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Genetic Expression of IGF-1 and IGF-2 in Nigerian Sheep Breeds: Implications for Growth and Productivity

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Abstract

This research investigates the genetic and phenotypic variability among three Nigerian indigenous sheep breeds (Balami, Uda, and Yankasa) by assessing morphometric characteristics and gene expression profiles of Insulin-like Growth Factors (IGF-1 and IGF-2). A total of 45 sheep (15 per breed), aged 1 to 5 years and comprising 20 males and 25 females, were analyzed for body weight, height at withers, heart girth, and body length. Sample distribution was as follows: Balami (9 males, 6 females), Uda (6 males, 9 females), and Yankasa (5 males, 10 females). Gene expression from muscle, kidney, and heart tissues was evaluated via real-time PCR. Significant breed, age, and sex effects ($P<0.05$) were observed across morphometric traits and gene expression levels. Balami sheep showed notably superior body measurements, suggesting a strong potential for meat production. IGF-1 expression exceeded IGF-2 across all breeds, with Balami having the highest IGF-1 levels. Notably, females exhibited increased IGF-2 expression, potentially influenced by hormonal factors. Age-related variation in gene expression indicates dynamic developmental phases. The integration of molecular and phenotypic evaluations enhances selection programs aimed at breed improvement and conservation of genetic diversity.

Keywords: Indigenous sheep breeds, Genetic diversity, Phenotypic variation, Morphometric traits, IGF-1 and IGF-2, Gene expression, Livestock improvement.

Introduction

Sheep are among the earliest domesticated animals and play a crucial role in the socio-economic livelihood of rural communities across Nigeria's diverse agro-ecological zones. Beyond serving as a source of meat, milk, and wool, they are also important as financial assets and carry cultural significance in many rural societies (Yami & Markel, 2017). The major indigenous sheep breeds in Nigeria include Yankasa, Uda, Balami, and the West African Dwarf (WAD). These breeds demonstrate substantial differences in morphological traits, productivity levels, and adaptability, reflecting the ecological diversity of the regions where they are found (Galal *et al*., 2005).

Despite their importance, the genetic potential of these native sheep breeds remains largely untapped (Mohammed *et al*., 2018). Limited application of advanced genetic techniques in breeding programs has hindered substantial improvement, mainly due to insufficient understanding of both phenotypic and genetic variation within and among breeds (Nottor, 2012). Morphometric traits, such as body length, height at withers, and heart girth, serve as critical parameters in evaluating growth performance, breed identity, and suitability for breeding objectives (Yakubu & Ibrahim, 2010).

Recent advances in molecular genetics have enabled the analysis of gene expression related to growth, notably the Insulin-like Growth Factor genes, IGF-1 and IGF-2 (Zhang *et al*., 2014). These genes play a fundamental role in cellular growth, differentiation, and tissue development. Evaluating their expression in different tissues offers valuable insights into breed-specific growth potential and supports marker-assisted selection in animal breeding (Sahoo *et al*., 2018).

This study was designed to evaluate the genetic and phenotypic variability among Nigerian sheep breeds through detailed morphometric analysis and quantification of IGF-1 and IGF-2 gene expression. The outcomes aim to guide selection strategies that enhance productivity while supporting conservation efforts of indigenous genetic resources.

Methods, Techniques, Studied Material, and Study Area

Study Area

The research was conducted in Maiduguri, the capital of Borno State in north eastern Nigeria. This city lies between latitudes 11°50′ and 11°85′ N and longitudes 13°09′ and 13°14′ E, with an elevation of approximately 354 meters above sea level. Maiduguri falls within the Sahelian ecological zone of West Africa, characterized by a short rainy season (typically 3–4 months) and a prolonged dry season (8–9 months). Temperatures are relatively low from December to January (15–19 °C), while the hottest months span from March to June, with temperatures ranging from 33–44 °C. The area also experiences low relative humidity, between 5% and 43.5% (Mohammed et al., 2018). Maiduguri covers around 3,000 km² and supports mixed agricultural systems including crop cultivation, livestock rearing, and fishing.

Experimental Animals

The study evaluated 45 sheep representing three indigenous breeds: Balami, Uda, and Yankasa. Each breed group included 15 animals aged 1 to 5 years, consisting of 20 males and 25 females. Breed-specific sex distribution was as follows: Balami (9 males, 6 females), Uda (6 males, 9 females), and Yankasa (5 males, 10 females). Samples were sourced from Maiduguri abattoir and nearby households during the period of August to September 2019.

Morphometric Trait Measurements

Four morphometric parameters were recorded: body weight (BW), heart girth (HG), height at withers (HW), and body length (BL). Body weights were measured using a spring dial mechanical scale manufactured by Avery Weigh-Tronix (United Kingdom/United States). Height at withers, the vertical distance from the ground to the top of the shoulder, was measured with a graduated tape measure while the animals stood upright. Body length, taken from the shoulder point to the pin bone, and heart girth, measured around the chest just behind the forelimbs, were also obtained using a flexible tape, following the protocol outlined by Khan *et al.* (2006). All animals were calm and restrained to ensure accuracy.

Tissue Collection and Preservation

Tissue samples from the heart, kidney, and skeletal muscle were collected from each of the 45 animals (15 per breed), resulting in 45 samples per tissue type. All samples were preserved in RNAlater solution at –20 °C to maintain RNA stability for subsequent molecular analyses.

RNA Extraction

Total RNA was extracted using the Geneaid Presto™ RNA Extraction Kit (Cat. No. DRP050/100), following the manufacturer's protocol and the method described by Sambrook *et al.* (2012). Approximately

25 mg of tissue was homogenized with DR buffer and β-mercaptoethanol using a TissueLyser. After mechanical disruption, the samples were processed through GD spin columns, and the RNA was purified, treated with DNase to eliminate DNA contamination, and quantified using a Nanodrop spectrophotometer. Only RNA samples with purity ratios (260/280 and 260/230) between 1.7–2.0 and concentrations ≥100 ng/µl were selected for further analysis.

cDNA Synthesis

Complementary DNA (cDNA) was synthesized using the FIREScript RT cDNA Synthesis Kit (Cat. No. 06-15-0000S). The reaction mix contained reverse transcriptase, reaction buffer, RNase inhibitor, primers, and RNA template, with nuclease-free water used to make up the total volume. The synthesis was carried out under the following thermocycling conditions: 25 °C for 10 minutes (annealing), 45 °C for 30 minutes (reverse transcription), and 85 °C for 5 minutes (enzyme inactivation).

Real-Time PCR (qPCR) for Gene Expression

Quantitative PCR (qPCR) was performed using the Bio-Rad My iQ™ Real-Time PCR system. The reactions were prepared using the HOT FirePol® qPCR Supermix in 25 µl volumes containing master mix, gene-specific forward and reverse primers (IGF-1 and IGF-2), nuclease-free water, and cDNA templates. Cycling conditions were: 95 °C for 12 minutes (initial activation), followed by 40 cycles of 95 °C for 15 seconds, 55–56 °C for 20 seconds (annealing), and 72 °C for 20 seconds (elongation). Samples with insufficient RNA concentrations were rerun after re-extraction.

Housekeeping Gene and Primer Details

Gene expression normalization was carried out using GAPDH as a reference gene. The same qPCR protocol was followed using specific primers:

IGF-1-F: ACCGAGGGGCTTTTACTTCA

IGF-1-R: TGGCTCACCTTTCCTTCTCC

IGF-2-F: CTGGTGGATGCTCTTCAGTTCG

IGF-2-R: TGCTTTTGTAGGCTTCAGTGGG

Melt curve analysis was used to confirm the specificity of amplification and absence of primer-dimer.

Gene Expression Analysis

Relative gene expression levels were calculated using the 2^−ΔΔCt method (Livak & Schmittgen, 2001). Comparative threshold cycle (Ct) values were analyzed using the Wilcoxon test to determine differences across breeds, sexes, and age groups.

Statistical Analysis

A General Linear Model (GLM) in SAS version 9.2 (SAS, 2002) was applied to assess the effects of breed, sex, and age on morphometric traits and gene expression. The model used was:
$Yijklm = \mu + Bi + Sj + Ak + eijkl,$

where Yijklm = phenotypic value, µ = overall mean, Bi = breed effect, Sj = sex effect, Ak = age effect, and eijkl = residual error. Mean comparisons were conducted using the Least Significant Difference (LSD) test.

Results

Table 1: Means (±SEM) of Body Weight and Linear Body Measurements as Affected by Breed, Age and Sex of Some Nigerian Sheep Breeds

Factors	**Body Traits**			
	BW	**HG**	**HW**	**BL**
Overall means	69.98±3.37	36.96±0.82	36.66±0.96	36.66±0.99
Breed (45)	*	*	*	*
Balami (15)	93.94±4.91	41.65±1.22	42.82±1.08	44.82±0.98
Uda (15)	71.50±2.88^{b}	38.00±1.11^{b}	37.50±1.55^{b}	37.83±1.39^{b}
Yankasa (15)	45.83±2.48^{c}	31.50±0.64^{c}	30.00±0.42^{c}	30.50±0.40^{c}
Age (years)	**	**	***	***
1 (7)	38.33±1.92^{d}	29.67±0.83^{c}	28.33±0.17^{d}	30.00±0.58^{e}
2 (7)	58.50±1.56^{c}	33.50±2.01^{b}	29.50±1.12^{d}	30.50±0.22^{e}
3 (7)	57.50±2.46^{c}	33.00±0.00^{b}	33.50±0.67^{c}	34.00±1.79^{d}
4 (9)	85.40±10.04^{b}	40.80±1.71^{a}	41.20±1.96^{b}	43.20±1.96^{b}
5 (15)	87.38±2.32^{b}	41.07±0.52^{a}	42.25±0.61^{b}	43.88±0.65^{c}
Sex	*	*	*	*
Male (20)	78.04±5.79	38.42±1.44	39.19±1.51	39.92±1.46
Female (25)	62.22±3.00^{b}	35.56±0.74^{b}	34.22±1.03^{b}	35.33±1.21^{b}

BW: Body Weight, HG: Heart Girth, HW: Height at Withers, BL: Body Length, ns: not significant, *= P<0.05; **= P<0.01; ***= P<0.001. a-e = means. Means in the same column within a subset bearing different superscripts are significantly different.

Table 2: Least Squares Means± SEM of Ct of IGF-1 and IGF-2 of Three Nigerian Sheep Breeds

Variables	**IGF-1**	**IGF-2**	**IGFs**
Overall means	23.10±0.68	25.33±0.68	24.21±0.54
Breed (45)	*	*	*
Balami (15)	21.96±1.14^{b}	23.38±1.14^{b}	22.67±0.78^{b}
Uda (15)	24.09±1.14^{a}	27.23±1.14^{a}	25.66±0.78^{a}
Yankasa (15)	23.25±1.19^{a}	25.38±1.19^{a}	24.32±0.81^{a}
Age (years)	*	*	*
1 (7)	25.64±1.46^{a}	28.01±1.46^{a}	26.82±0.99^{a}
2 (7)	20.18±1.93^{b}	23.51±1.93^{b}	21.85±1.32^{b}
3 (7)	25.28±1.93^{a}	27.15±1.93^{a}	26.21±1.32^{a}
4 (9)	24.13±1.95^{a}	25.61±1.95^{a}	24.87±1.33^{a}
5 (15)	19.89±0.97^{b}	22.04±0.97^{b}	20.97±0.66^{b}
Sex	ns	*	ns
Male 20	23.22±1.13^{a}	22.98±0.87^{b}	24.14±0.89^{ab}
Female (25)	25.06±1.31^{a}	25.56±0.87^{a}	24.29±0.59^{a}

Ct: Circle Threshold, IGF1: Insulin-Like Growth Factor-1, IGF2: Insulin-Like Growth Factor-2, *=P<0.05. a-b = means. Means in the same column within a subset bearing different superscripts are significantly different.

Morphometric Traits Across Breeds, Ages, and Sexes

Table 1 presents the least squares means and standard errors for body weight and linear body measurements as influenced by breed, age, and sex. The overall averages for body weight (BW), heart girth (HG), height at withers (HW), and body length (BL) were 69.98 kg, 36.96 cm, 36.66 cm, and 36.66 cm respectively.

Statistically significant differences (P<0.05) were observed across all traits, with Balami sheep consistently recording the highest body weight (93.94 kg), followed by Uda (71.50 kg) and Yankasa (45.83 kg). This pattern held across other measurements such as HG, HW, and BL.

Breed-specific differences were also significant for heart girth, with Balami leading (41.65 cm), followed by Uda and Yankasa. Similar patterns were noted for HW and BL, reinforcing Balami's superior body conformation.

Effect of Age on Morphometric Parameters

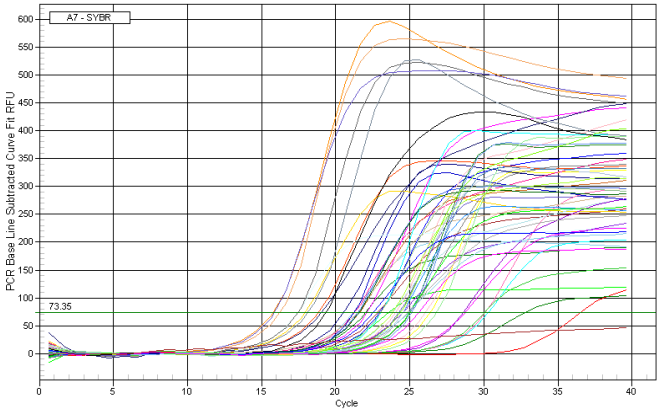
Age significantly influenced morphometric traits (P<0.01 to P<0.001). Body weight increased progressively from 38.33 kg in one-year-old sheep to 93.00 kg in five-year-old. A similar trend was observed for HG, HW, and BL.

Sex-Related Differences in Morphometric Traits

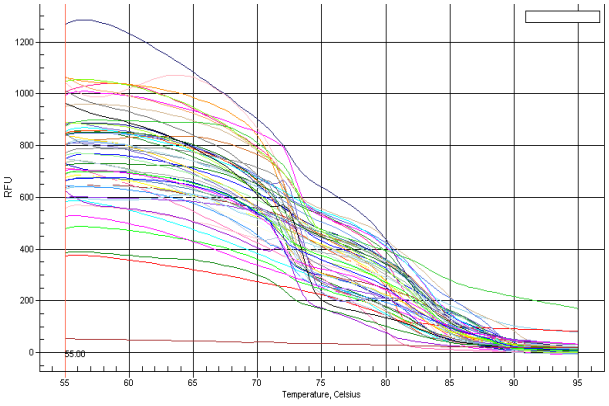
Sex had a significant effect (P<0.05) on all measured traits. Male sheep had higher BW, HG, HW, and BL than females across all three breeds.

PCR Amplification and Gene Expression of IGF-1 and IGF-2

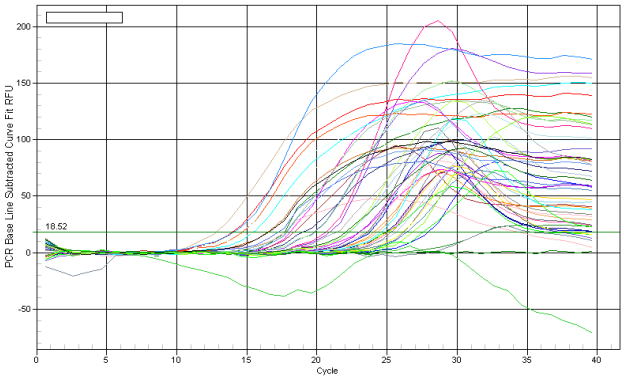
Figures 1 and 2 show amplification plots and melt curves for GAPDH, IGF-1, and IGF-2. All samples showed successful amplification. IGF-1 peaked at cycle 25, while IGF-2 peaked closer to cycle 30. Melt curve analysis confirmed single, specific peaks, validating primer specificity and reaction efficiency.



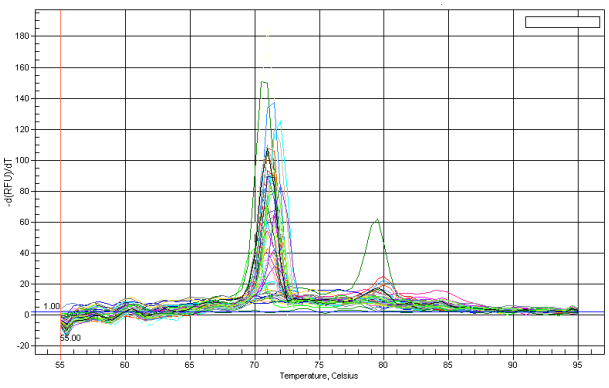
A1. Housekeeping gene (GAPDH) amplification plot



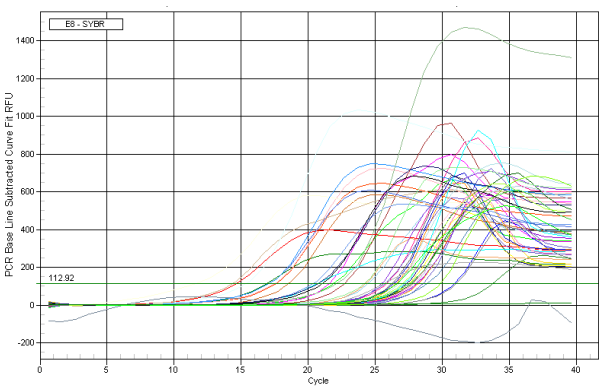
A2. House-keeping gene (GAPDH) Melt curve



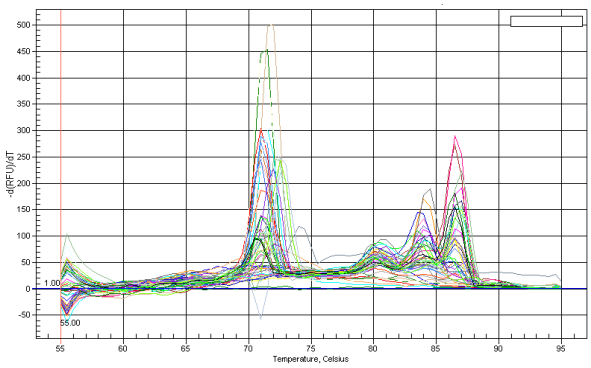
B1 IGF-1 amplification curve.



B2 IGF-1Melt Peak

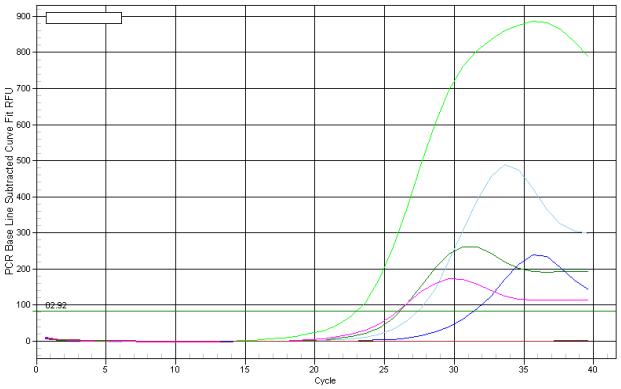


C1 IGF-2 amplification plot.

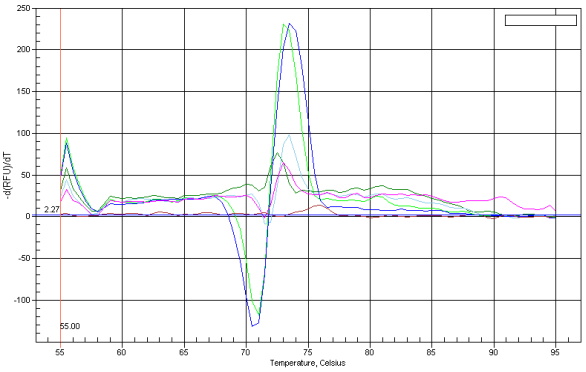


C2 IGF-2 Melt peak

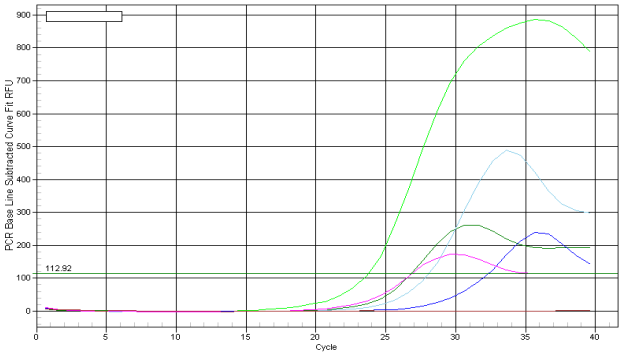
Figure 1. Amplification plot and dissociation curve of GAPDH, IGF-1 and IGF-2



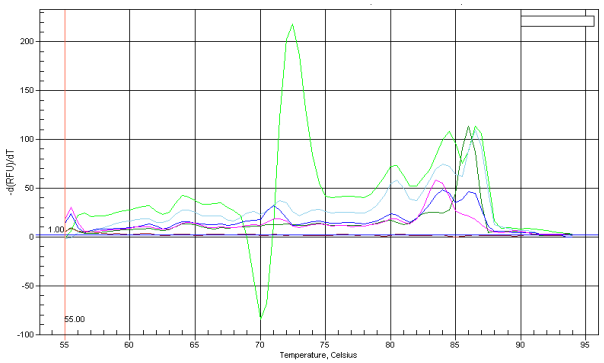
Amplification curve of samples with IGF-1 primer



Melt Peak IGF-1 after second run after the second run



Amplification plot IGF-2 run 2



IGF-2 run 2 melt peaks

Figure 2. The amplification curve of the two samples with IGF-1 and 2 with primer after the second run.

Ct-Based Expression Analysis of IGF Genes

Table 2 summarizes Ct values for IGF-1 and IGF-2 across breeds, ages, and sexes. IGF-1 exhibited higher expression (lower Ct value = 23.10) compared to IGF-2 (Ct = 25.33). Balami breed showed the highest expression, followed by Yankasa and Uda.

Discussion

The morphometric results observed in this study align with previous reports, particularly the superior performance of the Balami breed in terms of body weight and frame size (Fayeye *et al*., 2017; Dauda *et al*., 2018; Abbaya & Dauda, 2018). These breed-specific traits underscore the impact of genetic background on growth and physical development. Heart girth's association with overall mass further supports its utility as a reliable growth indicator in sheep breeding.

Age-related increases in BW and other traits are consistent with patterns reported in earlier studies, although occasional deviations (Ben-Hamouda & Megdiche, 2021) suggest environmental and management influences. The observed sex differences support the general principle that males tend to outperform females in growth due to androgenic hormones like testosterone (Carlos *et al*., 2015; Akpa *et al*., 2017).

Gene expression analysis confirms that IGF-1 is more actively expressed than IGF-2 across all groups, indicating its critical role in promoting postnatal growth. These findings are consistent with the amplification trends described by Pfaffl *et al.* (2002) and gene activity patterns reported by Sun *et al.* (2014) and Vishnuraj *et al.* (2023).

The Ct analysis revealed breed-dependent variation in IGF gene expression. Balami sheep again demonstrated higher expression levels, which may contribute to their superior growth traits. These echoes result from other species where IGF expression differed by genetic background (Rejduch *et al*., 2010; Dou *et al*., 2023).

Age also influenced IGF expression, with fluctuating levels across age groups, suggesting dynamic regulation of growth-related genes through different life stages (Sun *et al*., 2014; Huang & Xie, 2009). Younger animals showed higher IGF-1 levels, reflecting active tissue growth, while IGF-2 expression varied more subtly.

Sex-based differences were pronounced for IGF-2 but not for IGF-1. Higher IGF-2 expression in females could be due to estrogenic regulation, as supported by findings from Masoudzadeh et al. (2020) and Song *et al.* (2021). Such hormonal modulation may play a role in sex-specific growth dynamics and tissue development.

Overall, this study highlights the value of integrating morphometric and molecular approaches in small ruminant breeding, offering a pathway toward more informed and strategic livestock improvement efforts.

Conclusion

This study provides important insights into the genetic and phenotypic characteristics of three indigenous sheep breeds in Nigeria (Balami, Uda, and Yankasa). Significant variation was observed across body measurements and gene expression levels of IGF-1 and IGF-2, highlighting the distinct genetic makeup and growth potential of each breed. Balami sheep consistently exhibited superior morphometric traits and higher IGF-1 expression, making them particularly favorable for meat production.

The influence of age and sex on gene expression was evident, suggesting that developmental stage and hormonal regulation play important roles in modulating growth-related genes. IGF-1 expression was generally elevated in younger animals, reinforcing its role in active growth phases, while IGF-2 expression showed variation across sexes, likely reflecting underlying hormonal or epigenetic influences.

These findings underscore the utility of integrating traditional morphometric evaluation with molecular techniques to enhance breeding strategies. Such an approach can lead to more targeted selection, better conservation of genetic resources, and ultimately improved productivity in Nigeria's small ruminant sector.

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