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Research Paper

Effect of Polymorphism of Kappa Casein Geneon Milk Yield and Composition Traits in Friesian X BunajiCows

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ABSTRACT.
In the cattle population, milk protein genetic polymorphism has been investigated broadly to identify molecular markers for selecting, breeding and improvement of milk quantitative and qualitative traits. Polymorphism of kappa casein protein (κ-casein) in 30 Friesian X Bunaji cows was studied using Matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF) technique to assess the effects of the identified genotypes on milk yield and composition traits. Milk yield was calculated from the farm records while milk composition was analysed using an automatic Milkscan analyser. Three SNPs CSN3_136, CSN3_148 and CSN3-155 loci at the kappa-casein gene were genotyped. The results showed that at the CSN3 gene two genetic variants A (75.00 percent) and B (25.00 percent) were identified and variant E (0.00 percent) was absent. In addition, three genotypes AA, AB and BB occurred at frequencies 53.3, 43.3 and 3.3 percent respectively and were in accordance with HWE. Only one cow was carrying genotype BB and was not included in subsequent analysis. The most frequent variant was A and the most frequent genotype was AA. Cows carrying genotype AA produced a higher amount of milk while those carrying AB produced milk with the higher amount of fat, total solid and conduction. The CSN3 genotypes did not influence the contents of milk protein, lactose, solid-not fat and salts, milk pH, density, and freezing point. This study showed that the κ-casein genetic variants might be a tool to improve the milk production traits in the Friesian X Bunaji breed via increasing the frequency of desired genotypes.

KEYWORD: *Cows,Effects, Friesian X Bunaji, Genotypes, Kappa-casein, Milk, Polymorphism, Traits.*

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I. INTRODUCTION

The principal goals in dairy farming are to select animals with desired genotypes to improve milk yield and composition traits (Boro*et al.*, 2016; Desyibelew and Wondifraw, 2019). Recently, the genetic polymorphism of bovine milk proteins has been investigated to identify molecular markers for selecting, breeding and improvement of milk quantitative and qualitative traits as well as product quality parameters in dairy cattle breeding (Balteanu*et al.*, 2010; Ketto*et al.*, 2017).

Bovine milk contains six major milk proteins that include four caseins (α_{S1}-, αs2-, β-, κ-CN) and two whey (α-lactalbumin, β-lactoglobulin) coded by six genes *CSN1S1*, *CSN1S2*, *CSN2*, *CSN3*, *LAA* and *BLG* respectively; these genes are codominantautosomal and are inherited according to Mendelian inheritance; the genes exist in different allelic forms and code for about 95 percent of milk proteins (Martin *et al.*, 2013). The κ-Casein (κ-CN) protein comprises about 12 to15 percent of the total casein fraction in bovine milk (Contreras *et al*., 2011). Its reference protein is κ-CN A-1P (Holland, 2008) and was first established by Mercier and others in 1973 to contain 169 amino acid residues and a molecular weight of 19,007 Daltons (Mercier *et al*., 1973; Farrell Jr*et al*., 2004). In bovine milk, κ-CN exit in several forms with varied physicochemical properties due to the presence of missense mutations (genetic polymorphism), intermolecular disulphide linkages, glycosylation, and phosphorylation (Huppertz, 2013). So far, studies have identified in Bos's genus, 13 variants of the mature κ-CN gene: A, B, B^2 C, D, E, F^1, F^2, G^1, G^2, H, I and J which are located on the fourth exon that is 517bp in length plus A1 which is synonymous (Farrell *et al*., 2004; Gallinat*et al*., 2013; Martin *et al*., 2013). Up till now, several techniques have been applied for genotyping of polymorphisms in major milk proteins genes and the

iPlexmassARRAY genotyping technique is one of the techniques that relies on polymorphism identified at the DNA level regardless of age,sex, and physiological status of cattle (Teneva and Petrović, 2010).

In dairy cattle, the κ-CN variants A and B are the most common and variant E has been reported in lesser frequency (Farrell Jr., *et al*., 2004; Caroli*et al*., 2009; Martin *et al*., 2013; Awad*et al*., 2016). In addition, at the κ-CN locus, the variants A, B and E are situated around the C-terminal part (the caseinomacropeptide; CMP); here, two polar residues Thr at position 136 and Asp at position 148 in variants A are substituted in Variant B by hydrophobic Ile and Ala respectively; while at position 155 of the E variant, hydrophobic Gly substitute polar Ser in the A and B variants. In dairy cattle and their crosses, previous studies have shown that apart from the Jersey breed the variant κ-CN A is the most common (Awad*et al*., 2016; Neamt*et al*., 2017; Houaga*et al*., 2020). For example, Deb *et al*., (2014) found that in Frieswal cattle (Friesian x Sahiwal) at the κ-CN locus, the A allele (0.58) was higher than B allele (0.42). Several researchers have investigated the effects of *CSN3* genotypes on milk production and composition traits (Stipp*et al*., 2013; Singh *et al*., 2014) and the results are contradictory; some researchers have confirmed positive association (Bittante*et al*., 2012; Neamt*et al*., 2017) while others found non-association (Duifhuis-Rivera*et al*., 2014; Dogru, 2015) therefore, more studies are required for specific conditions (Neamt*et al*., 2017). For instance, studies have demonstrated that at the κ-casein gene locus, the A allele in both the homozygous (AA) and heterozygous (AB) genotypes is related with higher milk yield while the B allele, on the other hand, is associated with higher contents of fat and/or protein in milk which makes it a better material for cheese and yoghurt production (Alim*et al*., 2015; Neamt*et al*., 2017). Previous studies have shown that k-casein offers the best technological properties of milk and is considered as one of key markers in cattle selection and breeding.

The Bunaji breed is the main indigenous cattle used for dairy production in Nigeria and makes up 37 percent of the national cattle population (Alphonsus*et al*., 2012). Recent review has stated that the limitation of the Bunaji breed includes low milk production, long calving interval, delayed conception, late sexual maturity, and short lactation period (Kubkomawa*et al*., 2017).One of the strategies that has been used to improve their productivity is via crossbreeding schemes with the exotic breed (mostly Friesian sires) (Alphonsus, 2010).

In view that Friesian X Bunaji cattle is one of the main dairy cattle breeds in Nigeria and the role of kappa casein gene in milk related traits, this study was designed to identify alleles and genotype frequencies at the k-CN gene, determine the effects the genotypes on milk yield and composition traits in Friesian X Bunaji cows.

II. MATERIAL AND METHODS

2.1. Location of the Experiment: The current 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 situated 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 (Google earth, 2012). The average annual rainfall is 1,100 m (May to October); dry season (February-May) and dry and the cool weather harmattan (mid-October to January).

2.2. Ethical Statement: The study was realised based on an agreedguide by the Ahmadu Bello University (ABU) Committee on Animal Use and Care (ABUCAUC), Zaria, Nigeria.

2.3. Experimental Animals and Management: Thirty **(**30) Friesian X Bunaji cows raised at Dairy farm of NAPRI were utilised for this Investigation. The cows were raised under a semi-intensive system and nourished on the similar diet. They were allowed to graze on paddocks of established pasture containing various forages and grasses [Tanko *et al*., 2014] In the dry season, hay, silage, and cotton seed cake were offered to the cows. Furthermore, the cows had access to mineral salt blocks and fresh water *ad-libitum*. Milking was done twice daily with an automatic milking machine. Each cow was given about 4.0 kgs of concentrate feed daily. The cows were dipped against ectoparasitestwice and ones a week during the rainy season and dry season respectively.

2.4 Collection of Blood Sample: Five (5) ml of blood samples were taken from each Friesian X Bunaji cows through their jugular vein with sterile needle and syringe and put in a 5ml test tube containing an anticoagulant (EDTA). The blood was transported in an ice bag to a laboratory (Bioinformatics Services) at Ibadan, Oyo State, Nigeria; on arrival the samples were stored at 4 ^{o}C awaiting the extraction of the genomic DNA (gDNA).

2.5. 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 was followed. The purity and quality of each extracted gDNA was assessedvia a Nanodrop Spectrophotometer; protein contamination was measured via the ratio of absorbance at 260 nm and 280 nm. Gel electrophoresis was used to measure the integrity of the extracted gDNA. The samples that displayed an optical density (OD) ratio (260 nm/280 nm) ranging between 1.8 and 2.2 were reserved for

subsequent analyses. The 30 samples of gDNA were sent to InqabaBiotec West Africa Ltd for SNPs genotyping.

2.6. SNPs Genotyping SequenomMassARRAY® system (iPLEX GOLD Technique):Three non-synonymous missense SNPs CSN3_136, CSN3_148 and CSN3_155 identified by Ketto *et al*. (2017) were genotyped through the MassArray genotyping platform the SequenomMassARRAY® system (iPLEX GOLD; Sequenom, San Diego, CA, USA) guided by the producer'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) [Gabriel *et al*., 2009].

The targeted regions of gDNA comprising of the SNPs targeted were amplified using PCR in a 5µl total volume of reaction system consisting of 0.5 pmol of each primer, 20 ng of genomic DNA, 0.5µl 10×PCR buffer, 0.5U HotstarTaq (Qiagen), and 0.1µl dNTPs. PCR reactions were achieved in a PTC-100 PCR instrument (Eppendorf) under the subsequent 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 with 2µl shrimp Alkaline Phosphatase (SAP) (SEQUENOM). The single base extension made use of 2µl EXTEND Mix (SEQUENOM) containing 0.041µl iPLEX enzyme, 0.94µl Extend primer Mix, and 0.2µl iPLEX termination mix which was accomplished through the subsequent 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 were later analysed by means of MassARRAY Analyzer Compac (SEQUENOM) and software TYPER (SEQUENOM). Table 1, shows the marker IDs, primer IDs and their sequences adapted from previous publication (Ketto *et al*., 2017).

Table 1. Single nucleotide (SNIP ID) polymorphism of kappa casein gene (CSN3) and primer sequences for the genotyped markers

SNP ID	Position (bp)	Forward primer sequence	Reverse primer sequence	Extended primer sequence
CSN3_136	87390576	ACGTTGGATGACTTGGACTG TGTTGATCTC	ACGTTGGATGCCTACCATCAAT ACCATTGC	CTACAAGTACACCTA CCA
CSN3_148	87390612	ACGTTGGATGACTTGGACTG TGTTGATCTC	ACGTTGGATGCCTACCATCAAT ACCATTGC	GCACTGTAGCTACTC TAGAAG
CSN3_155	87390632	ACGTTGGATGCCTACCATCA ATACCATTGC	ACGTTGGATGACTTGGACTGTG TTGATCTC	GTGTTGATCTCAGGT GGGC

Source: Ketto et al. [2017]

2.7 Collection of Milk Samples: Twenty millilitres (20) of milk were taken from one of the morning milks of each of the 30 Friesian X Bunaji cows for analysis of milk composition traits. 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. Besides that, 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^{o}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 was utilized for duplicates analysis of contents of salts, fat, protein, lactose, solid-not-fat (SNF), density, freezing point, conductivity, and pHusingLactoscan milk Analyzer. The total solid (TS) was then assessedusingthe formula:

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

2.9. Statistical analyses:

Genotyped of three SNPs belonging to kappa casein gene *(CSN3_136, CSN3_148, CSN3_148)* through the IPLEX massARRAY analyses: The Matrix-assisted laser desorption/ionization, time-of-flight mass spectrometry (MALDI-TOF MS) mass spectra were used in plotting allelic peak intensity (*y*-axis) against mass (Daltons) (*x*-axis); the MassARRAYTyper software was used in analysing the allele peak intensities; cluster plots were generated which indicated the genotype calls at the SNPs CSN3_136, CSN3_148, CSN3_155 loci of the Friesian X Bunaji cows. Additionally, massARRAYTyper software allowed the recording of the resulting parameters on Microsoft excel format for each SNP: number of alleles; call rate, total number of cows; observed and expected heterozygotes genotypes, the alleles frequencies (p and q), and p-value for Hardy Weinberg Equilibrium.

Calculated Chi-square (χ^{2}) test to verify departure from Hardy-Weinberg proportion

Chi-square (χ^{2}) test was used to assess the departure from Hardy-Weinberg proportion at the significance levels of $P<0.05$ and $P<0.01$ using the following formular:

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

Calculated allele and genotypes frequencies for the most common genetic variants:
Data from the Microsoft Excel spreadsheet on the genotypes present at each SNP locus was used in estimating the genotypes frequencies for most common genetic variants: Variants A and B were estimated from a two-point mutation at SNPs CSN3_136C > T and CSN3_148A > C Alead to amino acid change Thr136 (157) Ile and Asp148(169) Ala or protein variation change from variant A to B (Alim*et al*., 2014); while the genetic variants A and E were estimated from a one-point mutation at SNP CSN3_155 locus where glycine substitutes serine (Martin *et al*., 2013).The allele and genotypes frequencies of the genetic variants A, B and E were noted.
The effects of kappa casein genotypes: The effects of kappa casein genotypes on the milk yield, milk pH, and composition traits were analysed through the MIXED procedure of Statistical Analysis System (SAS), Version 9.0 (SAS, [SAS 2002], where the effect of cow was treated as a random effect. Nevertheless, the effects of lactation stage and parity were non-significant and were not involved in succeeding statistical examination. Besides that, the less frequent genotype (<4 percent) of k-CN (BB) wasomitted from the statistical analysis. The fixed effect of the kappa casein genotypes(AA and AB) on the milk yield, milk pH and composition traits were tested usingtheLinear Mixed Model 1:

$$Y_{ijk} = \mu + CSN3gen_i + Cow_j + \varepsilon_{ijk}$$

Where: Y_{ijk} = dependent variables include milk yield, milk pH and composition traits;
μ= the overall mean; $CSN3gen_i$= the fixed effect of ith*CSN3* genotype (i=AA or AB);cow_l = 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})

All the means werenotedas least squares means and their differences were tested using Tukey-Kramer procedure of SAS [2002], which adjusted tests for unequal subgroup size at p≤0.05, p≤0.01,p≤0.001 levels as described in Kramer [1956]. The adjusted P-value will be used in separating the least square means.

III. RESULTS AND DISCUSSIONS

3.1. Allele and Genotype frequencies of kappa casein gene in 30 Friesian X Bunaji cows

Table 2, shows the results of allele and genotype frequencies, and Chi-square (χ^2) test for deviation from HWE for the most common genetic variants at the *CSN3* gene locus (A and B) in 30 Friesian X Bunaji cows.

Table 2.Distribution of genotype and allele frequencies of kappa casein gene in Friesian X Bunaji cows.

Gene	**Genotypic frequency (%)**				**Allelic Frequency (%)**			**Test HWE**
CSN3	AA	AB	BB	EE	A	B	E	
	53.3 (16)	43.3 (13)	3.3 (1)	0.0	75.0	25.0	0.0	0.92 ns

CSN3 = kappa casein;Note that the number of animals observed for each genotype are in brackets; χ^2 = Chi square test; NS= non-significant chi square

The genotypes and allele frequencies of the most common genetic variants at the kappa casein gene locus were determined from the allele frequencies of the three SNPs CSN3_136, CSN3_148 and CSN3_155 assayed. 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 MassARRAYTyper software analysed the allele peak intensities and generated cluster plots that showed genotype calls at the SNPs CSN3_136, CSN3_148 and CSN3_155 locus of the 30 Friesian X Bunaji cows. The results of this study indicated that the SNPs CSN3_136 locus was polymorphic and had two alleles C and T with frequencies 0.75 and 0.25 respectively that determined the genetic variants A and B respectively. In addition, three genotypes CC (16 cows), CT (13 cows) and TT (1 cow) were determined from the allele's peak intensities corresponding to genetic variants AA, AB, and BB respectively. Next, the SNPs CSN3_148 locus was also polymorphic and had two alleles A and C with frequencies 0.75 and 0.25 respectively that determined the genetic variants A and B respectively. Besides that, three genotypes AA (16 cows), AC (13 cows) and CC (1 cow) were determined from the allele's peak intensities corresponding to genetic variants AA, AB, and BB respectively. From the two-point mutation of SNPs CSN3_136 and CSN3_148 the genetic variants A and B at the CSN3 gene locus were determined. Furthermore, the SNPs CSN3_155 locus was found to be monomorphic and had one allele A with frequencies 1.00 that determined the genetic variants A; the G allele that determine genetic variants E was totally absent; in addition, only one genotypes AA (30 cows) was found here corresponding to genetic variants A. These results support the previous research that the differences between variants κ-CN B and A are two-point mutations at amino acid positions 136 and 148 where variant A displays Threonine (A**C**C) and Aspartic acid (G**A**T) at positions 136 and 148, respectively, while the B variant displays isoleucine (A**T**C) and Alanine (G**C**T) at the same positions; κ-CN E varies from the A variant at position 155 where glycine substitutes serine (Caroli *et al*., 2009; Martin *et al*., 2013; Alim *et al*., 2014; Awad *et al*., 2016).

Accordingly, at the CSN3 gene locus two genetic variants A and B were detected with frequencies 75.0 and 25.0 percent respectively; the allele A occurred more frequent than B. In addition, three genotypes identified at the CSN3 gene locus were AA (53.3 percent), AB (43.3 percent) and BB (3.3 percent) (see Table 2). The AA genotype was the most common followed closely by the AB and the BB occurred the least. The results of this study support previous research that have shown that apart from the Jersey breed the variant *κ-CN* A is the most common among dairy cattle breeds and their crosses (Awad *et al*., 2016; Neamt *et al*., 2017; Houaga *et al*., 2020). Similar results were recorded in Simmental and Holstein crossbred cattle (Trakovicka *et al*., 2012), in Friesian x Sahiwal cattle (Deb *et al*., 2014), Holstein Friesian cows (Adamov *et al*., 2020), and in White Fulani, N'dama and Muturu breed (Olanrewaju *et al*., 2018). On the contrary, some investigators reported that the B variant at the *CSN3* gene locus occurred most frequent than the A as in Holstein cows (Soyudal *et al*., 2018), and in Jersey cattle (Ren *et al*., 2011; Zepeda-Batista *et al*., 2015). Similarly, Hamza *et al*. (2010) studied the polymorphisms of κ–casein gene at exon IV and found that the T allele that represents variant B was more frequent in the population (0.60) than the C allele that represents variant A (0.441).

The result of chi-square test ($\chi 2$) was non-significant (0.92) and indicated that the Friesian X Bunaji cows were in HW equilibrium at the *CSN3* gene locus in Friesian X Bunaji cows.

3.2. Summary statistics for the random and fixed effects

Table 3, shows the means and variance component estimates (for random effects-cow and residual error) for the milk yield, milk pH and milk composition traits and significant of fixed effect (kappa casein genotypes) in Model 1.

Table 3. Means and variance (σ^2) estimates of random effects and significance of fixed effects (*k-CN*) included in the analysis for milk traits in Friesian X Bunaji cows (model 1)

Variable	N		σ^2 estimates		P-value
		Mean	Cow	Residual	*k-CN*
Average Daily Milk Yield (kg)	29	7.52±2.49	1.3862	0.8075	0.002**
Fat (%)	29	4.48±1.43	0.0000	0.6500	0.01 **
Protein (%)	29	3.23 ±0.23	0.0542	1.1400	0.83 NS
Lactose (%)	29	4.86 ±1.43	0.0000	0.1089	0.80 NS
Solid-not fat (%)	29	8.78±0.78	0.6448	0.4000	0.79NS
Total solid (%)	29	13.23±1.58	1.5322	0.0034	0.05 *
Salts (%)	29	0.73 ± 0.05	1.4600	0.0028	0.80NS
Milk-pH	29	6.67 ± 0.08	0.0001	0.0075	0.31 NS
Density	29	29.58±2.53	2.6180	0.8820	0.19 NS
Freezing point	29	-0.56 ± 0.03	2.5640	0.0028	0.74 NS
Electrical conductivity	29	4.74± 0.36	0.0070	0.0830	0.03 *

k-CN= kappa Casein; *= *k-CN*is significant at $P \leq 0.05$; **= *k-CN*is significant at $P \leq 0.01$; NS=*k-CN*is not significant at $P > 0.05$.

The results of this study indicated that the residual variance estimated within the cow were higher than the variation between cows (within fixed effects of the mode l) in contents of milk fat, protein, lactose, conductivity, and 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 average daily milk yield, contents of milk solid-not fat, total solid, salts, density, and freezing point. Additionally, there was zero cow variance component estimate for contents fat and lactose in milk. These results agree with the previous findings by Kettoet *al*. (2017)who found similar trends for milk pH, percentages of milk fat, protein, casein, and lactose; the cow had zero variance estimate for contents of fat and lactose. The current result suggests that larger variance (the residual variance estimated within the cow) indicate large variation from each other and from the mean; while small variance, indicate the opposite; a variance value of zero for average fat and lactose shows that all the values are identical.

The means recorded in this study (Table 3) were in accordance with previous reports [Alphonsus *et al*., 2010] and met the recommended standard conditions for cow's milk components [Anantakrishnan *et al.*, 1993].

3.3. Effects of kappa casein (*CSN3*) genotypes on milk yield, pH, and composition traits.

Table 4, represents the least square means and standard deviation of daily milk yield, milk pH, density, freezing point and conductivity; contents of milk fat, protein, lactose, solid-not fat, total solid, and salts for kappa casein genotypes (AA and AB) in Friesian X Bunaji cows.

Table 4. Effects of Kappa Casein genotypes on milk traits in Friesian X Bunaji cows

	Kappa Casein Genotypes		
Variable	AA	AB	P-value
No of cows	16	13	
Average Daily Milk Yield (kg)	8.81 ± 0.51^{a}	6.24 ± 0.57^{b}	0.002**
Fat (%)	4.15 ± 0.30^{b}	5.14 ± 0.35^{a}	0.01**
Protein (%)	3.21±0.10	3.23±0.11	0.83 NS
Lactose (%)	4.83±0.14	4.89±0.16	0.80 NS
Solid-not fat (%)	8.45±0.32	8.54±0.36	0.79 NS
Total solid (%)	12.90 ± 0.54^{b}	14.06 ± 0.61^{a}	0.05 *
Salts (%)	0.72±0.02	0.73±0.02	0.80 NS
Milk pH	6.69±0.04	6.65±0.04	0.31 NS
Density	27.99 ±1.06	29.26 ± 1.21	0.19 NS
Freezing point	-0.57±0.02	-0.57±0.02	0.73 NS
Electrical conductivity	4.34 ± 0.12^{b}	4.75 ± 0.13^{a}	0.02 *

*= *k-CN*genotypes are significant at $P \leq 0.05$; **= *k-CN*genotypes are significant at $P \leq 0.01$; NS=*k-CN*genotypes are not significant at $P > 0.05$; ab= Means with different superscript across roll differ significantly.

The results of the present study, indicated that the *k-Casein* genotypes (AA and AB) significantly affected daily milk yield (P=0.002), contents of milk fat (P=0.01), total solid (P=0.05) and electrical conductivity of milk (P= 0.02) but there had non-significant impacts on milk pH, density, freezing point as well as contents of protein, lactose, solid-not fat, and salts in milk. The cows carrying homozygous AA genotype produced a higher amount of milk per day (8.81 ± 0.51g/cow/day) than those carrying genotype AB. In addition, the cows carrying genotype AB produced milk with higher contents of fat, total solid and better conductivity than those carrying genotype AA. These results support the findings of previous researchers in White-backed cows (Barłowska *et al*., 2012), Holstein cows (Vidović *et al*., 2013), Slovak Pinzgau cattle (Miluchova *et al*., 2014), Holstein-Friesian and White-backed (Wolanciuk, 2015) and in Romanian Simmental cows (Neamt *et al*., 2017). On the contrary, the results of this study disagree with the reports of previous researchers who found that at the *CSN3* gene locus, the genotypes AA, AB, and BB had no significant effect on milk components (Strzalkowska *et al*. (2002)) and milk yield (Petrovska *et al*., 2017).

IV. CONCLUSION

The results of this study revealed the following conclusions:

In the Friesian X Bunaji cows the most common genetic variant at the kappa casein gene locus is A and the most frequent genotype is AA followed by AB while the BB genotype is the least occurred; cows carrying the AA genotype produced the highest amount of milk while those carrying the AB genotype produced milk with the highest amount of fat, total solid and have the better conductivity. It may be concluded that the kappa casein gene can be a candidate gene for selection to improve milk production traits in Friesian X Bunaji cows.

V. RECOMMENDATION

Considering that the milk from this farm is mostly used for yoghurt production, we recommend that the effects of the kappa casein genes on yoghurt parameters be investigated.

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