not significantly strong.

Association analysis revealed that certain SNPs were strongly associated with three milk production traits, as indicated by raw P-values <0.05. However, these associations did not remain statistically significant after applying the Bonferroni correction for multiple t-tests (Table 2). Notably, the g.17924 A>G polymorphism exhibited a moderate association with milk yield. Based on these findings, a structural and functional evaluation of the mutant protein formed by the polymorphism g.17924 A>G. Cows carrying the G allele at this locus showed dominance in milk yield within the population, potentially increasing production by 338.91 kg over a full lactation period (Table 2).

Structural and functional impact prediction of *FASN* mutant protein associated with milk traits

Structural impact prediction

The results from HHPred and the PROCHECK server indicated that most residues in the three-dimensional models, for both the wild-type and mutant forms, were

located in the most favored regions (Fig. 3).

Conservation profile analysis

The ConSurf web tool results were displayed as a structural representation of the protein sequence, highlighting the predicted structural and functional residues. In this analysis, the 2264th position in FASN showed an average conservation profile (Fig. 4).

Identification of domain

The FASN mutant T2264A was located within the thioesterase (TE) domain of the FASN protein (Fig. 5).

Table 1: Genotypic and allelic frequencies and Hardy-Weinberg equilibrium χ2 test of FASN genotypes

Polymorphisms	Genotypic frequency			Allelic frequency		Hardy-Weinberg equilibrium χ2 test
g.17924 G>A	GG	AG	AA	G	A	P>0.05
	0.61	0.34	0.05	0.75	0.25	

Table 2: Least squares mean (LSM) and standard errors (SE) for milk production traits of different *FASN* genotypes and Additive and allele substitution effects of SNPs on milk production traits in Bangladeshi local and Holstein cross cattle

Locus	Genotype	Milk yield (kg)	Fat percentage (%)	Protein percentage (%)	Additive (a), dominant (d) and allele substitution (α1) effects		
					Milk yield (kg)	Fat percentage (%)	Protein percentage (%)
g.17924 G>A	AA	2156.19±268.428	3.67±0.169	3.11±0.058	261.295 (a)* G>A	0.044 (a)	-0.007 (a)
	AG	1754.45±124.258	3.67±0.078	3.07±0.027	-140.447 (d)	0.041 (d)	-0.043 (d)
	GG	1633.60±94.903	3.59±0.059	3.12±0.020	338.906 (α1)*	0.021 (α1)	0.016 (α1)
P-value		<0.0001 (0.22-0.54 corrected)	<0.0001 (0.90 corrected)	<0.0001 (0.90 corrected)			

* Significant additive effect or allele substitution effect at P<0.05 level

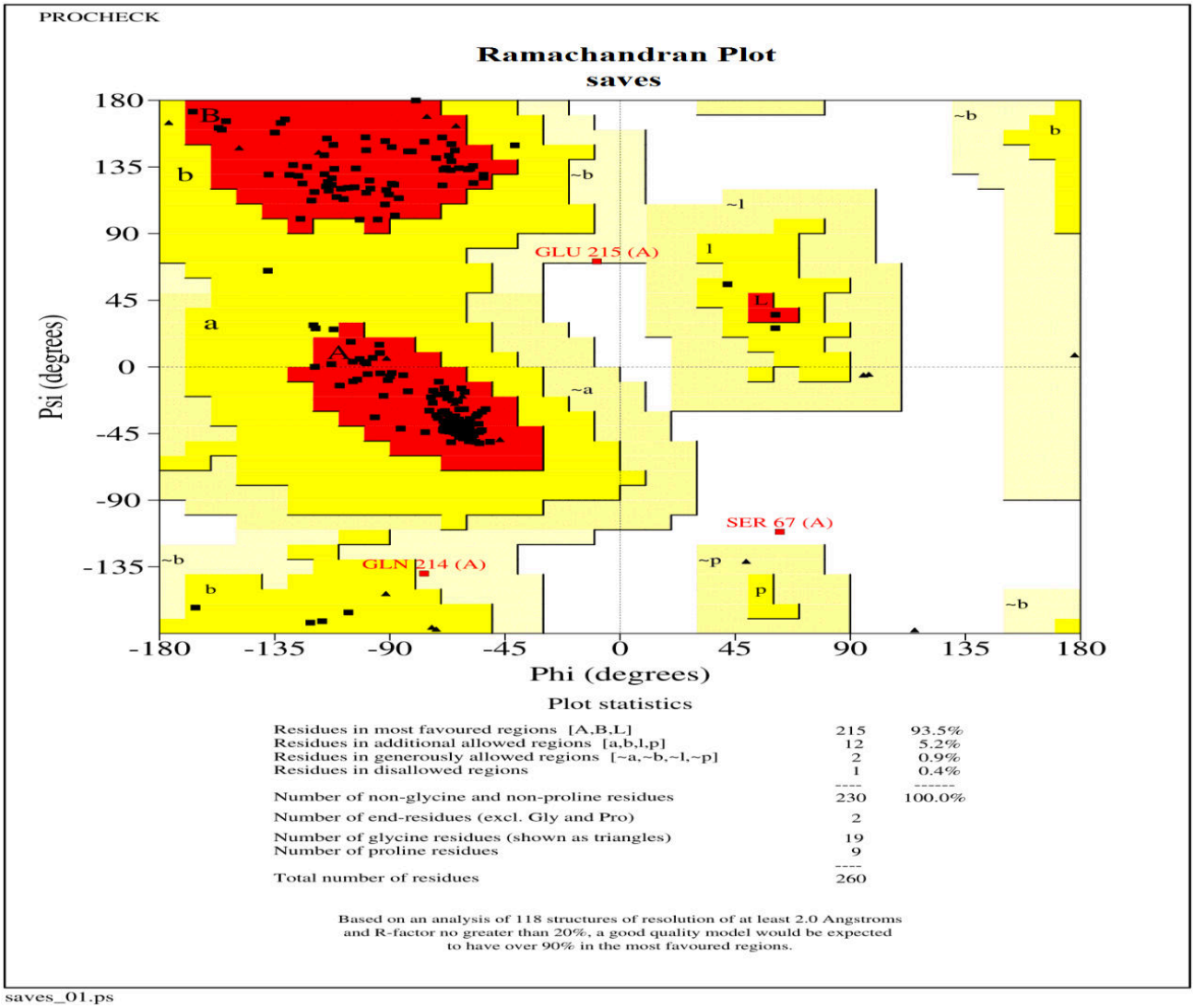

Fig. 3: Ramachandran plot of FASN mutant g.17924 G>A (T2264A) generated by PROCHECK

The TE domain is a structural and functional motif commonly found in enzymes involved in lipid metabolism, biosynthesis, and degradation.

Interatomic interactions prediction

MUpro analysis indicated that the T2264A mutation in FASN reduced protein stability, with a ΔG value of -1.4982588 kcal/mol. Similarly, DynaMut2 predicted a stability change (ΔΔGStability) of -0.13 kcal/mol for the same mutation. A negative ΔΔG value suggests that the mutation decreases protein stability (Fig. 6).

Molecular dynamic simulation

Variations in RMSD values indicate conformational changes in the protein. A significant increase in RMSD suggests major structural alterations, such as domain movements, loop motions, or unfolding events. The RMSD values of the wild-type and the T2264A mutant began to diverge at the 15 ns mark of the simulation. From that point onward, the T2264A mutant consistently exhibited a lower RMSD profile compared with the wild type (Fig. 7).

In our study, RMSF analysis revealed that in case of the wild FASN and the T2264A mutant different regions were more flexible. The region around the 25th and the 120th residues were significantly more flexible in the mutant compared with the wild type whereas the regions close to 60th, 90th, 210th, and 250th residues were more flexible in the wild type (Fig. 8). In molecular dynamics, RMSD gives insight into overall structural stability, while RMSF reveals local flexibility and dynamic behavior. In our study, we calculate RMSD and RMSF for better understanding of FASN T2264A mutant protein. Radius of gyration analysis revealed structural differences between the wild-type and mutant FASN (Fig. 9). A higher SASA value indicates an increased likelihood of protein destabilization due to greater solvent accessibility. Although the T2264A mutant initially maintained a lower SASA value up to 20 ns, it remained higher than the wild type thereafter, suggesting

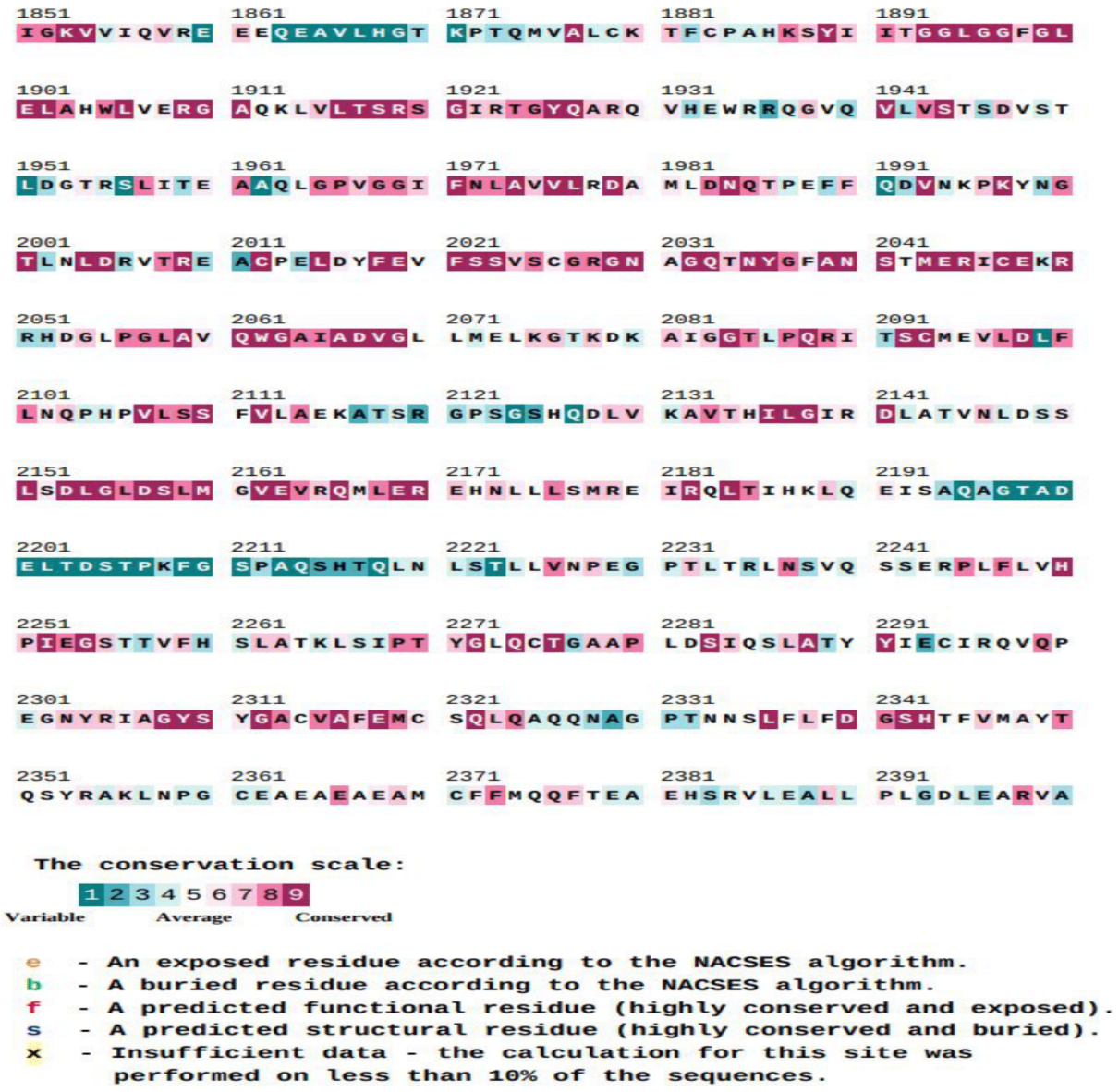

Fig. 4: Conservation profile analysis of FASN protein

a higher probability to solvent-induced disruption (Fig. 10).

Discussion

Since 1961, CCBS has conducted crossbreeding experiments with cattle across eight pure breeds: Bangladeshi Local, Sahiwal Kenyan, Sahiwal, Red Sindhi, Friesian, Jersey, Holstein-Friesian and Tharparker, along with their crosses. After the establishment of CCBS, cow information has been recorded consistently. The earmark numbers (1 to 9,999) of the first batch was completed by August 26, 1983, followed by a second batch starting the next day. All

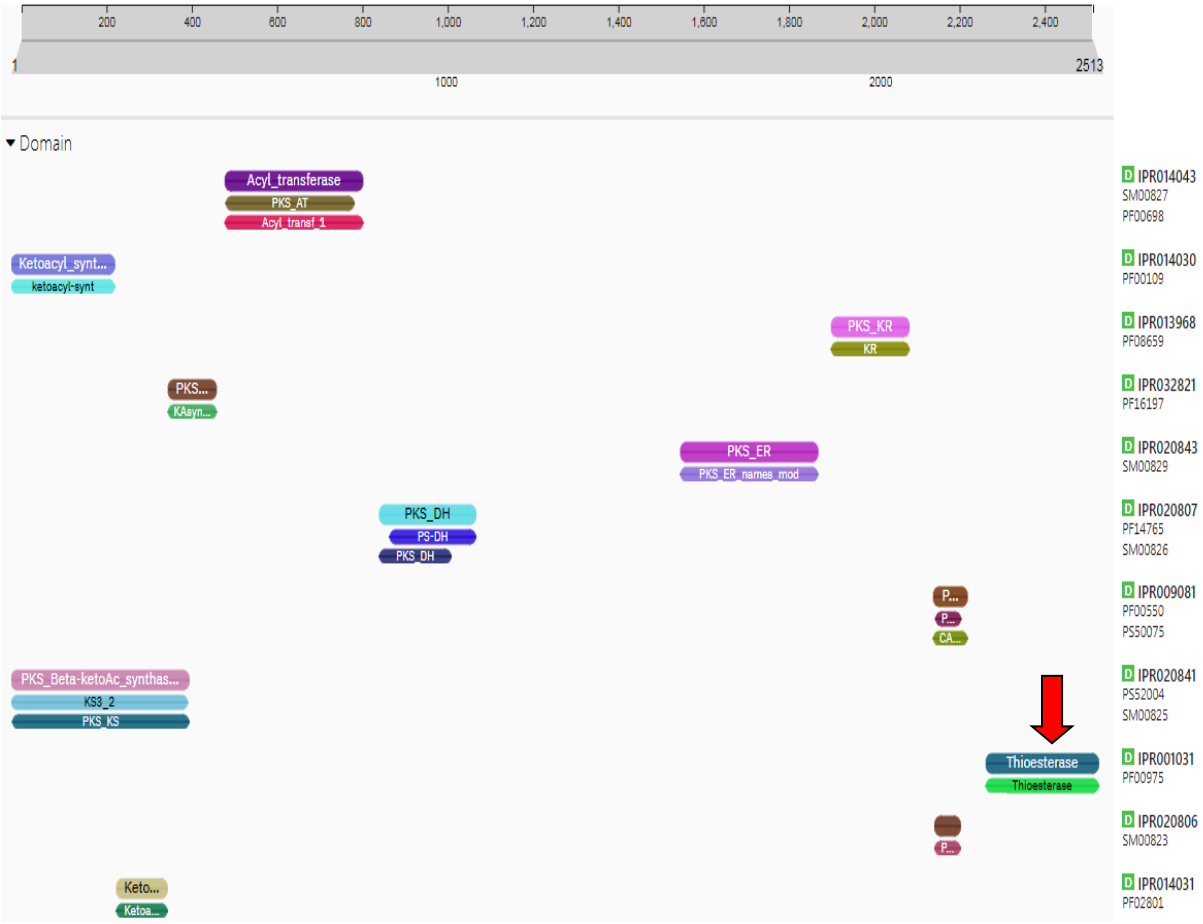

Fig. 5: FASN Domains

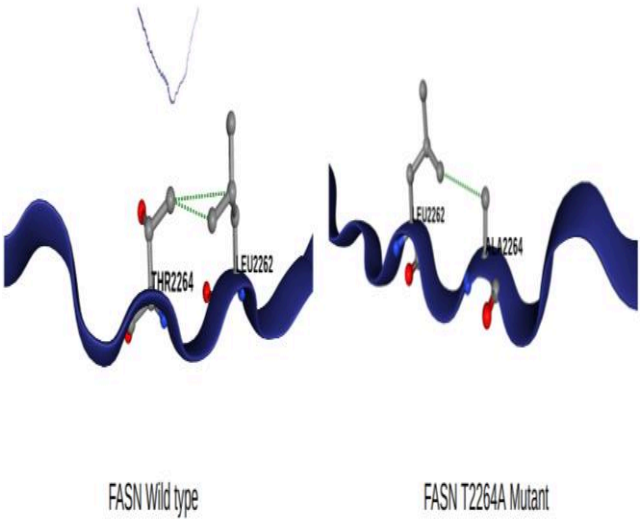

Fig. 6: Interatomic interactions of FASN wild-type and T2264A mutant revealed by dyanmute2 analysis

Fig. 7: RMSD of the wild-type (green) FASN and the T2264A mutant (red). The X-axis represents the time (ns) while the Y-axis represents the RMSD value (nm)

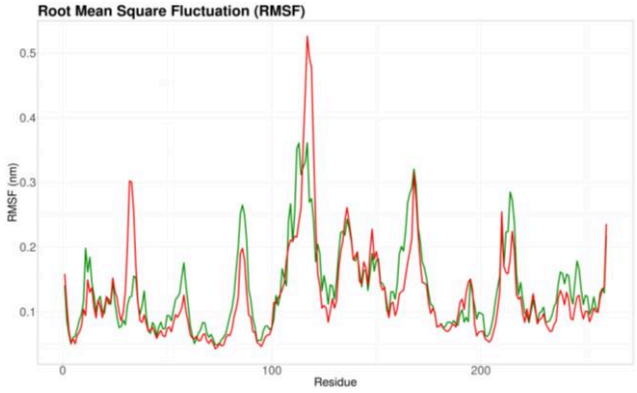

Fig. 8: RMSF of the wild-type (green) FASN and the T2264A mutant (red). The X-axis represents the amino acid residues while the Y-axis represents the RMSF value (nm)

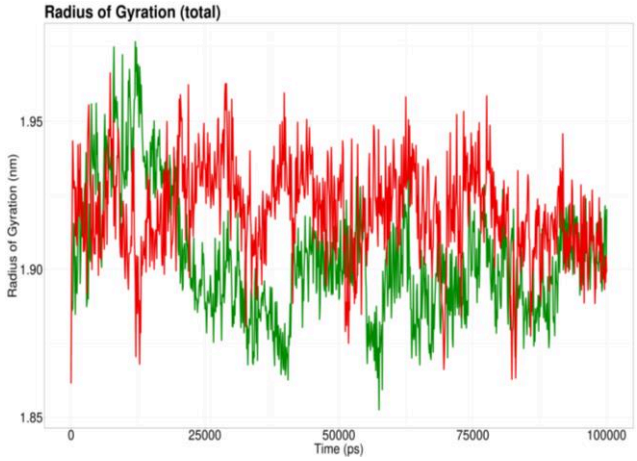

Fig. 9: Radius of gyration of the wild-type FASN (green) and the T2264A mutant (red). The X-axis represents the time (ps) while the Y-axis represents the Area (nm2)

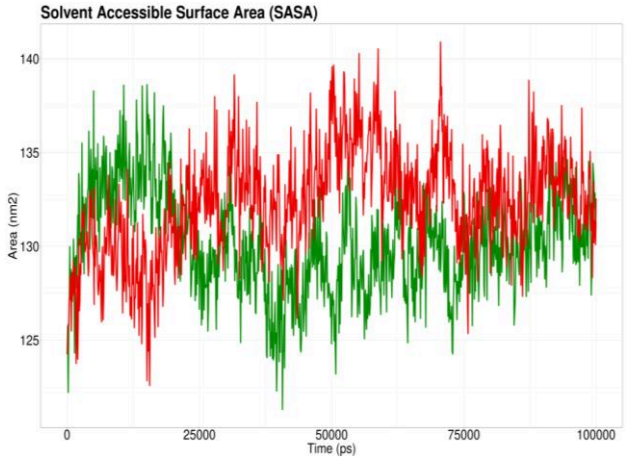

Fig. 10: SASA of the wild-type FASN (green) and the T2264A mutant (red). The X-axis represents the time (ps) while the Y-axis represents the SASA value (nm)

animals at CCBS are fed and managed under standard conditions throughout the year. Concentrate feeds, including rice polish, khesheri, wheat bran, till oil cake, and salt, are provided twice daily, before milking started at morning and in the evening, based on body requirements. Green grasses, like maize, napier, para, and oats, are supplied year-round. Cows are milked twice daily, machine milking for high-yielding cows and with hand-milking for low-yielding ones (Hossain *et al*., 2002). In our study, two conditions have been included:

a) Crossbred F1 generation (Bangladeshi Local X Holstein) cows

b) Reared in the same management situation

Although CCBS has a total of 14,512 records for all animals (Hossain *et al*., 2002; Hamid *et al*., 2017) but after setting the condition sample size become lower.

Since the 1930s, various scattered efforts have been made to improve cattle production by introducing foreign genes, but with no notable success. Key challenges include low-quality breeds, disease outbreaks, insufficient vaccines, feed scarcity and high costs and fluctuating market prices. Science-driven cattle breeding in Bangladesh remains underdeveloped, lacking a defined national strategy or vision (Hamid *et al*., 2017). Molecular genetics techniques are commonly applied to pinpoint genes associated with economically advantageous traits in dairy cattle. These genes are then used as selection markers in marker-assisted selection (MAS) breeding, thus driving advancements in the dairy cattle industry. In essence, breeding programs aim to pinpoint superior genotypes for economically valuable traits by leveraging data on animal performance, familial relationships, and molecular information. This enables the propagation of favorable genes throughout the population (Yudin and Voevoda, 2015; Miglior *et al*., 2017). In the present study, *FASN* gene had been chosen as positional candidate aiming to find out a suitable marker that could be used in cattle breeding. We identified mutation in *FASN* (SNP g.17924 A>G) that showed an association with milk yield trait in our population.

In this study, the frequency of A allele (0.25) at g.17924 A>G, in our population was higher than that found by (Bhuiyan *et al*., 2009) and (Maharani *et al*., 2012) in the Hanwoo population (0.16 and 0.19, respectively), but lower than those reported in Angus beef cattle (0.62) (Zhang *et al*., 2008), 0.31 in Friesian cattle (Morris *et al*., 2007) and 0.53 in Dutch Holstein-Friesian population (Schennink *et al*., 2009) 0.54 in Canadian Angus and Charolais-based commercial crossbred steers (Li *et al*., 2011). Very recent study on Polish Red (RP), Polish Red-and-White (ZR) and Polish Holstein-Friesian Red-and-White (RW) breeds, SNP g.17924 A/G, the GG genotype was most frequent in ZR and RP cows, with frequencies of 0.73 and 0.82, respectively ($P<0.01$) and RW cow had the highest frequency (0.30) of the A/G genotype ($P<0.01$) (Przybylska and Kuczaj, 2024). These discrepancies might be induced by long term cross breeding for milk yield, natural selection, and random drift. Many previous studies with QTL mapping and candidate gene approach found significant associations of *FASN* gene with milk fatty acid composition (Morris *et al*., 2007; Schennink *et al*., 2009; Stoop *et al*., 2009) and milk fat traits (Roy *et al*., 2006; Ordovas *et al*., 2008; Schennink *et al*., 2009)

in different cattle populations, but in our population the significant single locus associations showed a clear effect additive and allele substitution effects on milk yield trait, whereas it did not reach significance for other milk traits. The inconsistency may result from interactions with background genes in various cattle breeds. In general, the effects of polymorphism can vary across populations or breeds due to their unique genetic backgrounds. Bangladeshi Holstein cross cattle have developed through crossbreeding over the past 70 years, involving non-descriptive Deshi cattle and purebred Holstein bull semen from Australia, America, or Europe (NLDP, 2007). Morris *et al*. (2007) identified five SNPs in the *FASN* gene including g.17924 A>G, a non-synonymous SNP leading to a p.Tyr>Ala amino acid change. Our findings were consistent with the current literature on amino acid replacement.

The mutational impact analysis revealed that the mutation in the *FASN* (SNP g.17924 A>G) gene likely destabilizes the protein, affecting its functionality. The function, regulation and activity of a given proyein depend on how well its structure holds together. Proteins degrade, misfold, and clump when stability decreases, eventually becoming dysfunctional (Rozario *et al*., 2021). Analysis suggests the FASN mutant T2264A changes in interatomic interactions and protein stability. The FASN mutant T2264A was in the conserved thioesterase domain. The thioesterase domain is a conserved structural motif present in enzymes known as thioesterases. These enzymes essential for catalyzing the hydrolysis of thioesters, which are chemical compounds with a sulfur atom bonded to an acyl group that are involved in various biological processes, including fatty acid metabolism, polyketide biosynthesis, and protein modification. Molecular dynamics simulations show clear differences between the mutant and wild type FASN in terms of RMSF, RMSD, Radius of gyration, and SASA profile. In summary, the functional and structural analysis of SNP g.17924 A>G mutant protein showed clear differences from wild type protein and may have significant implications for protein structure and function, which could affect various biological processes.

Furthermore, our findings indicate that SNP in the bovine *FASN* gene is linked to variations in milk production traits, reinforcing previous evidence that *FASN* may be the causative gene for QTL associated with fat-related traits in dairy cattle. Structural and functional analysis of the mutation in *FASN* (SNP g.17924 A>G) also supports the association. However, additional experimental validation is required to confirm these findings.

The present study revealed that nsSNPs (SNP g.17924 A>G) was significantly associated with milk yield in the studied population. Such significant effect was possible due to amino acid replacement in FASN protein for this nsSNPs that might change the phenotypes. In comparison with G allele at g.17924 A>G position, an allele could increase the milk production 338.9 kg in a full lactation period. Structural prediction, domain identification, conservation profile, interatomic interactions and molecular dynamic simulation analysis also support the result. These results, along with the functional and structural evaluation of both the FASN wild-type and mutant proteins, provide valuable insights that, with proper validation, could be applied in selective breeding programs to enhance milk production performance in dairy cattle in Bangladesh.

Acknowledgements

The authors would like to acknowledge the Department of Livestock Services, Bangladesh for supporting this study. The study was funded by the Bangladesh Academy of Sciences under BAS-USDA program Code No. LS-15/2017. The funder has no role in the design and conduct of the study; collection, management, analysis and interpretation of the data, preparation, review and approval of the manuscript; or decision to submit the manuscript for publication. The authors also would like to thank a post-doctoral researcher (native speaker), Wuhan University, for his assistance with English expression and editing.

Conflict of interest

The authors declare no conflicts of interest.

References

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

Abraham, MJ; Murtola, T; Schulz, R; Pall, SR; Smith, JC; Hess, B and Lindahl, E (2015). GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers. SoftwareX. 1: 19-25.

Alim, MA; Wang, P; Wu, XP; Li, C; Cui, XG; Zhang, SL; Zhang, Q; Zhang, Y and Sun, DX (2013). Effect of *FASN* gene on milk yield and milk composition in the Chinese Holstein dairy population. Anim. Gen., 45: 111-113.

Apweiler, R; Attwood, TK; Bairoch, A; Bateman, A; Birney, E; Biswas, M; Bucher, P; Cerutti, L; Corpet, F and Croning, MDR (2001). The interPro database, an integrated documentation resource for protein families, domains and functional sites. Nucl. Aci. Res., 29: 37-40.

Ashkenazy, H; Abadi, S; Martz, E; Chay, O; Mayrose, I; Pupko, T and Ben-Tal, N (2016). ConSurf 2016: an improved methodology to estimate and visualize evolutionary conservation in macromolecules. Nucl. Aci. Res., 44: W344-W350.

Bhuiyan, MSA; Yu, SL; Jeon, JT; Yoon, D; Cho, YM; Park, EW; Kim, NK; Kim, KS and Lee, JH (2009). DNA polymorphisms in *SREBF1* and *FASN* genes affect fatty acid composition in Korean cattle (Hanwoo). Asi-Aus. J. Anim. Sci., 22: 765-773.

Chakravarty, B; Gu, Z; Chirala, SS; Wakil, SJ and Quiocho, FA (2004). Human fatty acid synthase: structure and substrate selectivity of the thioesterase domain. Proc.

Nat. Aca. Sci., 101: 15567-15572.

Chilliard, Y; Ferlay, A; Rouel, J and Lamberet, G (2003). A review of nutritional and physiological factors affecting goat milk lipid synthesis and lipolysis. J. Dairy Sci., 86: 1751-1770.

Conte, G; Mele, M; Chessa, S; Castiglioni, B; Serra, A; Pagnacco, G and Secchiari, P (2010). Diacylglycerol acyltransferase 1, stearoyl-CoA desaturase 1, and sterol regulatory element binding protein 1 gene polymorphisms and milk fatty acid composition in Italian Brown cattle. J. Dairy Sci., 93: 753-763.

DLS and Director, G (2021). https://www.dls.gov.bd/site/page/22b1143b-9323-44f8-bfd8-647087828c9b/Livestock-Economy. PP: 1-5.

Falconer, DS and Mackay, TFC (1996). *Introduction to quantitative genetics*. 4th Edn., New York, Longman Scientific and Technical. PP: 24-47.

Gibson, DM; Titchener, EB and Wakil, SJ (1958). Studies on the mechanism of fatty acid synthesis V. Bicarbonate requirement for the synthesis of long-chain fatty acids. Biochim. Biophy. Act., 30: 376-383.

Hamid, M; Rahman, A; Zaman, MA and Hossain, KM (2017). Cattle genetic resources and their conservation in Bangladesh. Asian J. Anim. Sci., 11: 54-64.

Hossain, KB; Takayanagi, S; Miyake, T; Moriya, K; Bhuiyan, A and Sasaki, Y (2002). Statistical genetic studies on cattle breeding for dairy productivity in Bangladesh: I. Genetic improvement for milk performance of local cattle populations. Asi-Aus. J. Anim. Sci., 15: 627-632.

Inoue, K; Shoji, N and Kobayashi, M (2008). Genetic relations among fat melting point, fatty acid composition and carcass traits in Japanese Black cattle. Anim. Sci. J. (Japan), 79: 1-8.

Kale, DS; Singh, J; Sathe, YB and Patil, DV (2021). *FASN* gene and its role in bovine milk production. Int. J. Biotech. Trends Technol., 11: 20-25.

Li, C; Aldai, N; Vinsky, M; Dugan, MER and McAllister, TA (2011). Association analyses of single nucleotide polymorphisms in bovine *stearoyl-CoA* desaturase and fatty acid synthase genes with fatty acid composition in commercial crossbred beef steers. Anim. Gen., 43: 93-97.

Li, C; Sun, D; Zhang, S; Yang, S; Alim, MA; Zhang, Q; Li, Y and Liu, L (2016). 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 Genet., 17: 1-16.

Lynch, M and Walsh, B (1997). *Genetics and analysis of quantitative traits*. 1st Edn., Sunderland, MA, USA, Sinauer Associates, Inc., PP: 35-48.

Maharani, D; Jung, Y; Jung, WY; Jo, C; Ryoo, SH; Lee, SH; Yeon, SH and Lee, JH (2012). Association of five candidate genes with fatty acid composition in Korean cattle. Mol. Bio. Rep., 1: 1-9.

Mannen, H (2011). Identification and utilization of genes associated with beef qualities. Anim. Sci. J., 82: 1-7.

Matsumoto, H; Inada, S; Kobayashi, E; Abe, T; Hasebe, H; Sasazaki, S; Oyama, K and Mannen, H (2012). Identification of SNPs in the *FASN* gene and their effect on fatty acid milk composition in Holstein cattle. Liv. Sci., 144: 281-284.

Mauriae, M; Masek, T; Beniae, M; Spehar, M and Stareeviae, K (2017). Effect of *DGAT1*, *FASN* and *PRL* genes on milk production and milk composition traits in Simmental and crossbred Holstein cattle. Ind. J. Anim. Sci., 87: 859-863.

Mauric, M; Masek, T; Ljoljic, DB; Grbavac, J and Starcevic, K (2019). Effects of different variants of the *FASN* gene on production performance and milk fatty acid composition in Holstein Simmental dairy cows. Vet. Med., 64: 101-108.

Miglior, F; Fleming, A; Malchiodi, F; Brito, LF; Martin, P and Baes, CF (2017). A 100-year review: Identification and genetic selection of economically important traits in dairy cattle. J. Dairy Sci., 100: 10251-10271.

Morris, CA; Cullen, NG; Glass, BC; Hyndman, DL; Manley, TR; Hickey, SM; McEwan, JC; Pitchford, WS; Bottema, CDK and Lee, MAH (2007). Fatty acid synthase effects on bovine adipose fat and milk fat. Mamm. Geno., 18: 64-74.

NLDP and Director, G (2007). National livestock development policy, Ministry of Fisheries and Livestock, Government of the People's Republic of Bangladesh. PP: 1-115.

Ordovas, L; Roy, R; Pampin, S; Zaragoza, P; Osta, R; Rodriguez-Rey, JC and Rodellar, C (2008). The g.763 G>C SNP of the bovine *FASN* gene affects its promoter activity via Sp-mediated regulation: implications for the bovine lactating mammary gland. Physio. Geno., 34: 144-148.

Pereira, PC (2014). Milk nutritional composition and its role in human health. Nutrition. 30: 619-627.

Przybylska, P and Kuczaj, M (2024). The effects of two selected single nucleotide polymorphisms of the fatty acid synthase gene on the fat content and fatty acid profile of cow's milk from the Polish Holstein Friesian Red-and-White breed versus two Polish Red-and-White and Polish Red conservation breeds kept in Poland. Animals. 14: 2268-2275.

Rodrigues, CHM; Pires, DEV and Ascher, DB (2021). DynaMut2: Assessing changes in stability and flexibility upon single and multiple point missense mutations. Prot. Sci., 30: 60-69.

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

Rozario, LT; Sharker, T and Nila, TA (2021). *In silico* analysis of deleterious SNPs of human *MTUS1* gene and their impacts on subsequent protein structure and function. PloS One. 16: e0252932-e0252945.

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

Stoop, WM; Schennink, A; Visker, M; Mullaart, E; Van Arendonk, JAM and Bovenhuis, H (2009). Genome-wide scan for bovine milk-fat composition. I. Quantitative trait loci for short- and medium-chain fatty acids. J. Dairy Sci., 92: 4664-4675.

Wickramasinghe, S; Rincon, G; Islas-Trejo, A and Medrano, JF (2012). Transcriptional profiling of bovine milk using RNA sequencing. BMC Geno., 13: 1-14.

Yudin, NS and Voevoda, MI (2015). Molecular genetic markers of economically important traits in dairy cattle. Rus. J. Gen., 51: 506-517.

Zhang, S; Knight, TJ; Reecy, JM and Beitz, DC (2008). DNA polymorphisms in bovine fatty acid synthase are associated with beef fatty acid composition. Anim. Gen., 39: 62-70.