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Polymorphism of β-Lactoglobulin Gene and their Effects on Milk Traits in Friesian X Bunaji Cattle

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***Abstract*— The dairy cattle breeding programs have been focused on the use of molecular genetics techniques to identify specific DNA markers that associate with economically important traits; these markers have been used to supplement conventional breeding methods and has resulted in the selection of young animals for future breeding stock as well as enhanced production. The present study was designed to identify the most common genetic variants of beta lactoglobulin gene and assess their significant effects on milk traits in Friesian X Buanji cows. The polymorphism of β-lactoglobulin geneswas detected via Matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF) in 30 Friesian X Bunaji cows. Two SNPs LGB_64 and LGB_118 at position 103303475 and 103304757 of the beta-lactoglobulin gene locus were genotyped. The results of this study revealed that the 30 Friesian X Bunaji cows were polymorphic and had two alleles A and B with frequencies of 68.3 and 31.7 percent respectively; allele B occurred more frequent than A. In addition, three genotypes homozygote AA(2 cows), heterozygote AB (15 cows), and homozygote BB (13 cows) were found with frequencies of 6.7, 50.0 and 43.3 percent respectively. The most frequent genotype was AB (50.0 percent) followed by BB (43.3 percent), while the least common was AA (6.9 percent). Furthermore, the β-LG genotypes (AA, AB, and BB) significantly affected daily milk yield (P<0.01), and content of milk fat (P<0.05). It might be concluded that in the Friesian X Bunaji cows the β-lactoglobulin may be documented as genetic marker in selection programs to enhanced milk production traits in dairy cattle.**

***Index Terms*— Genetic polymorphism, β-lactoglobulin gene, Friesian X Bunaji cow, Milk traits.**

I. INTRODUCTION

Currently, the dairy cattle breeding programs are focused on the use of molecular genetics techniques to identify specific DNA markers that associate with economically important traits and this has resulted in the selection of young animals for future breeding stock [1]. Molecular marker is defined as a fragment of DNA sequence related to specific region of the genome of an individual [2] and are inherited according to Mendel's laws [3]. Genetic markers research is focused on investigating mutations situatedwithin genes and associating them with economically important production traits [4]. Several molecular techniques have been established to identify alleles and genotypes frequencies within milk protein genes in cattle [5].The iPlex massARRAY genotyping technique is one of the techniques that takes advantage of polymorphism detected at the DNA level regardless of age, physiological status, and sex of the animal [3].

Cow milk contains all the nutrients that neonatal animals require for energy, growth, development, and repair of tissues and is a major raw material for human diet[6, 7]. Globally, about 13 percent of protein requirement of individual persons is gotten from milk and dairy products [8]. In Africa, dairy production and marketing contribute to the livelihoods of over one billion rural farmers through improved food and income security [9, 10].

The protein fraction of bovine milk contains about 95 percent of six major milk proteins: four caseins (alpha$_{S1}$-casein (αs1-CN), beta-casein (β-CN), alpha$_{S2}$-casein (αs2-CN), kappa-casein (κ-CN) and two whey proteins: α-lactalbumin (α-LA) and β-lactoglobulin (β-LG) (11). Beta-Lactoglobulinmakes up approximately 10 percent of the total milk protein and contains about 50 percent of the total whey protein (14, 15, 16]. The primary structure of β-LG was established by Braunitzer *et al*. (17) and its reference protein is β-LG B and it contains 162 amino acids residues with a molecular weight of 18,277 Daltons and is coded by the *LGB* gene[14, 15]. The *LGB* gene is mapped on bovine chromosome 11, it spans 4.7Kb and is organised in seven small exons and six introns. Polymorphism of B-LG gene was established in 1955 by Aschaffenberg and Drewry [18]. Up to date, 12 genetic variants of β-lactoglobulin (β-LG) have been discovered that include: A, B, C, D, Dr, E, F, G, H, I, J and W (14, 19, 20).However, in the dairy cattle, the most frequent genetic variants are A and B (8, 21). Variant A differs from B by two-point mutation that involves amino acid replacements at position 64 where variant B has Glycine (G**C**T) and variant A has Aspartic acid (G**A**T) (Gly64Asp) in the mobile surface loop (CD) and at position 118 where variant B has Alanine (G**C**C) and variant A has valine (G**T**C) (Ala118Val) in the hydrophobic core (8, 12, 22). In addition, variant A has a higher protein concentration compared to variant B (8); this difference is attributed to diverse levels of expression of the A and B variants (23). Several studies have been carried out to detect the polymorphisms of beta-lactoglobulin (*β-LG*) and their relationship with milk production traits, and processing properties [24, 25]. The resultshave been inconsistent; some studies confirmed significant association[26] while others have not [27, 28) therefore, more studies are required for precise situations. However, reasons for the disparities in the results might be due to non-additive interaction of alleles from various loci, or the interaction of genotypes with environmental factors (29).

In Nigeria, one of the strategies to enhancedomestic milk production on dairy cattle farms is through crossbreeding

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schemes between the indigenous and the exotic breeds; Friesian sires and their semen are among the key dairy breed of cattle used in cross breeding of the Bunaji cows [30]. Additionally, crossbreeding combines disease resistance, the hardiness and adaptability of the indigenous cattle with the early sexual maturity and high milk yield of European dairy breeds [31].

Considering that Friesian X Bunaji cattle is one of common dairy cattle breeds in Nigeria and the role of B-LG gene in milk related traits, this study was designed to detect β-LG gene polymorphisms and the influence of the variants on milk traits in Friesian X Bunaji cows.

II. MATERIAL AND METHODS

A. Location of the Experiment: The study was accomplished 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 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 (32). The average annual rainfall is 1,100 m (May to October). Dry, cool weather harmattan (mid-October to January) and dry season (February-May).

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

C. Experimental Animals and Management: Thirty **(**30) Friesian X Bunaji cows reared at Dairy farm of NAPRI were used for this study. Thecows were kept under semi-intensive system and fed on the same diet. They were allowed to graze on paddocks of established pasture consisting of assorted grasses [33]. In the dry season, hay, silage, and cotton seed cake were offered to the cows. Furthermore, the cows had access to fresh water and mineral salt blocks *ad-libitum*. Milking was done twice daily with a milking machine. Each cow was given about 2.0 kgs of concentrate feed at each milking. The cows were controlled against ectoparasites weekly in the dry season and twice a week during the rainy season.

D. Collection of Blood Sample: Five (5) ml of blood samples were collected from 30 Friesian X Bunaji cowsviatheir jugular veinwith sterile needle and syringe and put in test tubes containing an anticoagulant (EDTA). The blood wastransported in an ice bag to a laboratory (Bioinformatics Services) at Ibadan, Oyo State, Nigeria; on arrivalthe samples were stored at 4 ^{o}C awaiting the extraction of the genomic DNA (gDNA).

E. Genomic DNA extraction and quantification: Genomic DNA was extracted from 5ml of whole blood of 30 cows using a QUICK-DNA MINIPREP KIT Cat No. D3024 (Manufactured by Zymo Research) and the manufacturer's protocol were followed. The purity and quality of each extractedgDNA was evaluatedthrough a Nanodrop Spectrophotometer; protein contamination was assessedvia the ratio of absorbance at 260 nm and 280 nm. Additionally,gel electrophoresis was used to measure the integrity of the extracted gDNA. The samples that showed an optical density (OD) ratio (260 nm/280 nm) ranging from 1.8 to 2.2 were kept for further analyses. The 30 samples of gDNA were forwarded to Inqaba Biotec West Africa Ltd for SNPs genotyping.

F. SNPs Genotyping Sequenom MassARRAY® system (iPLEX GOLD Technique):

Two non-synonymous missense SNPs *LGB* _64, and *LGB* _118 documented by Ketto *et al*. (35) and 30 gDNA belonging to Friesian X Bunaji cows were genotyped via the MassArray genotyping platform the Sequenom MassARRAY® system (iPLEX GOLD; Sequenom, San Diego, CA, USA) following manufacturer's protocols. The method is based on the analysis of DNA products using matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) [34].

The PCR amplification of the targeted region of gDNA comprisingof the SNPs was attainedin a 5µl total volume of reaction system containing 20 ng of genomic DNA, 0.5µl 10×PCR buffer, 0.5U HotstarTaq (Qiagen), 0.5 pmol of each primer, and 0.1µl dNTPs. PCR reactions were achieved in a PTC-100 PCR instrument (Eppendorf) under 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, 2µl shrimp Alkaline Phosphatase (SAP) (SEQUENOM) was used to clean the PCR products. The single base extension used 2µl EXTEND Mix (SEQUENOM) consisting of 0.94µl Extend primer Mix,0.2µl iPLEX termination mix, and 0.041µl iPLEX enzyme and was realized via 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.The PCR products were cleaned with resin purification and werethen analysed via MassARRAY Analyzer Compac (SEQUENOM) and software TYPER (SEQUENOM). Table 1, represents the marker IDs, primer IDs and their sequences adapted from previous publication (35).

Table 1. Single nucleotide (SNIP ID) polymorphism of beta lactoglobulin gene and primer sequences for the genotyped markers

SNP ID	Position (bp)	Forward primer sequence	Reverse primer sequence	Extended primer sequence
LGB_64	103303475	ACGTTGGATGGCAATG ATCTTCTTCTGAGC	ACGTTGGATGATGAAA ATGGTCCATGCCCG	GTCTTTCAGGGA GAACG
LGB_118	103304757	ACGTTGGATGTGCTCTT CTGCATGGAGAAC	ACGTTGGATGAGGACC ACACAGCTGGTCTC	ACCCACCCAGGC ACTGGCAG

Source: Ketto *et al*. [35]

2.7Collection of Milk Samples: Twenty millilitres (20) of milk were collected once from one of the morning milks of each of the 30 Friesian X Bunaji cows for analysis of milk traits (two duplicates). Additionally, data on each cow's parity (1, 2-3 and ≥4) and lactation stage (early (7- 90 days), mid (91-180 days) and late (181-305 days) were collected. Moreover, the average daily milk yield of each cow was calculated from the farm records.

2.8 Laboratory Analysis of Milk Samples: Twenty millilitres (20 ml) of milk were collected from each cow, frozen at 4°C and transported in an ice bag to the laboratory at Centre of Excellence in Agriculture Development and Sustainable Environment (CEADESE) Central Laboratory, Federal University of Agriculture, Abeokuta, Nigeria. The 20 ml of milk from each cow was used for duplicates analysis of contents of salts, fat, protein, lactose, solid-not-fat (SNF), and pH usingLactoscan milk Analyzer. The total solid (TS) was then estimated via the formula:

$$\%\ Total\ solid = \%SNF + \%Fat$$

2.9. Statistical analyses: The effects of beta lactoglobulin genotypes on the milk yield and composition traits were analysed by using the MIXED procedure of Statistical Analysis System (SAS), Version 9.0 (SAS, [36], where the effect of cow was treated as a random effect and that of lactation stage, parity, and genotype were considered as fixed effects. However, the effects of lactation stage and parity were found to be non-significant and consequently, omitted from further statistical analysis. The fixed effects of the beta lactoglobulin genotypes on the milk yield and composition traits were tested in model 1:

$$Y_{ijk} = \mu + \beta LGgen_i + Cow_j + \varepsilon_{ijk}$$

Where:

Y_{ijk} = dependent variables include milk yield and composition traits;
μ= the overall mean;
$\beta LGgen_i$ = the fixed effect of ith *β-LG* genotype (i=AA or AB or BB)
Cow_j = the random effect of jth cow (j= 1 to 30) N ~ (0, σ^2_{cow}),
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 using Tukey-Kramer procedure of SAS [36], which adjusted tests forunequal subgroup size and multiple comparison at p≤0.05 and p≤0.01 levels as described in Kramer (37). In addition, the differences between the least squares means for each fixed effect were tested with the Probability of Difference (PDIFF) option of the mixed procedure of SAS.

III. RESULTS AND DISCUSSIONS

A. iPlex MassARRAY results for two SNPs genotyped at beta lactoglobulin gene locus

In the current study Matrix-assisted laser desorption/ionization, time-of-flight mass spectrometry (MALDI-TOF MS) mass spectra plotted allelic peak intensity (*y*-axis) against mass (Daltons) (*x*-axis). After which, the MassARRAY Typer software analysed the allele peak intensities and generated cluster plots that showed genotype calls at the SNP *LGB_64*locus (Plate 1a -d), and SNP *LGB_118*locus (Plate 2a-d) of the 30 Friesian X Bunaji cows.

At the SNP LGB_64 **locus**: The results of this study indicated that the SNP LGB_64 locus was polymorphic and had two alleles A and G that determined the genetic variants A and B respectively. Three genotypes AA (2 cows), AG (15 cows) and GG (13 cows) were determined from the allele's peak intensities corresponding to genetic variants AA, AB, and BB respectively (Plate 1a-d).

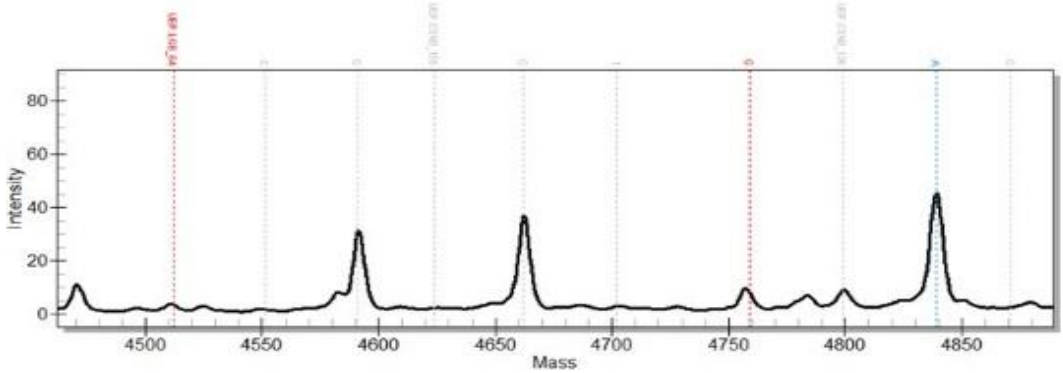

Plate 1a. MassARRAY spectrum plotting of allelic peak intensity versus mass (Daltons) indicated homozygosity of allele A at 4840 Da corresponding to genetic variant A at the SNP *LGB_64* locus in 30 Friesian X Bunaji cows.

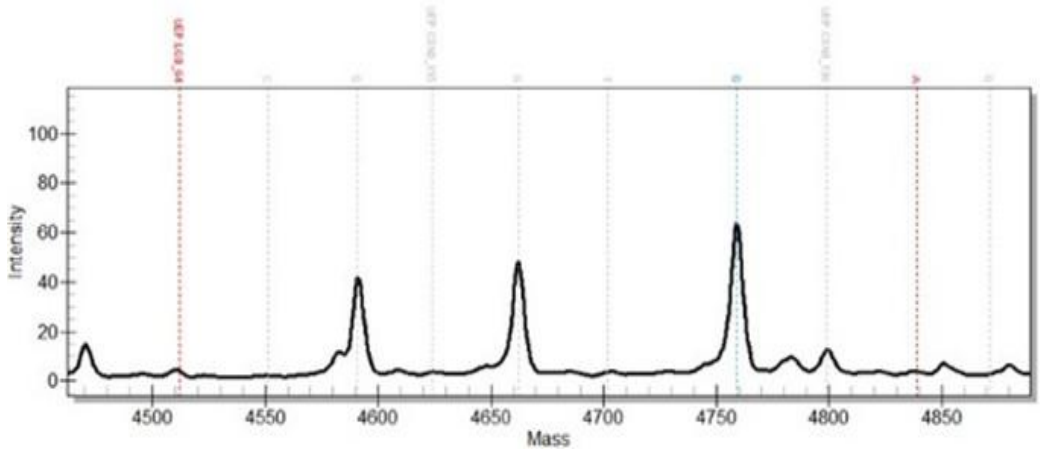

Plate 1b. MassARRAY spectrum plotting of allelic peak intensity versus mass (Daltons) indicated homozygosity of allele G at 4760 Da corresponding to genetic variant B at the SNP *LGB_64* locus in 30 Friesian X Bunaji cows.

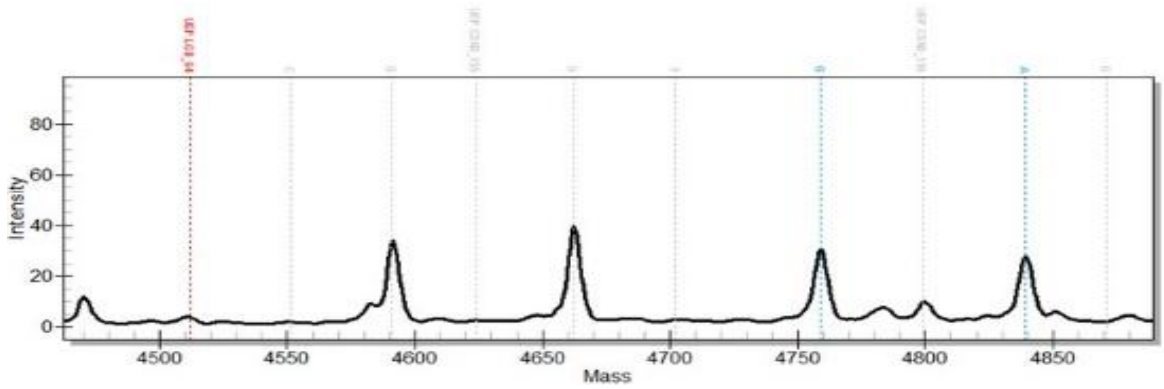

Plate 1c. MassARRAY spectrum plotting of allelic peak intensity versus mass (Daltons) indicated heterozygosity of allele G at 4760 Da and allele A at4840 Da corresponding to genetic variants B and A respectively at the SNP *LGB_64*locusin 30 Friesian X Bunaji cows.

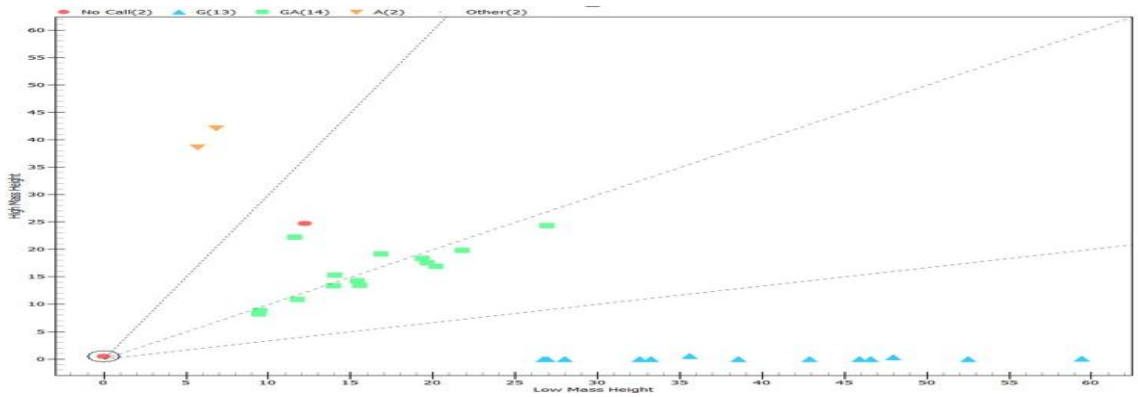

Plate 1d. The cluster plot indicated three genotypes of AA (2 cows), AG (14 cows) and GG (13 cows) corresponding to genetic variants AA, AB, and BB respectively at the SNP *LGB_64* locus in 30 Friesian X Bunaji cows.

At the SNP LGB_118 **locus**: The results of this study indicated that the SNP LGB_118 locus was polymorphic and had two alleles T and C that determine genetic variants A and B respectively. Three genotypes TT (2 cows), CT (15 cows) and CC (13 cows) were determined from the allele's peak intensities corresponding to genetic variants AA, AB, and BB respectively (Plate 1a-d)

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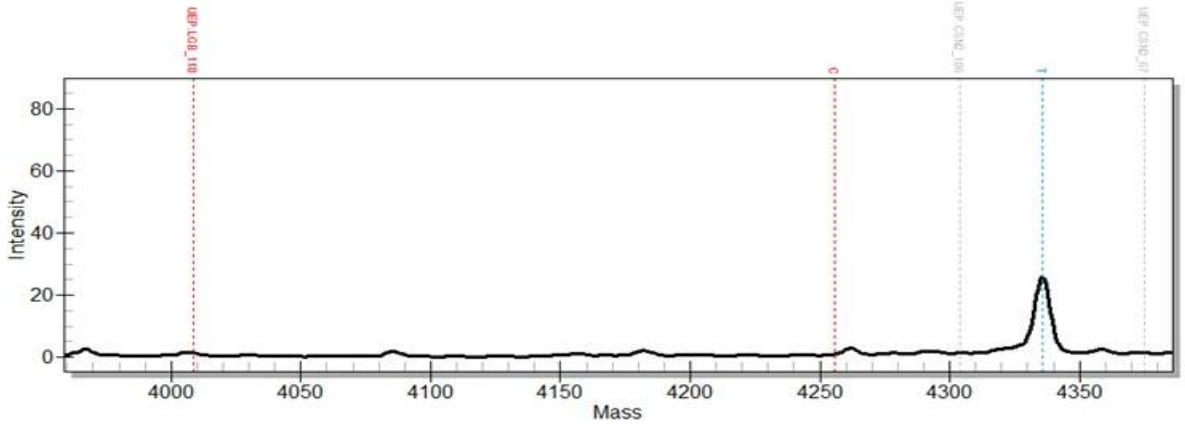

Plate 1a. MassARRAY spectrum plotting of allelic peak intensity versus mass (Daltons)showed homozygosity of allele T at 4335 Da corresponding to genetic variant Aat the SNP *LGB_118* locus in 30 Friesian X Bunaji cows.

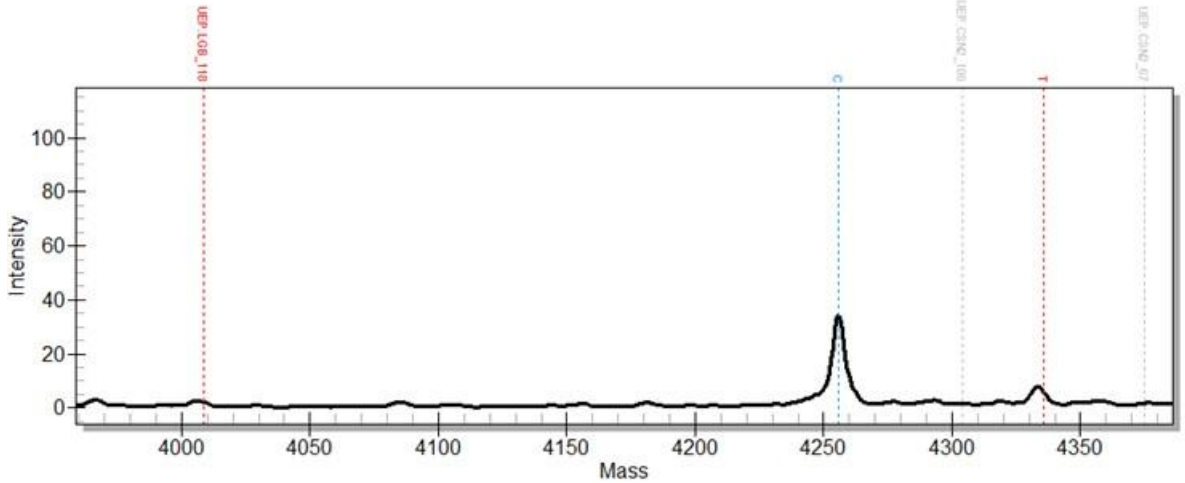

Plate 1b. MassARRAY spectrum plotting of allelic peak intensity versus mass (Daltons) indicating homozygosity of allele C at 4255 Da corresponding to genetic variant B the SNP *LGB_118*locus in 30 Friesian X Bunaji cows.

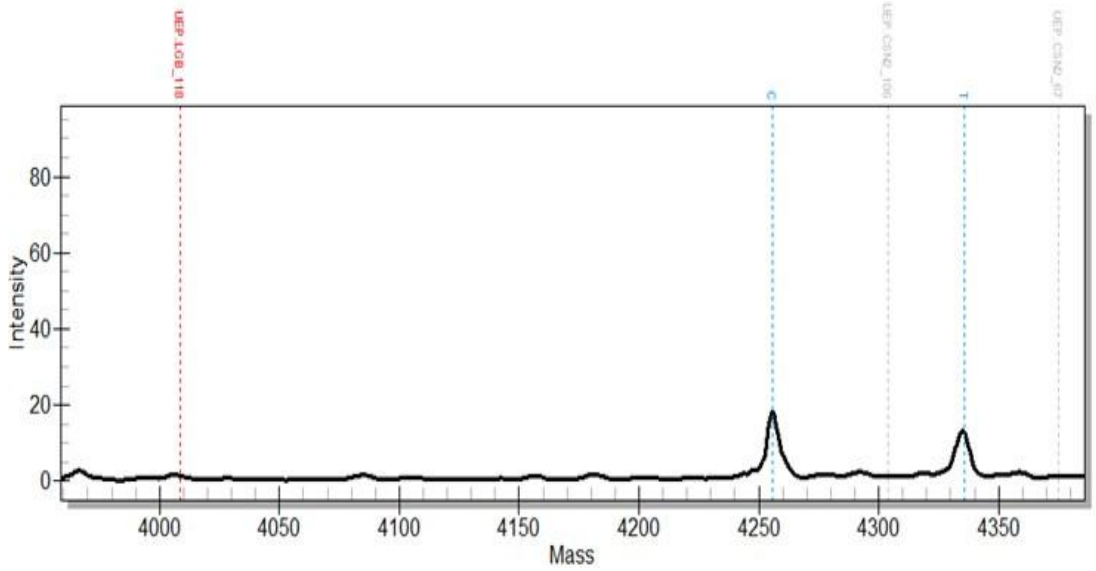

Plate 1c. MassARRAY spectrum plotting of allelic peak intensity versus mass (Daltons) indicated heterozygosity of allele C at 4255 Da and allele T at 4335 Da corresponding to genetic variants B and A respectively at the SNP *LGB_118* locusin 30 Friesian X Bunaji cows.

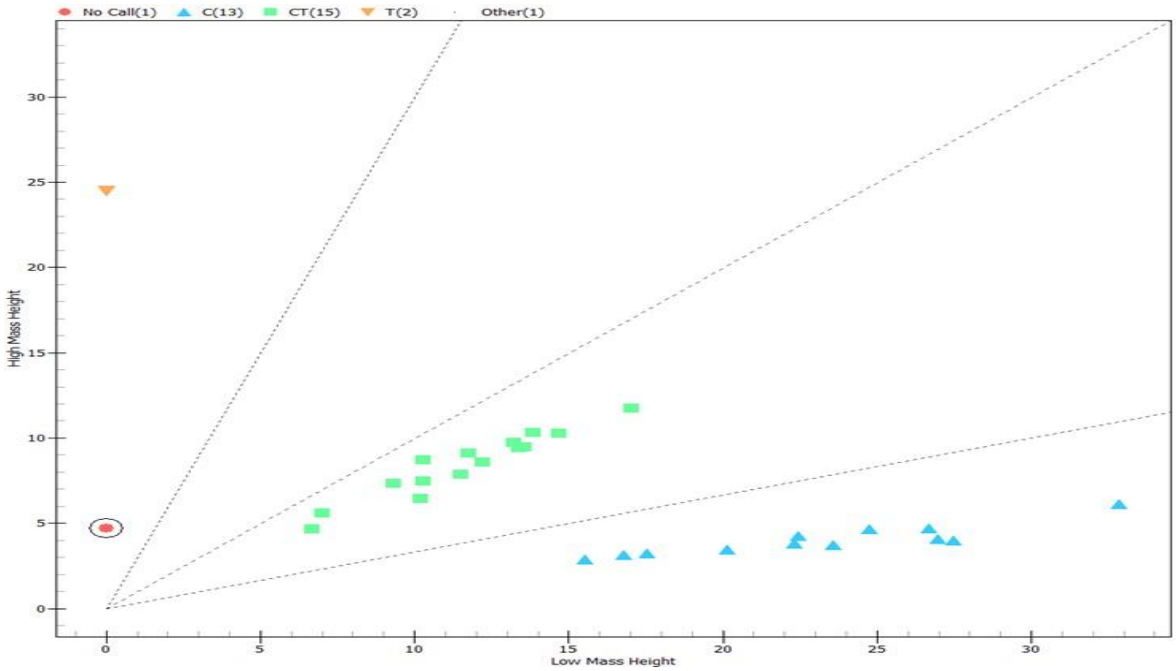

Plate 1d. The cluster plot indicated three genotypes of TT (2 cows), CT (15 cows) and CC (13 cows) corresponding to AA, AB, and BB respectively at the SNP *LGB_118* locus in 30 Friesian X Bunaji cows.

B. Characteristics of Genotyping Results for two SNPs at beta lactoglobulin locus

Table 2, shows the summary characteristics that includes SNP ID, polymorphism of the SNP, observed and expected heterozygosity, genetic variant involved, SNP call rate and Hardy- Weinberg Equilibrium (HWE) p-value for two SNPsat the beta lactoglobulin gene locus in 30 Friesian X Bunaji cows.

Table 2. Characteristics of two SNPs at beta lactoglobulin gene loci assayed by iPlex mass ARRAY.

Variables	*LGB_64*	*LGB_118*
Allele Number	2	2
Polymorphism	G/A	T/C
Genetic variants	B/A	A/B
SNP call rate	100	96.67
SNP genotype frequency	GG=44.8	CC=43.3
	AG=48.3	CT=50.0
	AA=6.9	TT=6.7
SNP allele frequency	**G** = 69.0	**C** = 68.3
	A = 31.0	T = 31.7
Heterozygosity Observed	0.483	0.500
Heterozygosity Expected	0.428	0.433
HWEp	0.49 ns	0.39 ns

At the *LGB_64* locus: The results of the current study indicated that the SNP *LGB_64*was polymorphic and had two alleles A and G with frequency 31.0 and 69.0 percent respectively corresponding to genetic variants A and B respectively. Three genotypes were identified AA, AG, and GG with frequency 6.9, 48.8 and 44.8 percent respectively corresponding to genetic variants AA, AB, and BB respectively. The call rate was 100 percent. The expectedheterozygosity (0.428) was lower than the observed value (0.483) resulting in heterozygote deficiency compared with HWE expectations. Heterozygote deficiency may be caused by certain degree of inbreeding in the population under study. A non-significant Hardy-Weinberg equilibrium P-value (0.49) was observed at the SNP *LGB_64*locus; this confirmed that the SNP*LGB_64*locus was at HWE.

At the *LGB_118* locus: The results of the current study indicated that the SNP *LGB_118*was polymorphic and had two alleles T and C with frequency 31.7 and 68.3 percent respectively corresponding to genetic variants A and B respectively. Three genotypes were identified TT, TC, and CC with frequency 6.7, 50.0 and 43.3 percent respectively corresponding to genetic variants AA, AB, and BB respectively. These results agree with previous findings in Holstein cattle [20] White Fuani and Borgou (38). The call rate was 96.67 percent. The expected heterozygosity (0.433) was less than the observed value (0.500) resulting in heterozygote deficiency compared with HWE expectations. Heterozygote deficiency may be caused by certain degree of inbreeding in the population under study. A non-significant Hardy-Weinberg equilibrium P-value (0.39) was observed at

the SNP *LGB_118*locus; this confirmed that the SNP*LGB_118* locus was at HWE.

C. Allele and Genotype frequencies of beta lactoglobulin gene in 30 Friesian X Bunaji cows

Table 3, indicates the results of genotype and allele frequencies, and Chi-square (χ^2) test for deviation from HWE for the most common genetic variants at the *β-LG* gene locus (A and B) in 30 Friesian X Bunaji cows.

Table 3.Distribution of genotype and allele frequencies of beta lactoglobulin gene in Friesian X Bunaji cows.

Gene	Genotypic frequency (%)			Allelic Frequency (%)		χ^2 (Chisquare)
LGB	AA	AB	BB	A	B	
	6.7 (2)	50.0 (15)	43.3 (13)	31.7	68.3	0.71 NS

LGB = beta-lactoglobulin;Note that the number of animals observed for each genotype are in brackets; χ^2 = Chi square test; NS= non-significant chi square

The 30 Friesian X Bunaji cows under this study were polymorphic and had two alleles A and B. The frequencies of the alleles A and B were 68.3 and 31.7 percent respectively; allele B occurred more frequent than A. These results agree with previous studies in Holstein-Friesian x Jersey crossbred [39], Bulgarian black pied [40], Sahiwal and Tharparkar (41,42], Mexican Jersey [43], Latvian Brown and Latvian Blue [44], Holstein [8,45] andCzech Fleckvieh cattle [19, 46]where at the β-LG gene locus variant B was more common than A. The results of the current study suggest the superiority of variant B at the beta lactoglobulin gene locus in Friesian X Bunaji cows.This is attributed to the fact that variant B is the reference variant at beta lactoglobulin gene locus.On the other hand, these results disagree with some previous investigations who reported that variant A at the β-LG gene locus occurred more frequent than B in Romanian Simmental [47], Girolando cattle [48].

Furthermore, three genotypes homozygote AA(2 cows), heterozygote AB (15 cows), and homozygote BB (13 cows) were found with frequencies 6.7, 50.0 and 43.3 percent respectively. The most frequent genotype was AB (50.0 percent) followed by BB (43.3 percent), while the least common was AA (6.9 percent) in the 30 Friesian X Bunaji cows. These results support the previous findings in Holstein-Friesian x Jersey crossbred [39], Bulgarian black pied [40], Sahiwal and Tharparkar (41, 42], Holstein [8, 45], and Czech Fleckvieh cattle [19, 46] where at the beta lactoglobulin gene locus genotype AB was the most frequent followed by BB. This is attributed to the fact that most selection program favoured the AB and BB genotypes which influenced milk yields or production in dairy cattle. On the contrary to the current results, some authors found that at the beta lactoglobulin gene locus genotype BB was the most frequent in Sahiwal and Tharparkar [41,42], Mexican Jersey [43], Latvian Brown and Latvian Blue, [44] and Holstein cattle [8, 45].

The result of chi-square test (χ_2) was non-significant (0.71) and indicated that the Friesian X Bunaji cows were in HW equilibrium at the *LGB* gene locus in Friesian X Bunaji cows.

D. Summary statistics for the random and fixed effects

Table 4, shows the means and variance component estimates for the milk yield, milk pH and milk composition traits and significant of fixed effect (β-LG genotypes) in Model 1.

Table 4. Means and variance (σ^2) estimates of random effects and significance of fixed effects included in the analysis for milk traits in Friesian X Bunaji cows (model 1)

Variable	N		σ^2 estimates		P-value
		Mean	Cow	Residual	β-LG
Average Daily Milk Yield (kg)	29	7.52±2.49	1.416	0.677	(0.01) **
Fat (%)	29	4.48±1.43	0.185	0.348	(0.07) *
Protein (%)	29	3.23 ±0.23	0.004	0.057	(0.38) NS
Lactose (%)	29	4.86 ±1.43	0.135	2.438	(0.33) NS
Solid-not fat (%)	29	8.78±0.78	0.267	0.400	(0.67) NS
Total solid (%)	29	13.23±1.58	0.000	1.926	(0.26) NS
Salts (%)	29	0.73 ±0.05	1.460	0.003	(0.34) NS
Milk-pH	29	6.67 ±0.08	0.007	0.009	(0.47) NS

M-pH= pH of milk; β-LG= beta-lactoglobulin; *= Fixed effect is significant at P ≤ 0.05; **= Fixed effect is significant at P ≤ 0.01; NS= Fixed effect is not significant at P > 0.05.

The results of this studyshowed that the estimates of variance within the cow were higher than the variance between the cows (within the beta lactoglobulin genotypes) for daily milk yield and contents of salts; whereas, contents of fat, protein, lactose, solid-not fat, and total solid indicated lower variance estimates within the cow than between the cows (within the beta lactoglobulin genotypes). Besides that, the content of milk total solid indicated zero variance estimate for the cow.

The means recorded in this study were daily milk yield (7.52±2.49kg/cow/day), contents of fat (4.48±1.43 percent), protein (3.23 ±0.23 percent), lactose (4.86 ±1.43 percent), solid-not fat (8.78±0.78 percent), total solid (13.23±1.58percent) and salts (0.73 ± 0.05 percent) in milk and the milk pH (6.67 ± 0.08). These values are in line with previous reports [30] and meet the suggested standard conditions for cow's milk components [49]. 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 [49].

E. Effects of Beta-lactoglobulin (β-LG /LGB) genotypes on milk yield, pH, and composition traits.

Table 5, represents the least square means and standard deviation of contents of daily milk yield, milk pH, contents of milk fat, protein, lactose, solid-not fat, total solid, and salts for beta-lactoglobulin genotypes (AA, AB, and BB) in Friesian X Bunaji cows.

Table 5. Effects of beta lactoglobulin genotypes on milk yield, pH, and composition traits in Friesian X Bunaji cows

	β-LG (Beta lactoglobulin)			
Variable (%)	AA	AB	BB	P-value
No of cows	2	15	13	
ADMY	9.13±1.07[a]	7.61±0.51[ab]	6.07±0.53[c]	(0.01) **
Milk pH	6.61±0.07	6.69±0.03	6.71±0.04	(0.47) NS
Fat	4.37±0.61[b]	4.28±0.29[b]	5.29±0.30[a]	(0.05) *
Protein	3.34±0.19	3.10±0.09	3.21±0.10	(0.38) NS
Lactose	5.05±0.29	4.66±0.14	4.83±0.14	(0.33) NS
Solid-not fat	8.26±0.64	8.48±0.31	8.74±0.32	(0.67) NS
Total solid	13.69±1.09	12.89±0.52	13.85±0.54	(0.26) NS
Salts	0.75±0.04	0.70±0.02	0.72±0.02	(0.34) NS

*= β-LG genotypes are significant at P ≤ 0.05; **= β-LG genotypes are significant at P ≤ 0.01; NS=β-LG genotypes are not significant at P > 0.05; abc= Means with different superscript across roll differ significantly.

The findings of the current study, showed that the *β-LG* genotypes (AA, AB, and BB)significantlyaffected daily milk yield (P<0.01), and content of milk fat (P<0.05) but there were non-significant effects on milk pH, contents of protein, lactose, solid-not fat, total solid and salts in milk. The cows carrying homozygote AAgenotype produced the highest amount of milk per day (9.13±1.07kg/cow/day) followed by those carrying AB genotype (7.61±0.51kg/cow/day) though there were not statistically different while those with genotype BB produced the least amount of milk; the ranking for daily milk yield was AA=AB>BB. Additionally, the cows carrying genotype BB produced milk with the highest fat content (5.29±0.30percent); the cows carrying genotype AB produced milk with the least fat content (4.28±0.29 percent); ranking recorded for content of fat was BB>AA=AB. These result support the report of Piątkowska*et al.* [50] who indicated that *BLG* genotypes AA, AB, and BB had significant (P<0.05) relationship with the milk traits in Holstein Frisian cattle; cows with *BLG* genotype AA produced the highest amount of milk compared to those carrying AB and BB genotypes (AA>AB>BB) while those with BB genotype produced milk with the highest content of fat (BB>AA=AB). Likewise, Wageh Zaglool *et al.*, (8) working with Holstein Friesian cows at the *LGB* locus found that cows carrying genotype AA indicated higher milk yield while those carrying the BB genotype, had higher content of fat than those with genotypes AA and AB.Similarly, Ren *et al.* [50] indicated that the BB genotype at the *LBG* locus correlated with higher contents of fat in milk, which are good properties for processing and milk quality improvement. According to the findings of Neamt *et al.* [47], the *AA* genotype of LGB gene related with higher milk production than *BB.*This is may be attributed to differences in the isoelectric point and molecular weight of the various variants. On the contrary with respect to yields of milk, Alim et al. [20] reported that in Chinese Holstein cattle at the LGB gene locus, the cows carrying genotype TT(BB) produced milk with the highest 305day milk yield, fat yield and protein yield compared with CC (AA) cows; CT(AB) cows were at the middle position. In the same way, Hristov *et al.* [26] reported that at the *LGB* locus, cows carrying the genotype BB influenced higher milk yield.

IV. CONCLUSIONS

Recently, the beta-lactoglobulin polymorphism has attracted interest among researchers, milk producers and consumers because of the possible association between certaingenetic variants and milk traits and technological properties of milk. It might be concluded that the Friesian X Bunaji cows produce milk that meet the recommended standard for contents of milk fat, protein, solid-not fat, total solid and milk pH for cow's milk. The beta lactoglobulin allele B is more frequent in the population of Frieisian X Bunaji cows. The cows carrying the beta lactoglobulin genotypesAA and AB produce the highest amount of milk per day per cow, while those cows carrying genotype BB produce milk with the highest amount of fat content. The LGB gene could be a useful marker for selection of cattle in favour of milk production. Considering the association of beta lactoglobulin variants with processing properties and most of the produced is used for yoghurt production; it is recommended that the effects of beta lactoglobulin genotype on yoghurt parameters be investigated.

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