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Full Length Research

Polymorphisms of the CTNNB1 gene in three breeds of sheep in Danbatta Local Government, Kano State, Nigeria

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ABSTRACT: The present study investigated the polymorphisms of the Catenin Beta 1 (CTNNB1) gene in three indigenous sheep breeds, namely Balami, Yankasa, and Uda sheep reared in Kano State, Nigeria, and evaluated their association with growth and reproductive traits. A total of 150 sheep, comprising 50 animals from each breed, were sampled from selected flocks across Kano State. Blood samples were collected for genomic DNA extraction, and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis was employed to detect genetic variations in the CTNNB1 gene. Three genotypes (AA, AB, and BB) were identified among the studied breeds. Genotype frequencies for Balami sheep were 0.42, 0.46, and 0.12 for AA, AB, and BB, respectively, while Yankasa sheep recorded frequencies of 0.38, 0.50, and 0.12. Uda sheep showed genotype frequencies of 0.34, 0.52, and 0.14, respectively. Significant associations ($p<0.05$) were observed between CTNNB1 genotypes and body weight, body length, heart girth, and litter size. Animals possessing the AA genotype exhibited superior growth performance and higher reproductive efficiency compared with BB genotype carriers. The A allele occurred more frequently in all three breeds, suggesting possible selective advantages associated with this allele under tropical production systems. The findings indicate that the CTNNB1 gene polymorphism may serve as a useful molecular marker for genetic improvement and marker-assisted selection in indigenous Nigerian sheep breeds.

Keywords: CTNNB1 gene, growth traits, PCR-RFLP, polymorphism, reproductive traits, sheep.

INTRODUCTION

Sheep production constitutes an important component of livestock agriculture in Nigeria, particularly in the northern region, where sheep contribute significantly to meat supply, household income, cultural ceremonies, and rural livelihoods. Indigenous sheep breeds such as Balami, Yankasa, and Uda are highly adapted to the harsh climatic conditions of the semi-arid regions of Northern Nigeria. These breeds possess valuable adaptive characteristics, including heat tolerance, disease resistance, and the ability to survive under low-input management systems. Despite these adaptive advantages, productivity in terms of growth rate and reproductive performance remains relatively low compared to improved exotic breeds (Silva *et al*., 2011).

Genetic improvement through molecular breeding has become increasingly important in livestock production because traditional selection methods based solely on phenotypic performance are usually slow and less efficient for quantitative traits. Recent advances in molecular genetics have enabled the identification of candidate genes associated with economically important traits in livestock species. Candidate gene approaches provide opportunities for marker-assisted selection aimed at improving growth, reproduction, and disease resistance in farm animals (Souza *et al*., 2001). Among the candidate genes associated with growth and reproductive traits, the Catenin Beta 1 (CTNNB1) gene has attracted considerable attention. The CTNNB1 gene encodes β-

catenin, an important intracellular protein involved in the Wnt signalling pathway. This signalling pathway regulates cell proliferation, tissue differentiation, embryonic development, follicular growth, and skeletal muscle development (Beecher et al., 2010). The CTNNB1 gene plays important roles in adipogenesis, muscle growth, ovarian follicle development, and reproductive functions in mammals.

Previous studies have reported significant associations between CTNNB1 gene polymorphisms and reproductive performance, litter size, growth traits, and carcass characteristics in sheep populations (Smołucha *et al*., 2021). Genome-wide association studies have also identified CTNNB1 as one of the candidate genes associated with prolificacy and body development in sheep breeds with superior reproductive performance (Zhang *et al*., 2011). Similarly, Wilson et al. (2021) reported that CTNNB1 contributes significantly to litter size and reproductive efficiency in indigenous sheep populations.

The ovine CTNNB1 gene, located on chromosome 6, consists of 16 exons and 15 introns and encodes the β-catenin protein, a key component of the canonical Wnt signalling pathway. The coding region extends from exon 2 to exon 15, while untranslated regions are present in exons 1 and 16. Exon 3 contains conserved phosphorylation sites that regulate β-catenin stability and cellular signalling. Due to its role in cell proliferation, differentiation, and tissue development, CTNNB1 has been investigated as a candidate gene for economically important traits in sheep, including growth performance, carcass characteristics, and reproductive efficiency (Davis *et al*., 2026).

In Nigeria, studies on the molecular characterisation of indigenous sheep breeds remain limited despite the economic importance of these animals. Balami sheep are recognised for their large body size and relatively fast growth performance, while Yankasa sheep are the most widely distributed breed in Nigeria because of their adaptability and meat production potential. Uda sheep are also valued for their hardiness and ability to thrive under extensive management systems. Understanding the genetic diversity and molecular characteristics of these breeds is important for conservation and genetic improvement programs.

Therefore, the present study was conducted to investigate the polymorphisms of the CTNNB1 gene in three breeds of sheep in Danbatta Local Government, Kano State, Nigeria, and to determine the association between CTNNB1 genotypes and selected growth and reproductive traits.

MATERIALS AND METHODS

Study area

The study was conducted in Danbatta Local Government Area (LGA), Kano State, Nigeria, located within the Sudan Savannah agro-ecological zone. The area experiences distinct wet and dry seasons, with annual rainfall ranging from 450–800 mm and an average temperature of about 33°C. Livestock production is a major agricultural activity, with cattle, sheep, goats, and poultry commonly reared under extensive and semi-intensive management systems. This environment makes the area suitable for animal science and livestock health studies.

Experimental animals

A total of 150 apparently healthy sheep comprising 50 Balami, 50 Yankasa, and 50 Uda sheep aged between 12 and 36 months were randomly selected from smallholder flocks and livestock markets across Danbatta Local Government. Animals were managed under semi-intensive production systems.

Blood sample collection

Approximately 5 mL of blood was collected aseptically from the jugular vein of each animal into EDTA-containing Vacutainer tubes. Samples were transported in ice packs to the molecular genetics laboratory for genomic DNA extraction (African Biosciences Ibadan).

DNA extraction

Genomic DNA extraction was carried out using the standard phenol-chloroform extraction protocol described by Sambrook and Russell (2001). DNA quality and concentration were evaluated using agarose gel electrophoresis and NanoDrop spectrophotometry.

PCR amplification of the CTNNB1 gene

Specific primers used for amplification of the CTNNB1 gene were designed based on published ovine CTNNB1 sequences available in GenBank.

Primer sequences

Forward primer: 5′-GCTTGGAATGAGACTGCTGA-3′
Reverse primer: 5′-TTCTCCAGGTTGGCATTTTC-3′
PCR amplification was performed in a 25 µL reaction mixture containing:
12.5 µL PCR Master Mix
1.0 µL forward primer
1.0 µL reverse primer
2.0 µL genomic DNA
8.5 µL nuclease-free water

PCR conditions included:

1. Initial denaturation at 95°C for 5 minutes
2. 35 cycles of:

Denaturation at 94°C for 30 seconds
Annealing at 60°C for 45 seconds
Extension at 72°C for 1 minute

3. Final extension at 72°C for 10 minutes

Restriction fragment length polymorphism (RFLP) analysis

Amplified PCR products were digested using HinfI restriction enzyme and separated on 3% agarose gel stained with ethidium bromide. Banding patterns were visualized under ultraviolet transillumination to identify genotypes.

Measurement of growth traits

The following growth traits were measured: Body weight (kg), Body length (cm), Heart girth (cm), Withers height (cm). Measurements were obtained using weighing scales and measuring tapes according to standard livestock measurement procedures.

Reproductive traits

Litter size and age at first lambing were obtained from farmers' breeding records and direct interviews.

Statistical analysis

Genotype and allele frequencies were calculated according to standard population genetic procedures. Chi-square analysis was used to test deviation from the Hardy-Weinberg equilibrium. The statistical model used for association analysis was:

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

Where: Y_{ijk} = observed trait, μ = overall mean, B_i = effect of breed, G_j = effect of genotype, e_{ijk} = residual error.

Data were analysed using SPSS version 25, and means were separated using Duncan's Multiple Range Test at a 5% significance level.

RESULTS

Genotype and allele frequencies of the *CTNNB1* gene

The distribution of *CTNNB1* genotypes among the three sheep breeds is presented in Table 1. The heterozygous genotype (AB) was the most frequently occurring genotype in Yankasa (0.50) and Uda (0.52) sheep, whereas the homozygous AA genotype had a relatively higher frequency in Balami sheep (0.42). The BB genotype recorded the lowest frequency across all breeds, ranging from 0.12 in Balami and Yankasa sheep to 0.14 in Uda sheep.

The allele frequencies of the *CTNNB1* gene are shown in Table 2. Allele A was more predominant than allele B in all the studied breeds. Balami sheep recorded the highest frequency of allele A (0.65), followed by Yankasa (0.63) and Uda (0.60). Conversely, allele B occurred at lower frequencies of 0.35, 0.37, and 0.40 in Balami, Yankasa, and Uda sheep, respectively.

Table 1. Genotype frequencies of the CTNNB1 gene.

Breed	AA	AB	BB
Balami	0.42	0.46	0.12
Yankasa	0.38	0.50	0.12
Uda	0.34	0.52	0.14

The heterozygous AB genotype was the most predominant in Yankasa and Uda sheep, while Balami sheep recorded relatively higher AA genotype frequency.

Table 2. Allele frequencies of CTNNB1 gene

Breed	A	B
Balami	0.65	0.35
Yankasa	0.63	0.37
Uda	0.60	0.40

The A allele occurred more frequently than the B allele in all studied breeds.

Association between *CTNNB1* genotypes and body weight

The association between *CTNNB1* genotypes and body weight is presented in Table 3. Significant differences ($p<0.05$) were observed among the genotypes across all breeds. Sheep possessing the AA genotype recorded the highest body weights, with values of 42.85 ± 1.12 kg, 36.74 ± 1.06 kg, and 39.31 ± 1.15 kg for Balami, Yankasa, and Uda breeds, respectively. Animals with the AB genotype showed intermediate body weight values, while the BB genotype had the lowest body weights across all breeds. The results indicate that the AA genotype was associated with superior growth performance compared to the BB genotype.

Association between *CTNNB1* genotypes and body length

Table 4 shows the relationship between *CTNNB1*

Table 3. Least squares mean of body weight (kg).

Genotype	Balami	Yankasa	Uda
AA	42.85 ± 1.12[a]	36.74 ± 1.06[a]	39.31 ± 1.15[a]
AB	39.20 ± 0.95[b]	33.68 ± 0.88[b]	35.84 ± 0.91[b]
BB	35.42 ± 0.83[c]	30.45 ± 0.79[c]	32.10 ± 0.82[c]

Different superscripts within columns indicate significant differences ($p<0.05$). Animals possessing the AA genotype recorded significantly higher body weights than BB genotype animals.

Table 4. Association between CTNNB1 genotypes and body length (cm), Heart girth (cm) and Litter size

Genotype	Balami	Yankasa	Uda
Body length			
AA	72.40 ± 1.28[a]	68.22 ± 1.14[a]	70.35 ± 1.20[a]
AB	68.15 ± 1.10[b]	64.85 ± 1.02[b]	66.71 ± 1.08[b]
BB	63.90 ± 0.98[c]	60.74 ± 0.94[c]	62.44 ± 0.96[c]
Heart girth			
AA	83.55 ± 1.45[a]	78.33 ± 1.21[a]	80.48 ± 1.33[a]
AB	79.62 ± 1.18[b]	74.95 ± 1.04[b]	76.80 ± 1.11[b]
BB	75.20 ± 1.01[c]	71.42 ± 0.93[c]	73.11 ± 0.97[c]
Litter size			
AA	1.82 ± 0.06[a]	1.76 ± 0.05[a]	1.79 ± 0.06[a]
AB	1.61 ± 0.04[b]	1.58 ± 0.04[b]	1.60 ± 0.05[b]
BB	1.42 ± 0.03[c]	1.39 ± 0.03[c]	1.41 ± 0.03[c]

genotypes and body length in the studied sheep breeds. Significant differences ($p<0.05$) were observed among the genotypes. In all breeds, animals with the AA genotype exhibited the highest body length measurements, recording 72.40 ± 1.28 cm in Balami, 68.22 ± 1.14 cm in Yankasa, and 70.35 ± 1.20 cm in Uda sheep. The AB genotype recorded intermediate values, whereas the BB genotype had the lowest body lengths. This trend suggests a positive association between the AA genotype and increased body conformation traits.

Association between *CTNNB1* genotypes and heart girth

The association between *CTNNB1* genotypes and heart girth is presented in Table 4. Significant variations ($p<0.05$) were observed among the different genotypes across the breeds. Sheep with the AA genotype recorded the highest heart girth values of 83.55 ± 1.45 cm, 78.33 ± 1.21 cm, and 80.48 ± 1.33 cm in Balami, Yankasa, and Uda sheep, respectively. The AB genotype showed moderate values, while the BB genotype consistently recorded the lowest heart girth measurements. These findings indicate that the AA genotype may contribute positively to improved body development traits in sheep.

Association between *CTNNB1* genotypes and litter size

Table 4 presents the association between *CTNNB1* genotypes and litter size. Significant differences ($p<0.05$) were observed among the genotypes in all breeds studied. The AA genotype recorded the highest litter size values of 1.82 ± 0.06, 1.76 ± 0.05, and 1.79 ± 0.06 in Balami, Yankasa, and Uda sheep, respectively. Intermediate litter size values were observed in animals with the AB genotype, while the BB genotype had the lowest litter size values across all breeds. The results suggest that the AA genotype may be associated with enhanced reproductive performance in the studied sheep breeds.

DISCUSSION

The present study revealed substantial genetic polymorphism within the CTNNB1 gene among Balami, Yankasa, and Uda sheep breeds in Kano State. The identification of three distinct genotypes (AA, AB, and BB) suggests the existence of considerable genetic diversity within these indigenous sheep populations. Genetic variability is essential for sustainable livestock improvement because it provides the basis for selection

and adaptation to changing environmental conditions.

The predominance of the A allele observed across all breeds may indicate that this allele confers adaptive or productive advantages under tropical production systems. Indigenous sheep breeds in Northern Nigeria are continuously exposed to harsh climatic conditions, nutritional stress, and endemic diseases. Consequently, favourable alleles associated with improved growth and reproductive efficiency may become more prevalent over time through natural and artificial selection.

The significant association between CTNNB1 genotypes and body weight observed in this study supports the important biological role of the CTNNB1 gene in growth regulation. β-catenin, encoded by the CTNNB1 gene, is a key component of the Wnt signalling pathway, which regulates skeletal muscle development, adipogenesis, and cellular proliferation (Roudbar *et al*., 2018). Animals possessing the AA genotype recorded significantly higher body weights, body lengths, and heart girths compared to BB genotype carriers. Similar findings were reported by Smołucha *et al*. (2021), who identified CTNNB1 among the candidate genes associated with prolificacy and body development traits in sheep populations.

The superior growth performance observed in AA genotype animals may be attributed to enhanced muscle cell proliferation and tissue development associated with favourable CTNNB1 variants. The Wnt/β-catenin signalling pathway has been widely recognised as an important regulator of skeletal growth and tissue differentiation in mammals. Variations in the CTNNB1 gene expression may therefore influence body conformation and growth performance in sheep.

The significant relationship between CTNNB1 polymorphism and litter size observed in the present study also confirms the involvement of this gene in reproductive physiology. Previous genome-wide association studies identified CTNNB1 as one of the genes influencing prolificacy and ovarian follicular development in sheep (Xu *et al*., 2017). Higher litter sizes recorded among AA genotype animals suggest that favourable CTNNB1 alleles may enhance reproductive efficiency through improved follicular maturation and ovulation processes.

The findings of this study are important for sheep breeding programs in Nigeria because indigenous breeds possess valuable adaptive traits that should be conserved while improving productivity. Marker-assisted selection involving favourable CTNNB1 alleles could accelerate genetic improvement for growth and reproductive traits without compromising environmental adaptability. Furthermore, molecular characterisation of indigenous sheep breeds contributes to the conservation of animal genetic resources and supports sustainable livestock production in tropical environments.

This study also provides baseline molecular genetic information for Nigerian indigenous sheep breeds, which remain underrepresented in genomic studies compared with exotic breeds. Future studies involving sequencing technologies and larger populations are necessary to validate the identified polymorphisms and explore additional candidate genes associated with economically important traits.

Conclusion

The present study demonstrated the existence of polymorphism within the CTNNB1 gene among Balami, Yankasa, and Uda sheep breeds in Kano State, Nigeria. Three genotypes (AA, AB, and BB) were identified, with the A allele occurring more frequently in all breeds. Significant associations were observed between CTNNB1 genotypes and economically important traits, including body weight, body length, heart girth, and litter size. Sheep carrying the AA genotype exhibited superior growth performance and reproductive efficiency compared with BB genotype animals. The findings suggest that the CTNNB1 gene polymorphism may serve as a useful molecular marker for marker-assisted selection programs aimed at improving growth and reproductive traits in indigenous Nigerian sheep breeds.

Recommendations

Based on the findings of this study, it is recommended that the CTNNB1 gene, particularly the AA genotype, be incorporated into marker-assisted selection programmes to improve growth and reproductive performance in indigenous Nigerian sheep breeds. Livestock breeders and relevant agencies should adopt molecular screening techniques to identify and select animals carrying favourable CTNNB1 alleles for breeding while maintaining the adaptive characteristics of Balami, Yankasa, and Uda sheep. In addition, further studies involving larger sample sizes, wider geographical coverage, and advanced genomic approaches such as DNA sequencing and genome-wide association studies are recommended to validate the observed associations and identify additional genetic markers that could enhance sustainable sheep breeding and conservation programmes in Nigeria.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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