EFFECTS OF LACTOFERRIN SNP GENOTYPE AND BREED ON MILK AND BIOMETRIC TRAITS OF BUNAJI AND SOKOTO GUDALI COWS

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ABSTRACT
This study was conducted on a total of 87 cows of two major Nigerian breeds (Bunaji and Sokoto Gudali) with the aim to identify single nucleotide polymorphisms (SNPs) at Lactoferrin (LTF) locus and to study association between the obtained LTF genotypes and milk yield, milk protein, milk fat, milk lactose, height at withers, body length, chest circumference, udder length and udder circumference. Polymorphism of bovine lactoferrin locus was determined by using restriction fragment length polymorphism (PCR-RFLP). Lactoferrin gene showed varied levels of polymorphism among Bunaji and Sokoto Gudali cattle. Three genotypes were identified (AA, AB and BB) in both cattle breeds. The mean analysis showed varying interaction effects of SNP genotype and breed on milk and biometric traits, with genotype AA of the Sokoto Gudali exhibiting distinct superiority. Variation at the LTF locus may prove to be important in possible selection and breeding for genetic improvement in milk quality and yield in the tropics. Therefore, the SNP genotype AA of the Sokoto Gudali be further investigated to exploit its full potential in milk yield and biometric advantage.
Keywords: Polymorphism, Milk traits, Morphometrics, Cattle, Nigeria

INTRODUCTION
Genetic polymorphisms of candidate genes have evoked considerable research interest in recent years because of possible association between their genotypes and important economic traits (Raschia *et al*., 2018; Čítek *et al*., 2022). Lactoferrin (Lf) is an iron-binding glycoprotein that is closely related structurally to transferrin (Tf) which is an iron-transport protein in the plasma which was discovered in 1939 as the 'red protein from milk' (Wojdak-Maksymiec *et al*., 2006). Nowadays, there is abundant evidence of the importance of lactoferrin and other 14 candidate genes in tracking the distribution of certain genotypes of several cow breeds in different geographical regions. The identification of variants which provide important tools for improving the yield and quality of milk and dairy production and association of single nucleotide polymorphisms (SNPs) with milk production parameters is desirable. This information is scarce in Nigerian indigenous breeds of cattle with respect to Lactoferrin gene. Therefore, this study aimed at determining the Lactoferrin SNP genotype and breed effects on milk parameters and biometric traits of Bunaji and Sokoto Gudali Cows in Nigeria.

MATERIALS AND METHODS
A total of eighty-seven (87) cows (43 of Bunaji and 44 of Sokoto Gudali genetic groups) in three lactation stages were randomly sampled in Zamfara and Kaduna States. A single milk sample from each animal included in the experiment was taken (from all four quarters) twice in a day (Morning and evening) manually. Height at withers (HW), body length (BL), chest circumference (CC), udder length (UL) and udder circumference (UC) of the animals were measured using standard procedure.
Isolation of genomic DNA from blood samples collected was done using the ZymoBead™ Genomic DNA kit (Zymo Research Corp. Irvine, CA, USA) at the Centre for Advanced Medical Research and Training, Usmanu Danfodio University Teaching Hospital, Sokoto. A 301-bp fragment of lactoferrin gene (intron 6) was amplified for polymorphism identification. Primers designed were identical to those described by Woydac-Maksimiec *et al.,* (2006). The forward and reverse primer sequences explored were LAC-FW: 5'-GCC TCA TGA CAA CTC CCA CAC-3' and LAC RV: 5'-CAG GTT GAC ACA TCG GTT GAC -3'. PCR amplification was done in a Kyratech Supercycler Trinity PCR machine. PCR cycling conditions were; initial denaturation at 95°C for 5 minutes, denaturation occurred at 95°C degrees for a period of 30 seconds, annealing then followed at 55 °C for 30 seconds, then extension at a temperature of 72 degrees for 1-minute, final elongation at 72 degrees for 5 minutes. Single nucleotide polymorphisms (SNPs) were detected in lactoferrin gene with the use of CodonCode Aligner software

and MEGA version 6 as described by Tamura *et al*. (2011). The restriction fragment length polymorphism (RFLP) analysis was done based on the identified SNPs and the associated enzymes. Analysis of data was carried out using the general linear model of R (2015) statistical software to test the fixed effects of SNP genotype, breed, lactation stage as well as their interactions on milk yield, milk protein, milk fat, milk lactose, somatic cell counts, height at withers, body length, chest circumference, udder length and udder circumference

RESULTS

The two alleles observed were A and B. The AA, AB, and BB genotype frequencies were 0.9, 0.00, and 0.06 (Bunaji); and 0.79, 0.12, and 0.09 (Sokoto Gudali). The respective A and B gene frequencies were 0.94 and 0.06 (Bunaji), and 0.85 and 0.15 (Sokoto Gudali) (Table 1).

Table 1. Distribution of lactoferrin genotypes and gene frequencies in Bunaji and Sokoto Gudali cows

		Genotype frequency			Gene frequency	
Breed	**No**	**AA**	**AB**	**BB**	**A**	**B**
Bunaji	32	30 (0.94)	0 (0.00)	2 (0.06)	0.94	0.06
Sokoto Gudali	33	26 (0.79)	4 (0.12)	3 (0.09)	0.85	0.15
Total	65	56 (0.86)	4 (0.06)	5 (0.08)	0.89	0.11

The mean analysis showed varying interaction effects on milk and biometric traits (Table 2). Interaction effect of SNP genotype and breed was significant in the Sokoto Gudali in both genotypes AA and BB on Milk yield (Litres per day) and udder length ($P < 0.05$). Interaction effect on biometric traits showed genotype AA of the Sokoto Gudali breed having better withers height and chest circumference.

Table 2. SNP genotype and breed interaction effect on milk and biometric traits (Mean±S.E.) of cattle

	AA		BB	
Parameters	**Bunaji**	**Sokoto Gudali**	**Bunaji**	**Sokoto Gudali**
Milk traits				
Milk yield (Litres per day)	2.61±0.06^{b}	3.56±0.06^{a}	2.45±0.21^{b}	3.275±0.18^{a}
Lactose (g/100ml)	10.86±0.44^{a}	11.63±0.43^{a}	12.65±1.55^{a}	12.50±1.34^{a}
Protein (g/100ml)	31.26±0.82^{a}	32.33±0.80^{a}	31.50±2.88^{a}	33.00±2.49^{a}
Triglycerides (g/100ml)	4.63±0.28^{a}	4.58±0.27^{a}	4.95±0.98^{a}	4.40±0.85^{a}
Biometric traits				
Withers height (cm)	123.12±1.05^{b}	129.27±1.02^{a}	120.00±3.66^{a}	128.00±3.17^{a}
Chest circumference (cm)	155.27±1.86^{b}	169.10±1.80^{a}	160.00±6.49^{a}	171.00±5.62^{a}
Body length (cm)	135.76±3.25^{a}	142.06±3.16^{a}	128.00±11.39^{a}	122.25±9.86^{a}
Udder length (cm)	14.35±0.38^{b}	17.82±0.37^{a}	14.75±1.32^{b}	19.00±1.15^{a}
Udder circumference	24.46±0.62^{a}	26.18±0.60^{a}	23.00±2.18^{a}	25.75±1.88^{a}

^{ab}Means within rows carrying different superscripts differ significantly ($P < 0.05$)

DISCUSSION

It is very important to unravel the genetic traits of Bunaji and Sokoto Gudali cattle, pinpoint genes advantageous for milk traits and muscle growth, and provide essential genomic data to support further analysis of genetic mechanisms tied to economic traits and the preservation of cattle breed genetic diversity. The AA, AB, and BB genotype frequencies obtained in the current study are in tandem with the findings of Seyfert and Kuhn (1994) who reported two alleles, A and B, in the Lactoferrin locus, with three realizable genotypes resolved: AA, AB, and BB. The alleles frequencies were, however, different with 0.755 and 0.245 for A and B, respectively. Also, Wojdak-Maksymiec *et al.* (2006) reported two alleles, A and B, in their work on Polish White and Black dairy cattle, with different frequencies of 67.74 % and 32.56

%, respectively. The interaction effect revealed separate ranking of each parameter under the SNP genotype and breed of cattle.

CONCLUSION

The occurrence and distribution of genotypes AA, BB, and AB in the study revealed the existence of polymorphism at intron 6 of the Lactoferrin gene locus of Bunaji and Sokoto Gudali breeds of cattle in Nigeria. Genotype AA of the Sokoto Gudali breed exhibited better withers height and chest circumference. The present information provides a guide to subsequent comprehensive genomic studies geared towards the management and conservation strategies for these extant cattle populations. This might constitute a first step towards the design of selection programs aimed at increasing profitability of dairy operations by improving milk and morphometric traits performance.

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