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MILK GENE EXPRESSION IN DIFFERENT TISSUES OF WHITE FULANI AND SOKOTO GUDALI CATTLE BREEDS IN NIGERIA

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ABSTRACT: *Milk production and composition are influenced by genetic factors, yet limited research has explored breed-specific differences in milk gene expression among indigenous Nigerian cattle. Understanding these variations is essential for improving milk quality and optimising dairy breeding strategies. This study investigated the expression of milk genes in different tissues of White Fulani and Sokoto Gudali cows. A total of 100 lactating cows (50 White Fulani and 50 Sokoto Gudali) in their second parity and mid-lactation (120–210 days in milk) were sampled from five locations in Oyo State and one in Ondo State. Milk samples were preserved at 3–4°C and analysed. Total RNA was extracted, treated with DNase, and reverse-transcribed into cDNA. Gene expression was assessed using qPCR, with GAPDH and β-Actin as reference genes. The experimental design followed a factorial arrangement. Results showed significant ($p<0.05$) variations in gene expression between breeds and tissues. Alpha Casein was highly expressed in milk (36.50 ± 0.29) and heart (34.58 ± 0.31) of White Fulani, while Sokoto Gudali exhibited higher expression in the kidney (32.56 ± 0.29) and mammary gland (34.65 ± 0.33). Leptin expression was higher in White Fulani milk (37.57 ± 0.30) but greater in Sokoto Gudali mammary gland (33.12 ± 0.12). These findings provided insights into breed-specific gene expression patterns, which may influence milk composition and quality improvement strategies.*

KEYWORDS: Gene expression, Milk composition, White Fulani, Sokoto Gudali, qPCR, Breeds.

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INTRODUCTION

The quantity and quality of milk available for human consumption from ruminant breeds remain a global concern due to the direct relationship between food composition and human health (Rafiq *et al*., 2016). Milk is widely regarded as a complete food for both humans and animals, playing an essential role in nutrition and the development of healthy societies. It serves as a crucial tool for rural development, especially in regions where diets are predominantly cereal based (Rafiq *et al*., 2015; Zhou *et al*., 2018).

Nutritionally, milk is often described as "the most nearly perfect food" (Alade *et al*., 2013), because it supplies essential nutrients in more significant amounts than most single foods. It is vital for reproduction, growth, maintenance, energy supply, tissue repair, and appetite satisfaction (Dandare *et al*., 2014; Fasae & Olusesan, 2015). Milk production is a cornerstone of global agriculture, contributing substantially to food security and nutrition. The ability of cattle to produce milk is shaped by a complex interaction of genetic, physiological, and environmental factors, with genetic composition playing a key role (Miglior *et al*., 2017).

Milk synthesis relies on the activity of several critical genes, such as those encoding casein proteins, alpha-lactalbumin, and prolactin. These genes are essential for mammary gland development, lactation, and milk composition (Osorio *et al*., 2016). Dairy production depends on cattle breeds with proven genetic capabilities. Milk production from cattle is highly dependent on the genetic capacity of the cow (Miglior *et al*., 2017). Standard dairy breeds were developed through decades of systematic selection for increased milk production among indigenous cattle populations in temperate regions (Olorunnisomo, 2017). This resulted in emergence of specialized dairy cattle with high milk yield and efficient reproductive capacity. Traits such as fast growth rate, early maturity, long lactation, regular calving interval and good milk-let down reflexes were incorporated into the genotypes (Gall, 2013).

Cow's milk is the most consumed and commercially important dairy product worldwide. In Nigeria, milk production from indigenous cattle breeds is a key component of the smallholder agricultural economy, providing significant financial, nutritional, and social benefits (Chagunda *et al*., 2016). Local cattle breeds account for approximately 90% of the country's total milk supply, with the White Fulani breed recognized as the leading milk producer (Fasae & Olusesan, 2015). Milk quality and composition are influenced by various factors, including milking techniques, lactation stage, season, environmental conditions, diet, feeding system, breed, and species (Dandare *et al*., 2014; Rafiq *et al*., 2016). Understanding these factors is crucial for enhancing milk production and quality in indigenous cattle breeds.

Cattle breeds vary significantly in their genetic potential for milk production due to evolutionary adaptations, selective breeding, and environmental influences. In Nigeria, two prominent cattle breeds, the White Fulani and Sokoto Gudali, play a vital role in livestock farming. The White Fulani breed, also known as Bunaji, is among the most widely distributed cattle breeds in West Africa, valued for its resilience to harsh climates and moderate milk production capacity (Oke *et al*., 2022). In contrast, the Sokoto Gudali breed, a taurine cattle type, is recognized for its strong body conformation, docile temperament, and dual-purpose suitability for both meat and milk production (Udeh, 2021). Despite the economic and nutritional importance of these breeds, there is limited research on the genetic factors that influence milk production in White Fulani and Sokoto Gudali cattle. Most studies on milk gene expression have focused on European dairy breeds such as Holstein Friesians and Jerseys, with

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minimal attention given to indigenous African breeds (Philipsson *et al*., 2011). The lack of data on gene expression patterns in these breeds represents a significant gap in understanding their genetic potential for milk production. Furthermore, milk-related gene expression is often studied exclusively in the mammary gland, neglecting the potential contributions of other tissues, such as the liver and adipose tissue, which are also critical to lactation biology (Qian & Zhao, 2014). This study aimed to investigate the expression of key milk-related genes across different tissues in White Fulani and Sokoto Gudali cattle breeds.

MATERIALS AND METHOD

Experimental site: Milk samples were collected from local farms in five Local Government Areas of Iseyin (7° 58' 0" North, 3° 36' 0" East) , Ibarapa (7° 32' 0" North, 3° 25' 0" East), Saki West,(8° 40' 0" North, 3° 23' 0" East) Atiba (7°33'28" North and 3°59'56" East) and Itesiwaju (8°12'25.74" North 3°31'49.04" East) in Oyo State and Akure (7° 15' 0" North, 5° 12' 0" East) region of Ondo State, Nigeria and transported on ice on 8th of February, 2018, which falls within the dry season in Nigeria. The cows were in their mild stage of lactation (120 -210 days in milk). The samples were packed in a controlled temperature container of 3°C to 4°C and extreme care was taken not to damage the cold chain. Laboratory analysis was carried out at the Biotechnology Laboratory of the Federal University of Technology, Akure (FUTA).

Experimental Animals: Fifty (50) White Fulani and fifty (50) Sokoto Gudali cows from different centres in the Southwest region of Nigeria were used for the experiment as stated above. The farmer was prior informed for their consent by the Friesland Campinas office in Iseyin. Most of the farms take their milk to Friesland Campinas collection centres. The animals weigh between 350 kg to 520 kg. They were all in second parity.

Sample Collection: Fresh milk samples were collected from the lactating cows in mild lactation and second parity into a 5ml tube collection bottle and preserved on ice to maintain cold chain while transporting.

Gene Expression Analysis: Total messenger RNA was extracted from the milk samples using Bioline® mini kit (USA) according to manufacturer's protocol. After extraction, the first DNase was treated with RQ1 RNase-Free DNase (Promega, USA) and heat inactivated according to manufacturer's protocol. Following RNA extraction, 12ul of total RNA was used to synthesize cDNA using SensiFast^{TM}cDNA Synthesis Kit, following manufacturer's instruction. The mixture was placed in a thermal cycler (Mastercycler$^{®}$ pro by Eppendorf, USA) programmed with the following reaction; 25^{0}C for 10 minutes, 42^{0}C for 15 minutes, 48^{0}C for 15 min, 85^{0}C for 5 minutes, and 4^{0}C to hold. Thereafter, the cDNA was kept in the freezer. qPCR assays were prepared and run in Roche Lightcycler^{R} 96 USA. The quantification cycle (cq) means were used as the criteria for gene expression analysis. Expression of the three milk genes (Alpha Casein, Alpha Lactalbumin and Leptin) were normalised by the mean value of the reference genes (GAPDH and β-Actin). Gene expression was calculated using the ΔCq method. The ΔCq-values were calculated as $\Delta Cq = meanCq^{targetgene} - meanCq^{refence\ gene}$. (Bustin, 2024). In order to avoid negative digits while allowing an estimation of a relative comparison between two time points, data are presented as ΔCq values subtracted from the arbitrary value 2 ($2 - \Delta Cq$). Thus, a high ΔCq-value resembles high transcript abundance. An increase of one ΔCq represents a two-fold increase of mRNA transcripts.

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Selected Genes and Primer Design: Forward and reverse primer sequences for the Alpha-Lactalbumin gene were designed based on the GenBank reference sequence (NC_032654.1) (available at http://www.ncbi.nlm.nih.gov). Primer sequences for other genes analyzed in this study, including Alpha-Casein, Leptin, Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH), and Beta-Actin (β-Actin), were obtained from relevant published literature, as presented in Table 1.

The experimental design

$Y_{ijk} = \mu + A_i + B_j + (AB)_{ij} + \varepsilon_{ijk}$

Where;

Y_{ijk} = Observed mean

μ = General mean

A_i = Effect of Milk genes

B_j = Effect of Breed

$(AB)_{ij}$ = Interaction between the Milk genes and breed effects.

ε_{ijk} = Experimental error

Data analysis: Data collected were subjected to analysis of variance (ANOVA) using General Linear Model of SAS, (2008). Means with significant differences were separated using Duncan Multiple Range Test (Duncan, 1955).

RESULTS

Expression of milk genes in different tissue samples of White Fulani and Sokoto Gudali

Expression of milk genes in different tissue samples of White Fulani and Sokoto Gudali is presented in Table 2. There was significance ($p<0.05$) in the expression of milk genes in White Fulani and Sokoto Gudali. Alpha casein was highly expressed in milk (36.50±0.29), heart (34.58±0.31) and liver (29.34±0.29) while it was highly expressed in kidney (32.56±0.29) and mammary gland (34.65±0.33) of Sokoto Gudali. However, the interactive effect shows significant ($p<0.05$) differences across all tissues evaluated. The mammary gland exhibited the highest expression (lowest Cq values) of α-casein in Sokoto Gudali (34.65 ± 0.33) compared to White Fulani (31.95 ± 0.05). Leptin expression in mammary gland was significantly lower in White Fulani (26.59 ± 0.30) compared to Sokoto Gudali (33.12 ± 0.12). α-Lactalbumin expression in the mammary gland was relatively similar between the breeds, with Sokoto Gudali (32.57 ± 0.30c) showing slightly higher Cq values than White Fulani (31.43 ± 0.29**)**. Leptin was highly expressed (lowest Cq) in the kidney (27.03 ± 0.03) and liver (27.36 ± 0.32) of White Fulani, while in Sokoto Gudali, it was slightly lower (29.31 ± 0.31, 27.56 ± 0.30). α-Lactalbumin showed the highest expression in the kidney of White Fulani (35.78 ± 3.22) and was lower in Sokoto Gudali (30.47 ± 0.29). The heart tissue exhibited significantly higher α-casein expression in White Fulani (34.58 ± 0.31) than in Sokoto Gudali (31.37 ± 0.31). Leptin

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expression in milk was higher in White Fulani (37.57 ± 0.30a) compared to Sokoto Gudali (35.56 ± 0.29c). α-Lactalbumin expression in the heart and milk was lower in Sokoto Gudali than in White Fulani.

Figures 1 and 2 show gene expression in milk and tissues of White Fulani and Sokoto Gudali. Leptin cq value in milk and tissue of Sokoto Gudali had higher cq value of Leptin in kidney, liver and mammalian gland (29.94, 27.69 and 33.36 respectively) White Fulani had higher cq value of leptin in milk and heart (37.50 and 32.74 respectively). The comparative result of Cq values for Alpha casein in White Fulani and Sokoto Gudali from milk, heart, kidney, liver and mammary gland shows that higher cq values were recorded in milk and heart in White Fulani(36.5 and 34.74 respectively). While higher cq values were recorded in kidney, liver and heart in Sokoto Gudali(29.4, 29.03 and 31.86 respectively). Also, the same trend was observed for alpha Lactalbumin where cq value of alpha lactalbumin was better in milk and heart for White Fulani (33.5 and 32.68 respectively). Sokoto Gudali had higher expression of alpha lactalbumin in kidney, liver and mammalian gland (30.4, 29.59 and 32.71 respectively).

DISCUSSION

Milk protein gene expression has a pivotal effect on milk protein composition (Osorio *et al*., 2016). The expression of milk genes across different tissue samples of White Fulani and Sokoto Gudali cattle breeds showed significant ($p<0.05$) variations, aligning with previous studies on gene regulation in ruminants and this variation could be due to genetic make-up of the animal. Alshamiry and Abdelrahman (2020) reported that alpha-casein is predominantly expressed in the mammary gland, which is consistent with the findings in Sokoto Gudali cattle, where the mammary gland exhibited the highest expression of α-casein compared to White Fulani and this could be attributed to the genetic make-up of the cows. Lactalbumin is an important gene controlling embryonic development and is also known as a bone morphogenic protein (BMP) antagonist, functioning to prevent differentiation of mammary epithelial cells (Jena *et al*., 2023). Similarly, Barnard *et al*. (2024) highlighted the role of α-lactalbumin in lactose synthesis, which was reflected in the higher α-lactalbumin expression in the mammary gland of Sokoto Gudali than White Fulani. Proportions of α-lactalbumin, which is involved in milk lactose synthesis, decreased with progress in lactation as a result of lowering milk yields (Gazi *et al*., 2024).

White Fulani had superior genes (Alpha Casein, Leptin and Alpha Lactalbumin) expressed in kidney, liver and mammalian gland. Therefore, some of the variations observed in gene expression may reflect the activity of a subgroup of cells, such as lactocytes or myoepithelial cells, whereas other variations may reflect the proportion of each cell type. White Fulani is widely known for its milk production, expression of alpha lactalbumin in milk of this lactating animal was low compared to Sokoto Gudali. Leptin expression differed significantly between breeds, with White Fulani exhibiting higher leptin levels in the liver and kidney, similar to findings by Vaidya *et al*. (2023), who suggested that leptin plays a vital role in energy metabolism during lactation. However, leptin expression in the mammary gland was significantly lower in White Fulani (26.59 ± 0.30) compared to Sokoto Gudali (33.12 ± 0.12), corroborating the observations of Haruna *et al*. (2021) that breed differences influence leptin's regulatory role in milk secretion. During lactation, leptin levels were usually lower than or similar to those observed during end of pregnancy (Childs *et al.*, 2021). Therefore, variation in

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leptin between Sokoto Gudali and White Fulani different tissues could be due to different genetic make-up. Leptin has been implicated in numerous roles, including modulation of reproduction, endocrine system, tissue metabolism, blood pressure, hematopoiesis, angiogenesis, brain and bone development, wound healing, and cell differentiation and proliferation (Casado *et al*., 2023).

Additionally, White Fulani had higher α-casein expression in milk (36.50 ± 0.29) and heart (34.58 ± 0.31), whereas Sokoto Gudali recorded higher values in the kidney (32.56 ± 0.29) and liver (29.34 ± 0.29), reinforcing the conclusions of Ledesma-Martínez *et al*. (2019) that casein expression extends beyond the mammary gland. The higher α-lactalbumin expression in White Fulanis heart (32.68 ± 1.11) and milk (33.52 ± 0.29) suggests a breed-specific adaptation for lactose synthesis, as previously noted by Ruvinskiy *et al*. (2024). Overall, the comparative analysis indicates that Sokoto Gudali may have greater potential for milk protein synthesis, while White Fulani demonstrates a stronger metabolic role in energy regulation. These findings of this study align with FAO (2019), which emphasized the importance of genetic diversity in dairy cattle selection.

CONCLUSION

This study demonstrated significant differences in the expression of milk genes between White Fulani and Sokoto Gudali cattle, highlighting breed-specific variations in lactation potential. Sokoto Gudali showed higher expression of α-casein and α-lactalbumin in the mammary gland, suggesting a stronger genetic predisposition for milk protein synthesis. White Fulani exhibited higher leptin expression in metabolic tissues, indicating a greater role in energy regulation during lactation. These findings provide insights that can be utilized for selective breeding programs aimed at improving milk production.

COMPETING INTERESTS

Authors have reported no competing interest exist

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APPENDIX

Table 1: Primer Sequences Used for the Study

Gene	Sequences (5'-3')	Tm (^{0}C)
Alpha Casein	F – TGCATGTTCTCATAATAACC	60
	R – GAAGAAGCAGCAAGC TGG	
Alpha Lactalbumin	F – CAGTCCTTTCGTCCCAGCAC	60
	R – GGACATCGAGCAAGGGTCAA	
Leptin	F – GGGCACGTCAGCATCTATTA	60
	R – CCTGTCTGCTGTTATGGTCTTA	
GAPDH	F – GAAGACTGTGGATGGCCCCTCC	60
	R – GTTGAGGGCAATGCCAGCCCC	
β-Actin	F – ACCGTGAGAAGATGACCCAG	60
	R – AGGAAGGAAGGCTGGAAGAG	

Source: Sigl *et al*. (2012)

Table 2: Expression of Milk Genes (Cq) in Different Tissue Samples of White Fulani and Sokoto Gudali

		Milk	Heart	Kidney	Liver	Mammary Gland
Breed	White Fulani	35.86±0.26^a	33.25±1.20^a	30.77±1.91	28.24±1.05	29.99±1.27^b
	Sokoto Gudali	34.56±0.26^b	29.50±1.20^b	30.78±1.91	28.65±1.05	33.45±1.27^a
Milk Gene	α-casein	35.78±0.39^b	32.98±1.81^a	31.04±2.88^b	29.27±1.57^a	33.30±1.91^a
	Leptin	36.57±0.27^a	30.55±1.28^b	28.17±2.04^c	27.46±1.11^c	29.86±1.35^b
	α-Lactalbumin	33.30±0.27^c	30.60±1.28^b	33.13±2.04^a	28.61±1.11^b	32.00±1.35^a
Breed x Milk Gene						
White Fulani	α-casein	36.50±0.29^b	34.58±0.31^a	29.51±0.29^c	29.34±0.29^a	31.95±0.05^c
	Leptin	37.57±0.30^a	32.62±0.31^b	27.03±0.03^d	27.36±0.32^c	26.59±0.30^e
	α-Lactalbumin	33.52±0.29^d	32.56±0.29^b	35.78±3.22^a	28.01±0.01^b	31.43±0.29^d
Sokoto Gudali	α-casein	35.06±0.06^c	31.37±0.31^c	32.56±0.29^a	29.20±0.20^a	34.65±0.33^a
	Leptin	35.56±0.29^c	28.48±0.29^d	29.31±0.31^c	27.56±0.30^c	33.12±0.12^b
	α-Lactalbumin	33.07±0.07^d	28.64±0.32^d	30.47±0.29^b	29.20±0.20^a	32.57±0.30^c

^{a,b,c,d,e} – Means on the same column having different superscripts are statistically ($p<0.05$) significant.

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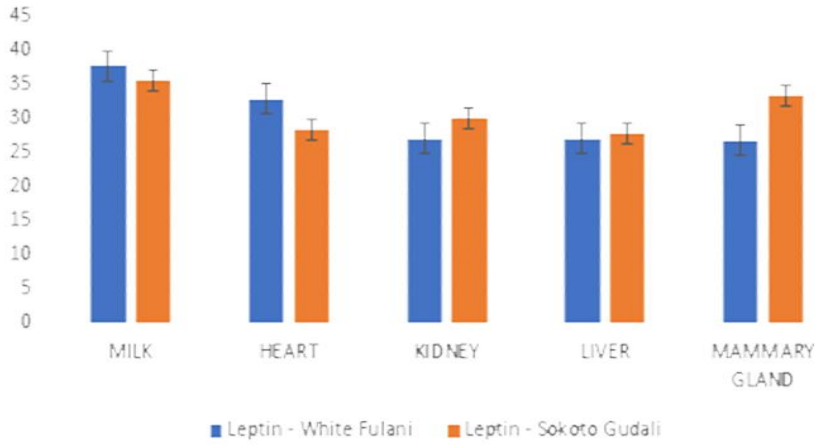

Figure 1: Leptin expression in milk and tissue of White Fulani and Sokoto Gudali

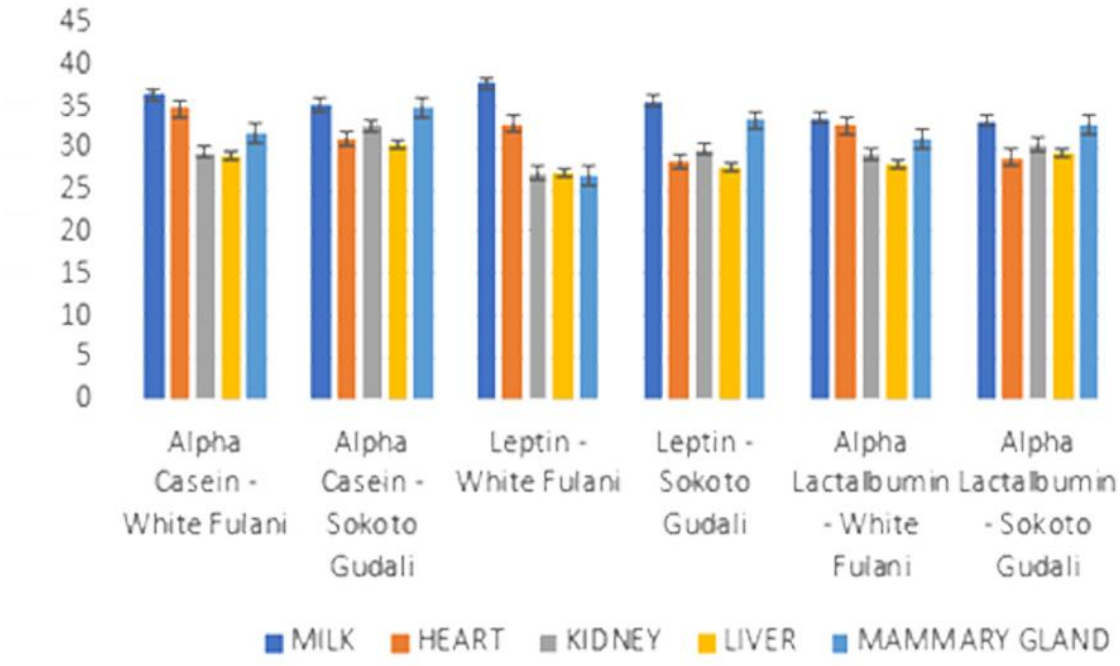

Figure 2: Expressions of Alpha Casein, Leptin and Alpha Lactalbumin in White Fulani and Sokoto Gudali Breeds of Cattle

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