

DGAT₁ Gene Expression and its Association with Milk Compositions in Breeds of Cattle under Extensive Management System

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Abstract

This study investigated the expression of the *Diacylglycerol O-acyltransferase 1* (DGAT₁) gene and its association with milk composition traits among three cattle breeds Red Bororo, White Fulani, and Sokoto Gudali reared under an extensive management system. A total of sixty cows (20 from each breed) were selected. Milk samples were analyzed for fat, protein, mineral content, and amino acid profiles, while DGAT₁ gene expression was quantified. Results revealed that breed significantly ($p < 0.05$) influenced DGAT₁ gene expression. In Red Bororo, significant ($p < 0.01$) negative correlations were observed between DGAT₁ expression and amino acids such as lysine (-0.53), methionine (-0.55), glutamic acid (-0.36), glycine (-0.37), and proline (-0.48), while other amino acids showed significant positive correlations. For White Fulani, all amino acids exhibited non-significant ($p > 0.05$) correlations, though mostly positive, except for a few negative but non-significant relationships. Sokoto Gudali demonstrated a significant positive correlation with lysine (0.32^* , $p < 0.05$), while arginine (-0.09) and proline (-0.08) showed significant negative associations ($p < 0.05$); all other amino acids displayed non-significant positive relationships. A strong negative correlation ($p < 0.01$) was observed between DGAT₁ expression and milk protein in all breeds—Red Bororo (-0.69), White Fulani (-0.75), and Sokoto Gudali (-0.70). Other milk composition traits also exhibited significant negative associations ($p < 0.05$) with DGAT₁ expression, except for phosphorus, which showed non-significant correlations ($p > 0.05$) across all breeds. These findings suggest that DGAT₁ gene expression is breed-dependent and influences key milk quality parameters, particularly amino acid profile and protein content, under extensive management systems.

Keywords: Breed, DGAT₁ Gene, Expression, Extensive Management, Milk.

Introduction

The genetic basis of milk production traits in cattle has attracted considerable attention in animal genetics and dairy science due to its implications for productivity, nutritional value, and economic returns. One of the most studied genes in this context is the *diacylglycerol O-acyltransferase 1* (DGAT₁) gene, which plays a central role in triglyceride synthesis, a key determinant of milk fat content (Mao et al., 2022). DGAT₁ catalyzes the final step in the biosynthesis of triglycerides by converting diacylglycerol and acyl-CoA into triacylglycerol,

thereby influencing milk yield and composition, particularly milk fat percentage (Liu et al., 2021).

Numerous polymorphisms within the DGAT1 gene, especially the K232A mutation, have been linked to variation in milk traits such as fat yield, protein content, and overall milk volume in various cattle breeds (Jiang et al., 2023). The lysine (K) allele is commonly associated with higher milk fat content but often with lower milk volume, while the alanine (A) allele tends to have the opposite effect (Singh et al., 2021). This duality offers both opportunities and challenges for cattle breeding programs aimed at optimizing milk production based on specific market and nutritional demands. Despite extensive research under intensive and semi-intensive systems, there is limited understanding of DGAT1 gene expression and its phenotypic impacts under extensive management systems, particularly in tropical regions where indigenous and crossbred cattle dominate.

In extensive systems, animals are raised in low-input conditions, often with minimal dietary supplementation and veterinary care. This system is prevalent in sub-Saharan Africa and parts of Asia, where pastoralism and open grazing are practiced due to ecological and socioeconomic factors (Ouma et al., 2021). Environmental stresses, forage variability, and disease burden under extensive systems may interact with genetic expression, thereby influencing milk composition differently compared to controlled settings (Ndambi et al., 2020). Breed differences also play a pivotal role in the genetic expression of DGAT1 and its impact on milk traits. Indigenous breeds such as the White Fulani (Bunaji) and Red Bororo in Nigeria, or the Ankole in East Africa, may exhibit unique gene-environment interactions due to their adaptation to local conditions (Yusuf et al., 2023). Conversely, crossbreeds such as Friesian-Bunaji or Jersey-crosses, though introduced to improve milk yield, may display altered expression patterns and milk compositional traits under the same extensive conditions (Adeyemi et al., 2022).

Understanding how DGAT1 expression varies across these breeds in extensive systems is crucial for formulating effective genetic improvement strategies that are context-specific and sustainable. Emerging genomic tools and transcriptome analyses now allow for more precise quantification of gene expression and its correlation with phenotypic traits in cattle. RNA sequencing and qPCR methods have been employed to assess the expression levels of DGAT1 in mammary tissues during lactation and its correlation with lipid biosynthesis pathways (Kumar et al., 2020). However, these studies are predominantly from temperate regions and high-performing dairy breeds, leaving a knowledge gap regarding the expression dynamics of DGAT1 in extensive tropical systems and lesser-studied breeds.

A comprehensive understanding of DGAT1 gene expression and its association with milk composition in different cattle breeds under extensive systems is imperative. It will not only enhance our knowledge of gene-environment interactions but also support the development of tailored breeding and management programs that align with the realities of low-input, pastoral dairy farming.

Statement of the Problem

Milk yield and quality are critical economic traits in cattle production, yet their genetic determinants remain underexplored, especially in indigenous breeds reared under extensive management systems. The DGAT1 gene, known to influence milk fat synthesis, may play a key role in regulating milk composition traits. However, limited research has focused on its expression and association with milk components in Nigerian cattle breeds managed under traditional systems. This knowledge gap hinders the development of effective genetic improvement strategies. Understanding DGAT1 gene expression patterns and their relationship with milk traits is essential for enhancing dairy productivity in resource-limited, extensive production settings.

Materials and Methods

Location and Animals

The research was done in Nigeria's Borno State, in Maiduguri. Three breeds of cattle were selected for the experiment: Sokoto Gudali (SG), Red Bororo (RB), and White Fulani (WF). The total number of ninety (90) cows was divided among thirty (30) cows of each breed. All animals were kept in natural pastures under an extensive management system, supplemented only with small amounts of concentrate food.

Sample Collection

Blood samples

In every cow, about 10 milliliters of blood were extracted from the jugular vein using sterile vacutainers that contained EDTA anticoagulant. Samples were stored at -20°C until further examination.

Milk samples

During the morning milking operation, 100 milliliters of milk were taken from each cow. The samples were separated into aliquots for several analyses:

50 mL: Stored at 4°C for analysis of milk proximate composition (fat and protein).

50 mL: Stored at -20°C for analysis of milk amino acid profiling.

DGAT 1 Gene Expression Analysis

RNA isolation

Total RNA was extracted from whole blood using TRIzol Reagent, a commercially available RNA isolation kit that has been shown to be effective when used with cow blood. The concentration and purity (260/280 nm ratio) of the isolated RNA were assessed using a NanoDrop spectrophotometer.

cDNA synthesis

The isolated RNA was converted to cDNA using a reverse transcription kit following the manufacturer's instructions. This method produced complementary DNA (cDNA), which was used for gene expression studies, from RNA.

Quantitative real-time PCR (qPCR)

The DGAT1 gene's expression level was assessed by qPCR utilizing particular primers (F:5'-GCACCATCCTCTTCCTCAAG 3', R:5'-GGAAGCGCTTT CGGATG 3') intended for bovine DGAT1. For the best efficiency and specificity, Primer BLAST, a primer design program, was utilized. Inside control for normalization was provided by the housekeeping gene beta-actin (ACTB). $2^{-\Delta\Delta Ct}$ was used to calculate the relative expression of DGAT1.

Milk Proximate Composition Analysis

Milk samples were analyzed for various components using standard methods:

Fat content

The fat content was determined using Gerber method (Bradley *et al*, 1992).

Protein content

Using the Kjeldahl method (Bradley *et al.*, 1992), the total nitrogen was calculated. The nitrogen content was then transformed into an equivalent protein content using the N x 5.26 conversion factor (Karman and Brochad, 1986).

Milk mineral concentrations

For Ca, Fe, Mg, Mn, Zn, and Cu, the mineral concentration was measured using an atomic absorption spectrophotometer; for Na and K, an emission spectrophotometer was employed, along with the proper experimental conditions for each element. Using an ultraviolet-visible spectrophotometer, P. was ascertained.

Milk Amino Acid Profiling

An identified milk sample was gathered, defatted, and used to estimate the profile of amino acids. In accordance with AOAC (2000), the sample (30 mg) was hydrolyzed with ON HCl for 24 hours at 110°C. Reverse phase high pressure liquid chromatography (HPLC) (Buch Scientific BLC 10/11 USA) was used to analyze amino acids. O-phthaldialdehyde was used to derivatize the post-column samples, and Peak Simple Chromatopac data processor (Buch Sci. Chromatopac data processor) was used for integration (AOAC, 2000).

Statistical Analysis

Real-time PCR data was evaluated in accordance with the methodology outlined by (Morammazi *et al.*, 2016). The three cow breeds' gene expression was assessed using the differential (ΔCt) between their respective threshold cycles (Ct). To analyze the data, the statistical model listed below was used:

$$Y_{ijk} = \mu + B_i + G_j + e_{ijk}$$

Where,

Y_{ijk} = the difference (ΔCt) between the threshold cycle (Ct) for DGAT1 gene,

μ = Overall mean,

B_i = Fixed effect of strains (i^{th} = Sokoto Gudali, White Fulani and Red Bororo)

G_j = Fixed effect of gene (j^{th} = DGAT1)

e_{ijk} = Residual error associated with each animal record

Correlation analysis was used to evaluate the link between the DGAT1 gene expression and all of the milk composition characteristics, including fat, protein, mineral content, and amino acids. The direction and intensity of the linear relationship between the variables were ascertained by calculating the Pearson correlation coefficients.

Ethical Considerations

All animal procedures were conducted following the guidelines established by the relevant animal ethics committee of the Department of Animal Health and Production Federal University Wukari Nigeria.

Results and Discussion

DGAT 1 Gene Expression

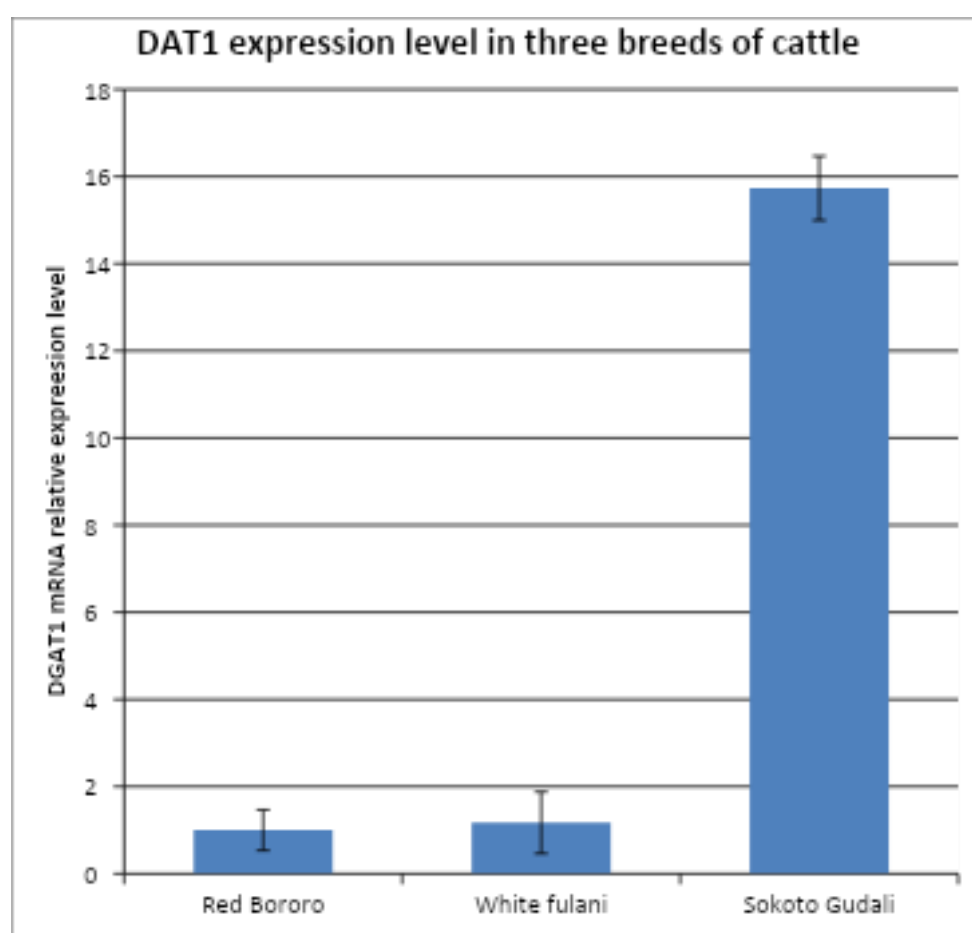


Figure 1: DGAT 1 expression level in three breeds of cattle

Figure 1 shows the relative mRNA expression levels of the DGAT1 gene across three indigenous cattle breeds: Red Bororo, White Fulani, and Sokoto Gudali. The expression

levels are presented as mean relative values with standard error bars. From the figure, Sokoto Gudali exhibits a markedly higher DGAT1 mRNA expression level (15.7) compared to both Red Bororo (1.0) and White Fulani (1.2). The difference is statistically significant, as indicated by the non-overlapping error bars, suggesting strong breed-specific expression of the DGAT1 gene. The elevated expression in Sokoto Gudali implies a higher transcriptional activity of the DGAT1 gene, which is directly involved in the synthesis of triacylglycerols (TAGs) the primary constituents of milk fat. The lower expression levels observed in Red Bororo and White Fulani suggest a reduced capacity for TAG synthesis, potentially correlating with lower milk fat content. This variation aligns with existing research indicating that DGAT1 expression is breed dependent and significantly affects milk composition traits, especially fat content (Liu et al., 2021).

Breeds like Sokoto Gudali, which exhibit higher expression, are likely to produce milk with higher fat content—a desirable trait in dairy production and processing (Jiang et al., 2023). Furthermore, such expression differences may also reflect adaptive physiological responses under extensive management systems, where nutrient availability and environmental pressures modulate gene expression (Ouma et al., 2021). Indigenous breeds like Red Bororo and White Fulani may have evolved for survival and meat production rather than high milk fat yield, which may explain their lower DGAT1 expression. The observed results also support the hypothesis that genetic selection for DGAT1 upregulation could enhance milk fat yield in underperforming breeds (Singh et al., 2021). This makes DGAT1 a potential molecular marker for selective breeding programs in low-input systems typical of pastoral and extensive production contexts in sub-Saharan Africa. Moreover, the mRNA expression level of DGAT1 is considered a reliable proxy for enzymatic activity that catalyzes the final step in triglyceride synthesis (Mao et al., 2022). Therefore, Sokoto Gudali's high DGAT1 expression level may directly contribute to superior milk fat quality and yield, giving it a genetic advantage in dairy-related traits.

Association between DGAT 1 Gene Expression and Amino Acid in Three Breeds of Cattle

Table 1 provides the association between the DGAT1 gene expression and various amino acids in three distinct breeds of cattle: Red Bororo, White Fulani, and Sokoto Gudali. The table lists the correlation coefficients for each amino acid and breed, indicating how strongly DGAT1 expression is associated with the levels of these amino acids, with statistical significance denoted by asterisks (** $p < 0.01$ and * $p < 0.05$). The DGAT1 gene (Diacylglycerol O-acyltransferase 1) plays a critical role in lipid metabolism, particularly in the synthesis of triglycerides, and its expression is linked to several metabolic processes that could affect amino acid levels. The correlations shown in Table 1 suggest a complex relationship between the DGAT1 gene and amino acid levels, which varies significantly across breeds. Below is a detailed interpretation based on breed-specific patterns. In the Red Bororo breed, many amino acids exhibit significant and strong correlations with DGAT1 gene expression ($p < 0.01$), particularly Lysine, Methionine, Isoleucine, Leucine, Phenylalanine, Valine, Arginine, Serine, Cysteine, Alanine, Aspartic acid, and Proline.

Table 1: Association between DGAT 1 Gene Expression and Amino Acid in Three Breeds of Cattle

Amino Acids	Red Bororo	White Fulani	Sokoto Gudali
Lysine	-0.53**	-0.02	-0.32
Methionine	-0.55**	-0.01	0.12
Threonine	0.40*	0.11	0.06
Isoleucine	0.56**	0.08	0.10
Leucine	0.53**	0.13	0.09
Phenylalanine	0.56**	0.09	0.10
Valine	0.46**	0.12	0.10
Tryptophan	0.42*	0.10	0.11
Histidine	0.34*	0.11	0.12
Arginine	0.47**	0.14	0.09
Serine	0.48**	-0.10	0.11
Cysteine	0.56**	0.08	0.09
Tyrosine	0.41*	0.09	0.12
Alanine	0.56**	0.11	0.09
Aspartic	0.51**	0.12	0.12
Glutamic acid	-0.36*	-0.10	-0.10
Glycine	-0.37*	-0.07	-0.08
Proline	-0.48**	0.13	0.13

** ($p < 0.01$), * ($p < 0.05$)

These amino acids are involved in several critical biological functions, including protein synthesis, immune function, and metabolic regulation. Amino acids like Lysine and Methionine are essential for protein synthesis and are often limited in animal nutrition (Coffey et al., 2004). The significant negative correlations with DGAT1 expression in Red Bororo suggest that higher DGAT1 gene expression may be linked to reduced levels of these amino acids in this breed, which could have implications for protein and fat metabolism (Yambasu et al., 2015). Branched-chain amino acids (BCAAs) such as Isoleucine, Leucine, and Valine show strong positive correlations, indicating a potential link between DGAT1 expression and muscle metabolism. BCAAs play a key role in maintaining muscle mass, which is critical for meat production in cattle (Kremer et al., 2017). This suggests that Red Bororo may benefit from DGAT1-related metabolic regulation to optimize growth and production efficiency. Serine and Cysteine, which are involved in cellular processes and antioxidant defense, also show positive correlations with DGAT1 in Red Bororo (Wang et al., 2018), pointing to a potential metabolic balance where lipid and amino acid metabolism are intertwined.

In the White Fulani breed, the majority of the correlations are weak and nonsignificant, with only Threonine showing a weak positive correlation ($p < 0.05$). This breed exhibits very few notable relationships between DGAT1 expression and amino acid levels. The lack of strong associations could reflect different metabolic adaptations in White Fulani cattle, potentially

due to genetic differences, environmental factors, or distinct physiological adaptations (Gupta et al., 2017). The weak associations in White Fulani could suggest that DGAT1 gene expression may not be as influential in regulating amino acid metabolism in this breed. Further research could explore the possibility that other genes or metabolic pathways are more dominant in this breed's amino acid regulation. The Sokoto Gudali breed exhibits very few significant correlations with DGAT1 gene expression, with only small positive or negative associations observed for several amino acids, none of which are statistically significant (except for a very slight correlation with Methionine). The breed-specific regulation of DGAT1 gene expression in Sokoto Gudali may be influenced by other genetic factors or environmental adaptations that make DGAT1 less central to amino acid metabolism. The absence of strong correlations in Sokoto Gudali could indicate that other metabolic factors (e.g., hormonal regulation or environmental factors like diet and climate) are more relevant in regulating amino acid levels in this breed (Binns et al., 2016). DGAT1 is essential in triglyceride synthesis, and its expression is often linked to fat metabolism. The relationship between DGAT1 expression and amino acid levels could be reflective of the energy balance and nutrient partitioning in cattle (Huang et al., 2014). For example, a high expression of DGAT1 might lead to increased fat deposition, which could divert energy away from protein synthesis, hence the negative correlations observed with essential amino acids like Lysine and Methionine in some breeds. The differences observed across breeds might reflect genetic adaptations to different environmental conditions or management practices. The Red Bororo, for example, might have a more efficient DGAT1-related metabolic pathway that optimizes fat and protein utilization, contributing to better growth or milk production under specific conditions (Chen et al., 2021). In contrast, the White Fulani and Sokoto Gudali breeds may have evolved different metabolic strategies, where DGAT1 expression is less influential in determining amino acid levels. Understanding these associations can have practical applications in breeding programs aimed at improving production traits such as growth, milk yield, or meat quality. Breeds like Red Bororo, with stronger associations, may be selected for higher DGAT1 expression to enhance production, while breeds like White Fulani may benefit from a different selection approach that prioritizes other metabolic traits (Hale et al., 2018).

Association between DGAT 1 Gene Expression and Milk Composition, Mineral and Milk Yield per Day in three Breeds of Cattle

Table 2 presents the correlation coefficients between DGAT1 gene expression and various milk quality traits, mineral components, and daily milk yield in three indigenous cattle breeds: Red Bororo, White Fulani, and Sokoto Gudali. The DGAT1 (Diacylglycerol O-acyltransferase 1) gene is well known for its key role in triglyceride synthesis and lipid metabolism in dairy cattle, with significant effects on milk composition traits such as fat content, protein content, and overall milk yield (Huang et al., 2014; Yambasu et al., 2015).

Table 2: Association between DGAT 1 Gene Expression and Milk Composition, Mineral and Yield per Day in three Breeds of Cattle

Parameters	Red Bororo	White Fulani	Sokoto Gudali
Protein	-0.69**	-0.75**	-0.70**
Fat	0.30*	0.73**	0.79**
Ash	0.71**	0.61**	0.56**
Moisture	0.57**	0.73**	0.38**
Calcium	0.68**	0.41**	0.30*
Iron	0.79**	0.88**	0.32*
Phosphorus	0.09	0.06	0.23
Sodium	0.75**	0.39**	0.38**
Milk Yield Per Day	0.17	0.42**	0.52**

** ($p < 0.01$), * ($p < 0.05$)

There is a strong negative correlation between DGAT1 gene expression and milk protein in all three breeds: Red Bororo (-0.69**), White Fulani (-0.75**), and Sokoto Gudali (-0.70**). This suggests that increased expression of DGAT1 may suppress milk protein synthesis, possibly due to resource allocation favoring fat synthesis over protein, as DGAT1 is heavily involved in lipid biosynthesis (Chen et al., 2021; Coffey et al., 2004). This inverse relationship aligns with previous studies that identified trade-offs between milk protein and fat concentrations (Binns et al., 2016). Conversely, DGAT1 expression showed a positive correlation with milk fat: Red Bororo (0.30*), White Fulani (0.73**), and Sokoto Gudali (0.79**). The association is strongest in Sokoto Gudali, confirming DGAT1's pivotal role in triacylglycerol accumulation and milk fat synthesis (Huang et al., 2014; Gupta et al., 2017). This supports the breed-specific metabolic efficiency in using fatty acids for milk fat synthesis, with Sokoto Gudali and White Fulani showing superior genetic potential for fat-rich milk.

The expression of DGAT1 was significantly positively correlated with ash (a proxy for total mineral content) across all breeds, especially in Red Bororo (0.71**), indicating enhanced mineral incorporation in milk (Hale et al., 2018). Moisture content also showed a significant positive correlation, particularly in White Fulani (0.73**), suggesting that higher DGAT1 expression may influence milk water content through osmotic adjustments due to increased fat and mineral content. Both minerals had significant positive correlations, notably iron, with values of 0.79**, 0.88**, and 0.32* for Red Bororo, White Fulani, and Sokoto Gudali, respectively. This implies that DGAT1 expression may affect micronutrient binding or transport in the mammary gland, as lipid vesicles often serve as carriers for such minerals (Wang et al., 2018). Showed non-significant associations, suggesting it might be regulated independently of DGAT1 pathways. Positively correlated across all breeds, aligning with increased milk osmolality accompanying high fat and moisture content (Kremer et al., 2017). DGAT1 gene expression is positively associated with milk yield, particularly in White Fulani (0.42**) and Sokoto Gudali (0.52**), while Red Bororo shows a non-significant relationship (0.17). These differences likely reflect breed-specific genetic potential for milk production, with White Fulani and Sokoto Gudali being better adapted

for high-yield performance (Chen et al., 2021). The positive association supports DGAT1's role in enhancing milk volume through efficient lipid utilization and secretion pathways (Yambasu et al., 2015).

Overall, the data reinforce the functional role of DGAT1 in regulating milk fat content, certain minerals, and yield, while potentially inhibiting protein synthesis due to metabolic trade-offs. These effects are breed-specific, with Sokoto Gudali and White Fulani exhibiting stronger positive correlations with beneficial traits like fat content, iron concentration, and milk yield. Such findings could inform selective breeding programs targeting DGAT1 polymorphisms to optimize dairy traits in indigenous cattle breeds (Gupta et al., 2017; Hale et al., 2018).

Conclusion

The results of this study reveal breed-specific variations in the association between DGAT1 gene expression and both amino acid profiles and milk composition traits in cattle. Notably, Red Bororo cattle exhibited the strongest and most significant correlations between DGAT1 expression and a wide range of amino acids, including essential amino acids such as leucine, isoleucine, phenylalanine, and valine, all with positive and significant correlations. Interestingly, lysine and methionine in Red Bororo showed significant negative associations, suggesting a complex regulatory mechanism of DGAT1 on protein metabolism in this breed. In contrast, White Fulani and Sokoto Gudali breeds showed weaker and mostly non-significant correlations with amino acids, indicating that DGAT1 may play a less dominant role in amino acid regulation in these breeds.

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