Quest Journals
Journal of Research in Agriculture and Animal Science
Volume 8 ~ Issue 8 (2021) pp: 22-29
ISSN(Online) : 2321-9459
www.questjournals.org

Research Paper

Polymorphism of Alpha$_{s1}$ Casein Gene and Its Effect on Milk Traits in Friesian X Bunaji Cows

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ABSTRACT
The genetic variants of milk protein genes that associate with milk traits are considered as useful markers for supplementing conventional selection methods in dairy cattle breeding. The objective of this study was to evaluate the genetic variant of alpha$_{s1}$ casein gene and the influence of detected genotypes on milk traits in Friesian X Bunaji cows. A total of 30 Friesian X Bunaji cows were used in this study. The SNPs Genotyping Sequenom Mass ARRAY® system (iPLEX GOLD Technique) technique was used to genotype SNP CSN1$_{S1}$_192 locus at the alpha casein gene. The analysis of milk contents and pH was achieved via a Lactoscan milk analyser. The results showed that SNP CSN1S1_192 was polymorphic and had two alleles (B and C), variant B occurred more frequently than C; two genotypes were identified BB and BC, genotype BB was more common than BC. The results indicated that the 30 Friesian X Bunaji cows were in Hardy-Weinberg equilibrium at the CSN1S1 gene. The results also indicated that the CSN1S1 genotypes (BB and BC) significantly influenced the daily milk yield (p=0.003), contents of milk fat (p=0.0003) and total solid (p=0.0349); cows carrying genotype BB produced milk with higher fat and total solid contents while those carrying BC produced milk with higher daily yield. It was concluded that the alph$_{S1}$ casein gene has significant influence on milk yield and composition traits and could be considered as candidate genes and marker for selection and breeding of Friesian X Bunaji cows.

KEYWORDS:*Friesian X Bunaji, cows, Alpha$_{s1}$-casein, Genetic Variants, Milk, Traits*

Received 21 August, 2021; Revised: 03 September, 2021; Accepted 05 September, 2021

I. INTRODUCTION

Milk yield and composition determine the nutritional and economic values of milk, its processing ability,andconsumers' acceptability of the products [1].The main goal of dairy cattle breeding is to identify an economically efficient way of selecting and breeding animals with desirable genotypes to improve production.

During the last decades, milk protein genetic polymorphism has received research interest because of the associations of their genotypes with milk production traits and its manufacturing properties proteinsthatinclude four caseins (alpha$_{S1}$-, beta-, alpha$_{S2}$-, kappa-) and two whey (alpha-lactalbumin, beta-lactoglobulin) proteins.These proteins are controlled by codominantautosomal genes according to Mendelianinheritance; the genes exist in different allelic forms (polymorphic) and code for 70 to 95 percent of milk proteins [2]. The four casein genes (CSN1S1, CSN2, CSN1S2 and CSN3)arecloselyconnected in a 250-kb cluster and are mapped on chromosome 6 [2]. While the twowhey genes (LAA and LGB) aremapped on chromosomes 5 and 11 respectively [2].Genetic polymorphism of milk proteins arises from substitutions, deletions, or insertion of some amino acids in the polypeptide chains. Previous studies have proven that an efficient way to improve milk yield and composition traits is to use the genetic variants that prove to associate with them for selection and breeding of the animals [3]. Several methods are usedforthegenotyping of polymorphicmajormilk proteins in cattle [4].TheiPlexMassARRAY genotyping technique is one of the modern molecular geneticmethods that take advantage of polymorphism detected at the DNA level regardless of age, physiological status,andsex of the animal [5].

Alpha$_{S1}$-casein protein makes up about 40 percent of total casein; it is highly phosphorylated and calcium sensitive; itsreference protein α_{s1}-CN B-8P is variant B and contains 199 amino acid residues and has a molecular weight of 23,614 Daltons[6]. Until now, 10 genetic variants of the α_{s1}-caseingene which includes: A, B, C, D, E, F, G, H, I and J exist in the genus *Bos*[7, 8]. Amongst these, B and C are most common and differing

in amino acid replacement (Glutamine/Glycine) at position 192 of the mature protein[9]. The frequencies of variant B at the alpha$_{s1}$-casein locus ranges from 0.600 to 1.00 in *Bostaurus* and 0.140 to 0.480 in*Bosindicus* breeds [8]. On the other hand, variant C (0.510) was higher than B (0.490) in Sahiwal cattle [1].
These variants have been characterized and their associations with milk traits and processing parameters have been reported [10, 11,12]. The results so far are contradictory, consequently more studies are required to resolve the situations.

Presently, the Friesian X Bunaji cattle is one of the common dairy breeds used in Nigeria; it has improved productivity compared to the Bunaji cow [13]. Besides that, crossbreeding combines early sexual maturity and high milk yield of European dairy breeds with the adaptability, disease resistance and hardiness of the indigenous cattle [14]. So far, there is no published information on α_{s1}-casein polymorphism and the effects of the genotype on milk quantitative traits in Friesian XBunaji cattle breed in Nigeria. Considering that Friesian X Bunaji cattle is one of common dairy cattle breeds in Nigeria; the aims of this study were to identify the occurrences of alpha$_{S1}$ variants B and C and their genotypes in Friesian X Bunaji cows; investigate how the identified genotypes affect milk yield and composition traits. The output may help to understand the biological significance of the alpha$_{S1}$casein genetic variants identified in the population of Friesian X Bunajicattle

II. MATERIALS AND METHODS

Location of the Experiment: The study was carried out at the Dairy Research Programme farm of the National Animal Production Research Institute (NAPRI), Ahmadu Bello University (ABU), Shika, Zaria, Kaduna State, Nigeria. Shika is geographically located in the Northern Guinea Savanna between latitude 11^{o} 12'59''N and longitude 07^{o} 33'40''E at an elevation of 702 m above sea level [15].

Ethical Statement: The study was implemented following an approved guideline by the Ahmadu Bello University (ABU) Committee on Animal Use and Care (ABUCAUC), Zaria, Nigeria.

Experimental Animals and Management: Thirty (30) Friesian X Bunaji cows were used in this study that variedin their lactation stage and parity. These cows were reared under semi-intensive system and same diet was given to them. The research spanned through months (January - November) in the dry and wet season; that involved feeding, follow-up and collection of milk and blood sample. The cows were permitted to graze on paddocks of established pasture [16]. The cows were offered cotton seed cake, silage,and hay in the dry season. The cows had access to mineral salt blocks and fresh water *ad-libitum*. An automatic milking machine was used in milking the cows twice daily at 7:00 am and 4:00 pm and each cow was given 2.0kgs of concentrate feed at each milking.

Collection of Blood Sample: About 5 ml of blood samples were from 30 Friesian X Bunaji cows throughvenipuncture of *v. coccygea*with a 23-gauge sterile needle and syringe and placed in tubes containing an anticoagulant (EDTA). The 30 blood samples were transported in an ice bag to Bioinformatics laboratory Services at Ibadan, Oyo State, Nigeria and stored at 4 ^{o}C pending the extraction of the genomic DNA.

Genomic DNA Extraction and Quantification: The genomic DNA was extracted from 5ml of whole blood using a QUICK-DNA MINIPREP KIT Cat No. D3024 (Manufactured by Zymo Research) and the manufacturer's protocol were followed. The quality or purity of each extracted genomic DNA (gDNA) was evaluated using a Nanodrop Spectrophotometer; protein contamination was evaluated using the ratio of absorbance at 260 nm and 280 nm. In addition, gel electrophoresis was used to evaluate the integrity of the extracted genomic DNA [17]. The samples that indicated an optical density (OD) ratio (260 nm/280 nm) of between 1.8 and 2.2 were set aside for subsequent analyses. The 30 samples of gDNA were forwarded to InqabaBiotecWest Africa Ltd for SNP CSN1S1s1_192genotyping.

SNP Genotyping SequenomMassARRAYSystem(iPLEX GOLD Technique): The SNP CSN1S1_192 identified by Ketto*et al*. [19] and 30 gDNA belonging to Friesian X Bunaji cows were genotyped using the MassARRAY genotyping platform of the SequenomMassARRAYsystem (iPLEX GOLD; Sequenom, San Diego, California, USA) following manufacturer's protocolswith modifications [18].
The PCR amplification of the targeted region of gDNA containing the SNP was done in a 5µl total volume of reaction system comprising of 20 ng of genomic DNA, 0.5U HotstarTaq (Qiagen), 0.5µl 10×PCR buffer, 0.1µl dNTPs and 0.5 pmol of each primer. PCR reactions were performed in a PTC-100 PCR instrument (Eppendorf) with the following conditions: 4 minutes of denaturation at 94°C, 35 cycles of 20 seconds at 94°C, 30 seconds at 56°C and 1 minute at 72°C and a final extension at 72°C for 3 minutes. After which the PCR product was cleaned using 2µlshrimp alkaline phosphatase (SAP) (SEQUENOM). The single base extension used 2µlExtend Mix (SEQUENOM) consisting of 0.2µl iPLEX termination mix, 0.94µl Extend Primer Mix and 0.041µl iPLEX enzyme and was accomplished through the following steps: initial denaturation at 94°C for 30 seconds, followed by 40 cycles of three steps amplification profile of 5 seconds at 94°C, additional 5 cycles of 5 seconds at 52 °C and 5 seconds at 80°C and a final extension at 72 °C for 3 minutes. Resin purification was used to clean the PCR products and then it was analysed by means of MassARRAY Analyzer Compac (SEQUENOM) and

software TYPER (SEQUENOM). Table 1, represents the marker IDs and primer IDs and their sequences adapted from previous publication [19].

Table 1: Single nucleotide (SNIP ID) polymorphism and primer sequences for the genotyped Markers at the alpha casein gene locus

SNP ID	Position (bp)	Forward primer sequence	Reverse primer sequence	Extended primer sequence
CSN1S1_192	87157262	ACGTTGGATGCACACAATAC ACTGATGCCC	ACGTTGGATGTTACCA CCACAGTGGCATAG	CAGTGGCATAGTAGT CTTT

Source: Kettoet *al*. [19]

Collection of Milk Samples: Twenty millilitres (20) of milk were collected from each of the 30 Friesian X Bunaji cows for analysis of milk traits (two duplicates). Information on cow's parity (1, 2 – 3and ≥4) and lactation stage (early (7 – 90 days), mid (91 – 180 days) and late (181 – 305 days) were recorded. The average daily milk yield of each cow was calculated from farm records.

Laboratory Analysis of Milk Samples: Twenty millilitres (20 ml) of milk were collected from each cow, frozen at 4^{o}C and transported to the laboratory at Centre of Excellence in Agriculture Development and Sustainable Environment (CEADESE) Central Laboratory, Federal University of Agriculture, Abeokuta, Nigeria. The milk was used for duplicates analysis of contents of fat, lactose, protein, fat,andsolid-not-fat (SNF), salts and pHusingLactoscan milk Analyzer.

Statistical Analyses

Genotyped of the SNP *CSN1S1_192 locus* via the IPLEX massARRAYassays:The Matrix-assisted laser desorption/ionization, time-of-flight mass spectrometry (MALDI-TOF MS) mass spectra plotted the allelic peak intensity on *y*-axis against mass (Daltons) on the *x*-axis; while the MassARRAYTyper software analysed the allele peak intensities; the homozygous A and G allele and the heterozygous A G alleles were recorded; from the alleles, cluster plots were generated indicating the number of cows in each genotype calls. Besides that, the massARRAYTyper software recorded the following parameters on Microsoft excel format: number of alleles present; the alleles frequencies (A and G); call rate; observed and expected heterozygosityand the p-value for Hardy Weinberg Equilibrium (HWEp).

Calculated Chi-square (χ^{2}) test to verify departure from Hardy-Weinberg proportion: Then, the above-mentioned data from Excel spreadsheet was used to test the differences between observed and expected genotype frequencies for each SNP using Chi-square (χ^{2}) test to verify departure from Hardy-Weinberg proportion at the significance levels of P<0.05 and P<0.01 using the following formular:

$$\chi^2 = \sum \frac{(Observed - Expected)^2}{Expected}$$

Calculation of allele and genotypes frequencies for the most common genetic variants:The information from the Microsoft Excel spread sheet was used to calculate the genotypes frequencies for the most common genetic variants at the *CSN1S1* gene locus; the Variants B and C were calculated fromaone-point mutation at the SNP CSN1S1_192 A > G locus leading to amino acid change Glu192 Gly and/or protein variation change from B to C; the allele frequencies and genotype frequencies of the genetic variants present in the 30 Friesian X Bunaji cows were recorded.

Calculated allele and genotypes frequencies for the most common genetic variants: The Microsoft Excel spreadsheet gave summaries of the genotypes present at each SNP locus; this information was used to calculate the genotypes frequencies for the following genetic variants: Variants B and C were calculated from SNP CSN1S1_192 (one point mutation; the allele frequencies and genotypes frequencies of the genetic variants present in the 30 Friesian X Bunaji cows were recorded.

Analysed the Effects of AlphaS1-Casein genotypes (BB and BC) on milk yield and composition traits: These effects were evaluated via the MIXED procedure of Statistical Analysis System (SAS), Version 9.0 [20], where the effect of cow was treated as a random effect; while the Alpha$_{S1}$ casein genotypes were considered as fixed effects. However, the effects of lactation stage and parity were found to be non-significant and consequently, were omitted from further statistical analysis. Based on the above, the fixed effects of the Alpha$_{S1}$casein genotypes on the milk yield, milk pH and composition traits were tested in a Linear Mixed Model 1:$Y_{ijk} = \mu + \alpha S1CNgen_i + Cow_j + \varepsilon_{ijk}$ Where: Y_{ijk} = dependent variables include milk yield, pH, and composition traits, μ= the overall mean, $\alpha S1CNgen_i$= the fixed effect of ithα_{S1}-*CN* genotypes (i=BB or BC), Cow_j = the random effect of jth cow (j= 1 to 30) N ~ (0, σ^2_{cow}) and ε_{ijk} = the random residual effect N ~ (0, σ^2_{ε}).

Furthermore, the values were presented as least squares means and their differences were tested with adjusted P-value of Tukey-Kramer procedure of SAS (2002), which adjusted tests for unequal subgroup size for the fixed effect (AlphaS1-casein genotypes (BB=17 cows and BC =11cows) at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$ levels as described in Kramer [21].

III. RESULTS AND DISCUSSION

iPlexMassARRAYResultsfor CSN1S1_192 GenotypedatAlphaS1 Casein Gene Locus Theresultsof this study showed that the SNP CSN1S1_192 locus was polymorphic and had two alleles A and G that determined the genetic variants B and C respectively. Two genotypes AA (17 cows) and AG (11cows) were estimated from the allele's peak intensitiesthat corresponded to genetic variants BB and BC respectively(Figures 1 – 3) The findings of this study agreed with previous outputs [22, 23] that the SNP CSN1S1_192 is polymorphic with two alleles A and G. The allele A and G had frequency of 0.804 and 0.196 respectively corresponding to genetic variants B and C respectively. Two genotypes were detected AA, and AG with frequency 0.617 and 0.393 respectively corresponding to genetic variants BB and BC respectively. This result agrees with the report of Isidore*et al.*[24] who found that the most frequent genotype was the homozygous genotype AA (76) in White Fulani and AA (75) in Borgou. On the other hand, this result does not support the report of Asmarasari*et al*. [25] who found that the most frequent genotype was heterozygous genotype AG (0.67) in Holstein Friesian cows. Allele A (61.7 percent) occurred more frequently than G in Friesian x Bunaji cows. This result agrees with the reports of Asmarasari*et al*. [25] who found that the most frequent allele was A (0.66) in Holstein Friesian; it also agrees with the report of Isidore*et al.* [24] who found that the most frequent allele was A (0.99) in White Fulani and A (0.96) in Borgou cows. The call rate for the SNP was 93.30 %. The expected heterozygositywas 0.316 less than the observed value of 0.393 resulting in heterozygote deficiency. The result of this study agrees with the findings ofHohmann*et al*. [23] who observed heterozygote deficiency at the Alpha$_{S1}$ casein gene locus in different German cattle lines. Heterozygote deficiency in this study may beattributed to slow rate selection against inbreeding depression. A non-significant Hardy-Weinberg equilibrium (P-value 0.20) was observed and confirmed that the CSN1S1_192 locus is at HWE. The findings of this study are not consistent with the previous reports by Isidore*et al*. [24]who found that, the genotypic frequencies at the *CSN1S1* loci deviated from Hardy–Weinberg Equilibrium in Borgou and White Fulani populations ($p<0.05$).

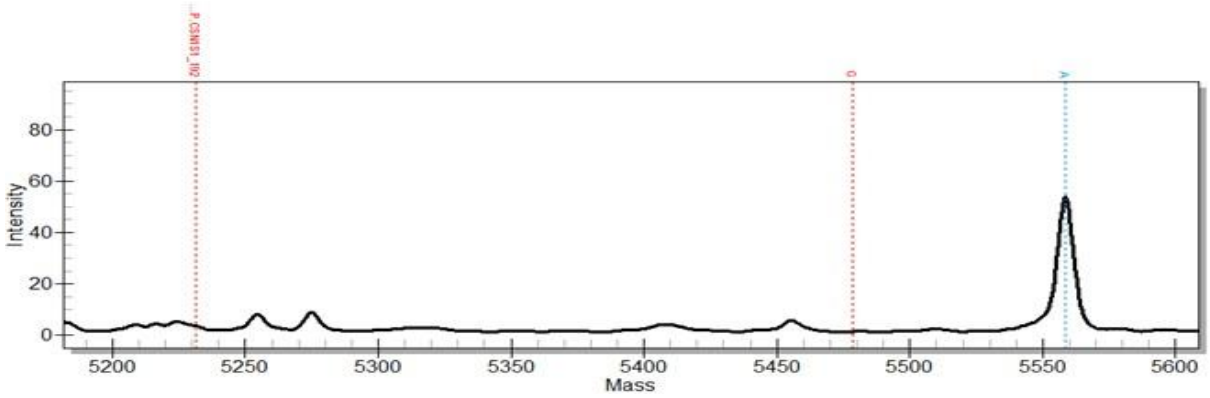

Figure 1: MassARRAY spectrum plotting of allelic peak intensity versus mass (Daltons) indicated homozygosity of allele A at 5560 Da that is corresponding to genetic variant B at the SNP CSN1S1_192 locus

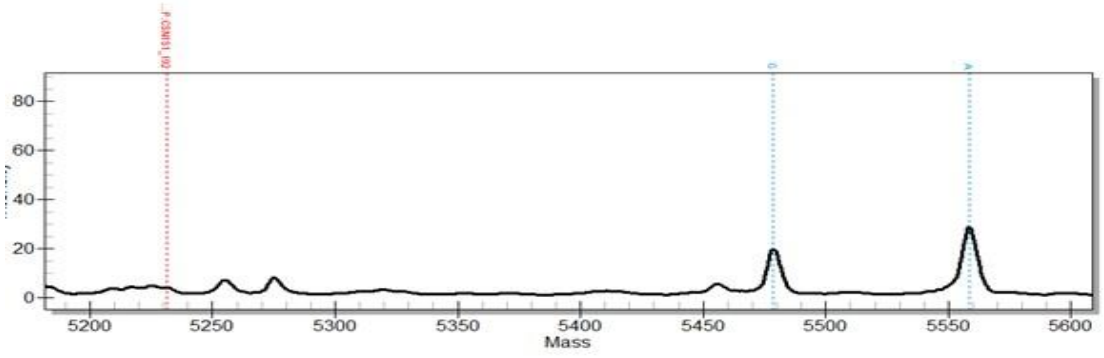

Figure 2: MassARRAY spectrum plotting of allelic peak intensity versus mass (Daltons) indicated heterozygosity of allele A at 5560 Da and allele G at 5480 Da that are corresponding to genetic variants B and C at the SNP CSN1S1_192 locus in 30 Friesian X Bunaji cows

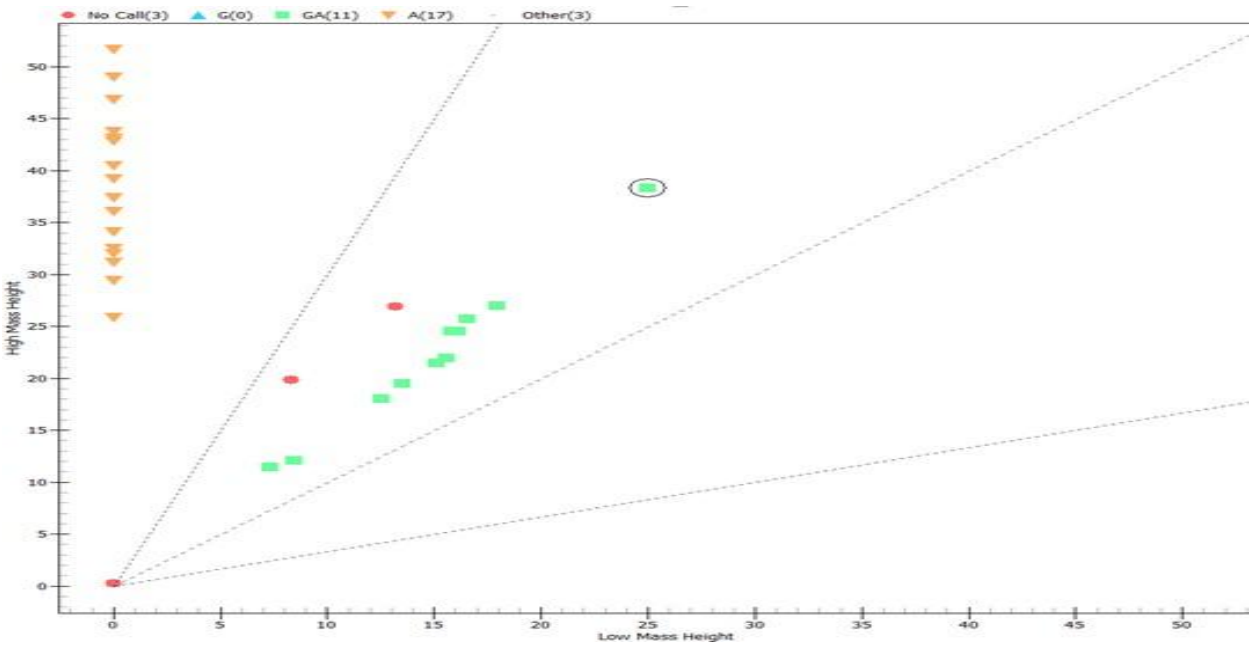

Figure 3: The cluster plot indicated two genotypes of AA (17 cows) and AG (11 cows) corresponding to genetic variants BB and BC respectively at the SNP CSN1S1_192 locus.

Allele and Genotype Frequencies of Beta Casein Gene in 30 Friesian X BunajiCows: The results of genotypic and allelic frequencies and Chi-square ($\square^2$) test for the most common genetic variants of CSN1S1/α_{S1}-CN (B, C) gene in 30 Friesian X Bunaji cows indicated a one-point mutation at the SNP CSN1S1_192 A>G locus was used to determine the genetic variants B > C at the CSN1S1 gene locus (Table 2).

Table 2: Distribution of genotype and allele frequencies of major milk protein genes in Friesian X Bunaji cows

Gene	Genotypic frequency (%)			Allelic Frequency (%)		Test HWE	
CSN1S1	BB	BC	CC	B	C	$\square^2$	HWEp
	60.7 (17)	39.3 (11)	0.0 (0)	80.4	19.6	1.50 NS	0.2Ns

CSN1S1 = alpha$_{S1}$-casein; $\square^2$= chi square test; HWp = Hardy– Weinberg Equilibrium p-value; Note that the number of animals observed for each genotype are in brackets; $\square^2$ = Chi square test; HWp= Hardy-Weinberg equilibrium; NS= non-significant chi square and HWEp

The Friesian X Bunajicows under study were polymorphic at the SNP CSN1S1_192 locus and had two alleles B and C with frequencies of 80.4 and 19.6 percent respectively; allele B occurred more frequent than C in the 30 Friesian X Bunaji cows. In addition, two genotypes homozygote BB (17 cows) and heterozygote BC (11 cows) were detected and their frequencies were 60.7 and 39.3 percent respectively and the most frequent genotype was BB. These results agree with previous researchers in CzechFleckvieh[26], Black-and-White and Jersey Cows [27], Holstein breed [11],Agerolesecattle breed [28], BorgouandWhiteFulani cattle [10], German cattle selection lines[23]. This may be attributed to the fact that selection favoured CSN1S1 variant B because of its superiority over the C allele in milk production [9]. On the other hand, this result disagreed with the findings of Hristov*et al*. [12]who found that the genotype BC occurred more frequent than BB in native Bulgarian Rhodopean cattle breed. Besides that, Mir *et al*. [1] reported that variant C (0.510) was higher than B (0.490) in Sahiwal cattle.

The result for Chi-square test wasnotsignificant (1.50) and indicated that the observed and the expected genotype frequencies were of similar values and confirmed that the 30 Friesian X Bunaji cows were in Hardy-Weinberg equilibrium at the CSN1S1 gene locus [12]. The results of this research further suggest that the Friesian X Bunaji cows were not under selection pressure [29]. Moreover, these results confirmed that there were no technical problems during genotyping.

Summary statistics for the Random and Fixed Effects: Table 3, represents the means and variance component estimates for average daily milk yield, milk pH and milk composition traits and the significance of fixed effect (Alpha$_{S1}$-casein genotypes-BB and BC)intheLinear Mixed Model 1.

Table 3: Means and variance components (σ2 estimates) and significance of fixed (Alpha$_{S1}$ genotypes BB and BC) effects included in the analysis for overall milk traits in Friesian X Bunaji cows (Linear Mixed model 1)

Variable	N	Linear Mixed Model 1			
		Mean	Variance (σ^2) Estimates		Fixed Effect (αs1-CNgenotypes (BB and BC)
			Cow	Residual	
ADMY (kg)	28	7.44±2.50	0.0000	5.1581	P=0.003 **
Fat (%)	28	4.41±1.44	0.0643	0.5480	0.0003 ***
Protein (%)	28	3.23 ±0.23	0.0032	0.0549	0.4661 NS
Lactose (%)	28	4.86±0.34	0.1300	0.0001	0.5224 NS
Solid-not fat (%)	28	8.77 ±0.80	0.2811	0.4079	0.3873 NS
Total Solid (%)	28	13.21±1.64	0.0000	2.2505	0.0349 *
Salts (%)	28	0.73±0.05	1.4600	0.0029	0.5351 NS
Milk-pH	28	6.67±0.08	0.0001	0.0083	0.8351 NS

αs1-CN=Alpha$_{S1}$-casein; *= Alpha$_{S1}$-casein genotypes BB and BC are significant at $P \leq 0.05$; **= Alpha$_{S1}$-casein genotypes BB and BC are significant at $P \leq 0.01$; ***= Alpha$_{S1}$-casein genotypes BB and BC are significant at $P \leq 0.001$; NS=Alpha$_{S1}$-casein genotypes BB and BC are not significant at $P > 0.05$.

The results showed that the residual variance estimated within the cow were higher than the variation between cows (within fixed effects of the mode l) in average daily milk yield (ADMY), contents of milk Fat, Protein, Solid-not fat and Total Solid as well as milk pH; on the other hand, the residual variance estimated within the cow were lower than the variation between cows (within fixed effects of the mode l) for contents of lactose and salts in milk. Besides that, the current study did not find cow variance component estimate for average daily milk yield and total solid. These results agree with the findings of Ketto*et al*. [19] for milk pH, percentages of milk fat, protein, casein and lactose; however, the authors reported that had no cow variance for contents of fat and lactose. The current result implies that larger variance (the residual variance estimated within the cow) are far from the mean as well as far from each other; on the other hand, the small variance, shows the opposite; a variance value of zero for average daily yield and total solid shows that all the values are identical.

Furthermore, the means recorded in this study were daily milk yield (7.44±2.50 kg/cow/day), contents of fat (4.41±1.44 percent), protein (3.23 ±0.23 percent), lactose (4.86±0.34 percent), solid-not fat (8.77 ±0.80 percent), total solid (13.21±1.64 percent) and salts (0.73±0.05 percent) in milk and the milk pH (6.67±0.08) (Table 3). These values were in line with the report of Alphonsus*et al*. [30] who found that in Friesian X Bunaji cows the daily milk yield ranged from 4.04 to 12.00kg/cow/day. The contents of milk fat (ranged =3.74 to 4.71 %; mean =4.30 ± 0.10 %), protein (ranged= 3.89 to 4.45 %; mean=4.16 ± 0.07 %) and lactose (ranged=3.88 to 4.66 %; mean=4.29 ± 0.06 %). Moreover, the values recorded for milk composition traits were within the recommended standard requirement for cow's milk components: for contents of fat (range 3.25 to 5.0 %), solid-not fat (range 8.25 to 9.5 %), total solid (range 12.5 to 14.5 %), pH (range 6.5 – 6.7) [31]. The pH ranged of 6.5 – 6.7 reported in this study for cow milk indicated that the cows were free from bacterial contamination or mastitis [31. The contents of fat and protein in this study were within the recommended standard of CODEX for production of fermented products such as yoghurt [32, 33]

Effects of Alpha$_{S1}$-Casein (αS1-CN /CSN1S1) genotypes on milk yield, pH, and milk composition traits: Table 4, shows the least square means and standard deviation for average daily milk yield, milk pH, contents of milk fat, protein, lactose, solid-not fat, total solid, and salts for Alpha$_{S1}$-casein genotypes (BB, and BC) in Friesian X Bunaji cows.

Table 4: Effects of Alpha$_{S1}$ casein genotypes on milk yield, pH and composition traits in Friesian X Bunaji cows

Variables	αS1-CN (AlphaS1 Casein)		
Genotype	BB	BC	Adjust P-value
Number of cows	17	11	
ADMY (kg)	6.973 ± 0.568^{b}	8.839±0.718^{a}	P=0.003**
Milk pH	6.674 ±0.023	6.666±0.029	0.8351 NS
Fat %	5.026 ± 0.275^{a}	3.173 ± 0.348^{b}	0.0003 ***
Protein %	3.190 ± 0.060	3.262 ± 0.076	0.4660 NS
Lactose %	4.806 ±0.090	4.900 ±0.114	0.5224 NS
Solid-not fat %	8.629 ±0.208	8.924 ±0.263	0.3873 NS
Total solid %	13.586 ±0.375^{a}	12.272 ±0.474^{b}	0.0349 *
Salts %	0.718 ±0.018	0.732 ±0.017	0.5351 NS

*= αS1-CN genotypes (BB and BC) are significant at $P \leq 0.05$; ***= αS1-CN genotypes (BB and BC) are significant at $P \leq 0.001$; ab= Means with different superscript across roll per differ significantly.

The results of the current study indicated that the α_{s1}-CN genotypes (BB and BC) had significant effect on average daily milk yield (P=0.003), contents of fat (p=.0003) and total solid (P=0.03) but had non-significant effects on contents of protein, lactose, solid-not fat and salts in milk as well as milk pH (Table 4). Cows carrying the BB genotype produced milk with a higher content of fat (5.026 ± 0.275 percent)than those with the BC (3.173 ± 0.348 percent) (BB>BC); similarly, cows carrying the BB genotype (13.586 ± 0.375 percent) produced milk with a higher total solid content than those carrying BC genotype (12.272 ± 0.474 percent) (BB>BC). While cows carrying the BC genotype produced milk with a higher daily milk yield (8.839±0.718 percent) compared to those with BB (6.973 ± 0.568 percent) (BC>BB). These results agree with the findings of previous researcheswhofoundthat milk yield was (BC>BB) and content of milk fat was (BB>BC) in native Bulgarian Rhodopean cattle breed [12], in Holstein [11] and in Agerolese Cattle Breed [28]; while content of milk total solid was (BB>BC) in Agerolese Cattle Breed [28]. On the contrary, the results of current study disagree with those of previous findings who reported that milk yield was higher in cows carrying the BB genotype than in hose with BC;BB>BC in Holstein and Jersey breed [27] and in Holstein cattle [11]; the content of milk fat was BC>BB in Holstein and Jersey [27]. The differences in amino acid composition and sequence for genetic variants B and C might partly explain changes in the properties of genotypes BB and BC via net charge, isoionic points and molecular weights; glutaminein variant B is substituted with glycinein variant C; thus, variant B carries one more negative charge than variant C [34]. The lesser net negative charge in variant C compare to variant B results in decrease in the repulsive forces among casein micelles compared to those forces in the B variant. On the other hand, the results of this study disagree with the findings of Ardicli*et al*. [11] who reported that in Holstein cows at the CSN1S1 gene locus, the genotypes BB and BC had non-significant effect on milk yield, contents of fat, and total solid in milk.

IV. CONCLUSION

It may be concluded at the CSN1S1 gene locus, the genetic variant B occur more than C in Friesian X Bunaji cows; the cows carrying genotype BC produce higher amount of milk while those carrying genotype BB produced milk with higher contents of fat and total solid. The alph$_{S1}$ casein genes has significant influence on milk traits and are considered as candidate genes.

REFERENCES

[1]. Mir, S. N., Ullah, O. and Sheikh, R. (2014). Genetic polymorphism of milk protein variants and their association studies with milk yield in Sahiwal cattle. *African Journal of Biotechnology, 13*(4): 555-565.

[2]. Martin, P., Bianchi, L., Cebo, C. and Miranda, G. (2013). Genetic polymorphism of milk proteins. Pages 463 – 514. *In*: MCSWEENEY, P. L. and FOX, P. F. (Eds.). *Advances in Dairy Chemistry*.Volume 1A: Proteins: Basic Aspects, 4thEdition**,** Springer Science + Business Media, New York, USA.

[3]. Morkūnienė, K., Baltrėnaitė, L., Puišytė, A., Bižienė, R., Pečiulaitienė, N., Makštutienė, N., Mišeikienė, R., Miceikienė, I., and Kerzienė, S. (2016). Association of Kappa Casein Polymorphism with Milk Yield and Milk Protein Genomic Values in Cows Reared in Lithuania. *Veterinary Medicine and Zoo technology 74*(96): 27-32.

[4]. Koopaee, H. K. and Koshkoiyeh, A. E. (2014). SNPs genotyping technologies and their applications in farm animals breeding programs: review. *Brazilian Archives of Biology and Technology, 57*(1): 87-95.

[5]. Teneva A. and Petrovic M. (2010): Application of molecular markers in livestock improvement. *Biotechnology in Animal Husbandry, 26* (3-4): 135-154.

[6]. Farrell, Jr H. M., Jimenez-Flores, R., Bleck, G. T., Brown, E. M., Butler, J. E. and Creamer, L. K. (2004). Nomenclature of the proteins of cows' milk-sixth revision. *Journal of Dairy Science, 87*: 1641-1674.

[7]. Caroli, A. M., Chessa, S. and Erhardt, G. J. (2009). Invited review: milk protein polymorphisms in cattle: effect on animal breeding and human nutrition. *Journal of Dairy Science, 92* (11): 5335-5352.

[8]. Gallinat J. L., Qanbari, S., Drögemüller, C., Pimentel, E. C. G., Thaller, G. and Tetens, J. (2013). DNA-based identification of novel bovine casein gene variants. *Journal of Dairy Science, 96* :699–709.

[9]. Hristov, P.I., Teofanova, D.R., Mehandzhiyski, I.D., Zagorchev, L.I. and Radoslavov, G.A. (2013). Significance of milk protein genes polymorphism for Bulgarian Rhodopean cattle: comparative studies. *Biotechnology and Biotechnological Equipment, 27*: 3659-3664.

[10]. Kishore, Amit.,Manishi, Mukesh., Ranbir, C. Sobti., Bishnu, P. Mishra. and Monika, Sodhi. (2013). Variations in the Regulatory Region of Alpha S1-Casein Milk Protein Gene among Tropically Adapted Indian Native (*BosIndicus*) Cattle.International Scholarly Research Notices, Volume 2013 |Article ID 926025 | https://doi.org/10.5402/2013/926025

[11]. Ardicli, Sena.,Bahadir, Soyudal., Hale, Samli., Deniz, Dincel. andFaruk, Balci. (2018). Effect of *STAT1, OLR1, CSN1S1, CSN1S2,* and *DGAT1* genes on milk yield and composition traits of Holstein breed. *Brazilian Journal of Animal Science, 47:* 1-9.

[12]. Hristov, J. Peter., Denitsa, Teofanova.,AniGeorgieva. andGeorgiRadoslavov. (2018). Effect of genetic polymorphism of *αS1-casein* gene on qualitative and quantitative milk traits in native Bulgarian Rhodopean cattle breed. *Genetics and Molecular Research 17* (1): gmr16039868.

[13]. Alphonsus, G.N., Akpa P.P., Barje B.I., Nwagu M., Orunmuyi I.A., Adeyinka M., Alphonsus R.J., Tanko A.M. and Hassan N.P. (2016). Genetic evaluation of fertility traits of Friesian x Bunaji dairy cows using multivariate milk composition-based models. *Journal of Animal Production Research, 28*(1): 112-122.

[14]. Roschinsky, R., Kluszczynska, M., Sölkner, J., Puskur, R., and Wurzinger, M. (2015). Smallholder experiences with dairy cattle crossbreeding in the tropics: from introduction to impact. *Animal,* 9(1), 150-157

[15]. Google Earth (2012). *Shika, Zaria, Kaduna State, Nigeria.* Google Earth. https://earth.google.com/web/search/shika,+Zaria,+Kaduna,+Nigeria/

[16]. Tanko, R.J., Kallah, M. S., Adamu, A. M., Lakpini, C. A. M. and Adeyinka, I.A. (2014). Weight Responses of Bunaji Cattle Grazing Native Rangelands Strip-Sown to Three Different Forage Legumes in the Northern Guinea Savanna of Nigeria. *Journal of Animal Production Research 26:*90-98.
[17]. Black, J. A. andFoarde, K.K. (2007). Comparison of four different methods for extraction of Stachybotryschartarum spore DNA and verification by real-time PCR. *Journal of Microbiology Methods 70*: 75–81.
[18]. Gabriel, S., Ziaugra, L. and Tabbaa, D. (2009). SNP genotyping using the SequenomMassARRAYiPLEX platform. *Current Protocol of Human Genetic, 60*(1):2–12.
[19]. Ketto, I. A., Knutsen, T. M., Øyaas, J., Heringstad, B., Ådnøy, T., Devold, T. G. and Skeie, S. B. (2017). Effects of milk protein polymorphism and composition, casein micelle size and salt distribution on the milk coagulation properties in Norwegian Red cattle. *International Dairy Journal*, *70*: 55-64.
[20]. SAS, (2002). Statistical Analysis System, Version 9.0 SAS Institute Inc. Cary North Carolina, USA.
[21]. Kramer, C.Y. (1956). Extension of Multiple Range Tests to Group Means with Unequal Numbers of Replications. *Biometrics*, *12*: 309–310.
[22]. LIRÓN, J. P., RIPOLI, M. V., DE LUCA, J. C., PERAL-GARCÍA, P. and GIOVAMBATTISTA, G. (2002). Analysis of genetic diversity and population structure in Argentine and Bolivian Creole cattle using five loci related to milk production. *Genetics and Molecular Biology*, 25(4): 413 – 419.
[23]. Hohmann, L. G., Scheper, C.C., Erhardt, G. and König, S. (2021). Genetic diversity and population structure in divergent German cattle selection lines on the basis of milk protein polymorphisms. *Archives Animal Breeding, 64* : 91 -102.
[24]. IsidoreHouaga, Anne W.T. Muigai, Chakirath F.A. Salifou, Souradjou O.G. Idrissou, Kévin S. Kassa, SouleySidi, Emmanuel Hogbonouto, Chabi T.S.R. Biobou and Issaka A.K. Youssao (2018). Effect of Herd, Age, Parity and Stage of Lactation on Milk Production Traits in Indigenous Borgou Cattle Breed in Benin. *International Journal of Advanced Research* 6(5): 92-100.
[25]. Asmarasari SA, Sumantri C, Gunawan A, Taufik E, Anggraeni A. (2020). Genetic variants of milk protein genes and their association with milk components in Holstein Friesian cattle. *Indonesian Journal of Animal and Veterinary Sciences (IJAVS)*, 25(3): 99 – 111.
[26]. Kucerova J, Matejicek A, Jandurova O, Sorensen P, Nemcova E, Stipkova M, Kott T, Bouska J, Frelich J (2006) Milk protein genes CSN1S1, CSN2, CSN3, LGB and their relation to genetic values of milk production parameters in Czech Fleckvieh. *Czech Journal of Animal Science* 51:241-247.
[27]. Miciński, Jan. and Klupczyński, Janusz. (2006). Correlations Between Polymorphic Variants of Milk Proteins, and Milk Yield and Chemical Composition in Black and White and Jersey Cows. *Polish Journal of Food and Nutrition Sciences, 15/56* (1): 137–143.
[28]. Albarella, S., SELVAGGI, M., D'ANZA, E., COSENZA, G., CAIRA, S., SCALONI, A., FONTANA, A., PERETTI, V. and CIOTOLA, F. (2020). Influence of the casein composite genotype on milk quality and coagulation properties in the endangered Agerolese cattle breed. *Animals*, 10(5): 892.
[29]. Falconer, D.S., and Mackay, T.C. (1996). *Introduction to quantitative genetics, 4 Edition.* London, UK: Pearson, London. 480pp.
[30]. Alphonsus, Cyprian, Akpa, Gerald Nwachi, Barje, Peter Pano, Ayigun, Elijah Ade, Zanna, Mustapha, Olaiya, Oluwakemi, Olayinka, IfeoluOluseyi, Anoh, Kevin Usman, Opoola, Emmanuel and Abdulahi, Idris. (2014). Effect of Post-Partum Body Condition Score on Milk Yield and Composition of Friesian X Bunaji Dairy Cows. *Animal Research International 11*(3): 2048 – 2056.
[31]. Anantakrishnan, C. P., Khan, A. Q. and Padmanabhan, P. N. (1993). The Technology of Milk Processing, ShriLakshmi Publications, Kilpauk, Madras, 1-25 pp.
[32]. FAO and WHO. (2010) Food and Agriculture Organization/World Health Organization CODEX standard for fermented milks. CODEX STAN 243-2003. CXS_243e.pdf. Accessed 19 September 2012.
[33]. Bettoni, R.W., and Burlingame, B.)2013(. Milk and dairy product in human nutrition. factor affecting milk composition. Cow milk. 63:51- 59. (FAO) Rome Italy.
[34]. Żukiewicz, A., Grzesiak, W., Szatkowska, I., Błaszczyk, P. and Dybus, A. (2012). Genetic factors of milk yield in dairy cattle. *Israel Journal of Veterinary Medicine*,67(2): 82 –91.