EFFECT OF AGE ON MORPHOMETRIC TRAITS AND EXPRESSION OF IGF-1 AND IGF-2 IN BALAMI SHEEP BREED

Mohammed, A[1]*., Modu-Kagu, H.A., Abdulraheem, A.O., Shettima, M.M[1]., Owie, P.A[2]., Raji, A.O., Aliyu, J. and Alade, N.K.

[1]Department of Animal Science, Borno State University P.M.B. 1122 Maiduguri, Borno State, Nigeria.
Department of Animal Science, University of Maiduguri P.M.B 1069 Maiduguri, Borno State Nigeria.
[2]National Biotechnology Development Agency, Lugbe, Abuja. Nigeria.
Corresponding author: banayeyya@yahoo.com; +2348033413930

ABSTRACT

Balami sheep are the largest native breed in Nigeria, primarily found in the semi-arid north but also used as a stall-fed breed throughout the country. The study explores the genetic variations of Balami, focusing on the Insulin-like Growth Factor 1 and 2 (IGF-1and IGF-2) genes, which plays a crucial role in growth and development. IGF-1 levels are linked to various growth traits in sheep, such as muscle growth, body weight, and development. The study analyzed the expression patterns of IGFs and its relationship with growth traits in Balami sheep, using molecular genetic techniques. Forty-five samples were collected from Balami sheep in Maiduguri, Nigeria, and analyzed using various molecular biology techniques. Morphometric traits measured include body weight, height at withers, body length, and heart girth. Ribonucleic Acid (RNA) was extracted and converted to cDNA for gene expression and analysis using quantitative real-time PCR (qPCR). The study found significant (P<0.05) effects of age on body weight and other measurements. Older sheep generally had higher body weights and measurements. The expression levels of IGF-1 and IGF-2 varied with age. It showed significant (P<0.05) fluctuations that corresponded to growth phases. The knowledge of IGF-1 gene expression can be valuable for selecting fast-growing animals and enhancing production traits through marker-assisted selection.

Keywords: Sheep, IGF genes, Expression, Biometric traits, Selection

INTRODUCTION

The age of an individual can have significant effects on various morphometric traits and the expression of insulin-like growth factor 1 (IGF-1) and insulin-like growth factor 2 (IGF-2) in sheep. As sheep age, they typically experience changes in their growth and body size characteristics (Teixeira *et al.,* 2021). Younger sheep typically have a faster growth rate and can attain a larger overall body size compared to older sheep (Teixeira *et al*., 2021). The expression of IGF-1, a growth factor involved in cell proliferation, differentiation, and survival, can be affected by the age of the sheep (Gluckman *et al.,* 1992). Younger animals often have higher levels of IGF-1, which is associated with their increased growth and development (Gluckman *et al*., 1992). The expression of IGF-2, another growth factor that plays a role in embryonic and fetal development, can also be influenced by the age of the sheep (Fowden, 1995).

Balami is the largest bodied native sheep in Nigeria. As a pastoral animal, it is confined to the semi-arid north but it is favoured as a stall-fed breed throughout Nigerian. It is white and hairy with pendulous ears, long-leg and a long-thin tail. Rams are horned but ewes are polled. Another feature that makes the Balami distinctly recognizable is its, bulbous nose that distinguishes it from Yankasa. It has good potential as a meat producer. The weights of mature males range from 40 to 80 kg while those of females are between 30 and 40 kg (Yunusa *et al*., 2013).

Molecular genetics techniques are of great interest in the identification of genetic variations in genetic markers which are associated with different production and reproduction traits in farm animals (Jiang *et al*., 2002; Arora and Bhatia, 2006). These genetic variations affect the physiological pathways that consequently lead to quantitative variations in different phenotypic characteristics (Schwerine *et al*., 1995; Lan *et al*., 2007). In quantitative genetics, there are a number of single genes associated with muscle growth, development and function which were studied as excellent candidates for linkage relationships with quantitative traits of economic importance (Lan *et al*., 2007). A few studies have been carried out on IGF-1 and its relationship with growth and development traits of Balami sheep breed.

MATERIALS AND METHODS

Experimental Location and Animals

The animals used for the study were reared at the Teaching and Research Farm, Borno State University Maiduguri in the year 2023. Fourteen animals, seven each of the male and female Balami sheep breed were used for the research. The morphometric measurements were done prior to tissue sample collection. The laboratory analysis was carried out at the Biotechnology Centre, University of Maiduguri.

Morphometric Traits Measured

Body Weights (BW) were taken using weighing balance (200kg manual weighing scale). Height at Wither (HW) were measured using graduated measuring tape (cm). Body Length (BL) and Heart Girth (HG) were measured using measuring tape (cm).

Animal Tissue Collection

Samples collected after slaughter from three organs (heart, kidney and muscle) from each of the two (2) sexes, seven (7) male and eight (8) female i.e., 15 x 3 = 45 tissue samples. The samples were preserved in RNALater solution before isolation.

RNA Extraction/Separation

Total RNA was isolated using Geneaid (Presto^{TM} DNA/RNA/Protein Extraction Kit) according to the method of Sambrook *et al.* (2012) and preserved at -20^{0}C before gene expression analyses. The extracted RNA was treated with RNase-free DNase to remove contamination. Concentration and quality of RNA extracted was assessed on Nanodrop spectrophotometer (ND-1000).

Purified RNA with optical density between 1.7 - 1.8 (260/280) and 1.9 - 2.0 (260/230) as well as a minimum concentration of 100 ng/µl were selected as templates for gene expression analysis. The isolated RNA was synthesized to cDNA before amplification, then qPCR was performed to ascertain gene expression differences.

cDNA Synthesis

The extracted RNA was converted to cDNA using the FIREScript RT cDNA Synthesis KIT.

Quantitative Real Time Polymerase Chain Reaction (qPCR)

The synthesized cDNA was amplified using the Biorad My iQ Real Time PCR machine®. The qPCR mix used was Solis Biodyne 5x HOT FirePol qPCR supermix.

Amplification for housekeeping gene (GAPDH)

The synthesized cDNA was amplified using the My IQ single-color real-time cycler®. The qPCRmix used was Solis Biodyne 5x HOT FirePol qPCR supermix plus. The reaction was done in 25 µl reactions consisting of 4 µl of the 5x HOT Firepol qPCR Mix, 0.4 µl each of the forward and reverse primers and specific probe which had a concentration of 250 nM, 18.2 µl of Nuclease free water and 2 µl cDNA template (100 ng).

Gene Expression Analysis

Two-step Quantitative Polymerase Chain Reactions (qPCR) protocol was performed using first strand reverse transcription of extracted total RNA into cDNA. The resulting cDNA were then used as the template for the qPCR reactions using the SYBR green qPCR master mix kit. Primers were designed from the mRNA reference sequences of National Centre for Biotechnology Information (NCBI) using the Primer plus and optimized by performing a standard PCR with the cDNA as the template. Appropriate reference gene GADPH was selected as positive control due to stable expression. The comparative Circle Threshold (CT) method for relative quantification were performed for the qPCR. The generated CT values were analysed using the $2^{-\Delta\Delta CT}$ method.

Statistical and Data Analyses

The procedure used for the analysis of the association between IGF-1 gene expression and morphometric traits is the General Linear Model of the statistical package SAS Version 9.2 (2002) using the following model: $Y_{ijk} = \mu + A_k + e_{ijk}$

Where Y_{ijk} is information on the phenotypic value of growth trait (body weights, body length, heart girth and Height at withers) measured on ijk_{th} animal. µ is population mean, A_k is fixed effect associated

with age and e_{ijk} is the random residual effect of each observation. Significant means were compared by the Least Significant Difference method using the same statistical package.

RESULTS AND DISCUSSION

Linear Body Measurements of Body Weight, Heart Girth, Height at Withers and Body Length of Balami sheep breed.

Table 1 shows the Least Squares Means and standard errors of body weight and linear body measurements as affected by age in Balami breed of sheep. The overall means of Body Weight (BW), Heart Girth (HG), Height at Withers and Body Length were 69.98 Kg, 36.96 cm, 36.66 cm and 36.66 cm respectively. In this study, significant effects (P<0.05) on age were observed. Balami sheep had body weight of 93.94 kg. This is similar to the studies of Fayeye *et al*. (2017) who showed that Balami sheep had significantly higher values. Dauda *et al.* (2018) and Abbaya and Dauda (2018) observed similar results in Nigerian sheep breeds.

Effects of Age on Morphometries

The effect of age on linear body measurements was significant (P<0.01). The present study indicated a continuous increase in body weight and other morphometries as the animal progress in age. The mean BW at the lowest age group (38.33 Kg) among three (3) years of age and the mean for the highest age group was 87.38 Kg among eight (8) years of age. The sequence cut across the HG, HW and BL. Age had effect on morphological parameters of Balami sheep, as increase in age lead to increase in the value of morphological parameters. This is also in line with the findings of Dauda *et al.* (2018) who found the effect of age on body traits of Balami breed of sheep, the value of the BL for less than three, seven and eight years were 30.00, 43.20 and 43.88 cm respectively. Abbaya and Dauda (2018), also showed that age significantly influenced body length (BL), height at wither (HW), TL RL and rump wide (RW), with the older animals having higher values than the younger ones in Balami sheep breed. However, Ben-Hamouda and Megdiche, (2021) did not observed regular pattern across the age transitions among Barbary breed sheep in Tunisia.

Table 1: Least Squares Means and Standard Errors of Body Weight and Linear Body Measurements according to age of Balami Sheep Breed

Factors	Traits			
	BW (kg)	**HG** (cm)	**HW** (cm)	**BL** (cm)
Overall means	69.98±3.37	36.96±0.82	36.66±0.96	36.66±0.99
Balami (15)	93.94±4.91^{a}	41.65±1.22^{a}	42.82±1.08^{a}	44.82±0.98^{a}
Age (years)	**	**	***	***
3 (9)	38.33±1.92^{d}	29.67±0.83^{c}	28.33±0.17^{d}	30.00±0.58^{e}
5 (9)	58.50±1.56^{c}	33.50±2.01^{b}	29.50±1.12^{d}	30.50±0.22^{e}
6 (9)	57.50±2.46^{c}	33.00±0.00^{b}	33.50±0.67^{c}	34.00±1.79^{d}
7 (9)	85.40±10.04^{b}	40.80±1.71^{a}	41.20±1.96^{b}	43.20±1.96^{b}
8 (9)	87.38±2.32^{b}	41.07±0.52^{a}	42.25±0.61^{b}	43.88±0.65^{c}

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.

Expression of IGF-1 and IGF-2 based on Ct values

Table 2 provides an analysis of the levels of Insulin-Like Growth Factor-1 (IGF-1) and Insulin-Like Growth Factor-2 (IGF-2) in Balami sheep, categorized by age and sex. The overall means showed that IGF-2 levels are slightly higher than IGF-1 across the studied population. The Balami breed showed high levels of IGFs, suggesting a possible genetic predisposition or better adaptability that influences IGF expression. There is variability in IGF levels with age, with significant differences. IGF levels tended to peak around three and six years, with a noticeable drop at ages five and eight. This fluctuation could be linked to growth phases and metabolic changes over the lifespan of the sheep.

Expression of *IGF-1* and *IGF-2* genes according to Age

The mean IGF-1 and IGF-2 circle threshold values for ages 3,5,6,7 and 8 were 25.64±1.46, 20.18±1.93, 25.28±1.93, 24.13±1.95 and 19.89±0.97, 28.01±1.46, 23.51±1.93, 27.15±1.93, 25.61±1.95 and 22.04±0.97 respectively. Generally, due to relatively higher Ct values, lower level of expression was observed at the early ages of 3-7 years compared with the late ages of 7-8 years. These indicated that there were fluctuations by age in the expression of these genes in the study area. The effect of age on

the expressions of *IGF-1* and *IGF-2* was significant (P<0.05). This is in line with the findings of Sun *et al.* (2014), who observed that age had significant effect on the expressions of *IGF-1* and *IGF-2* genes. Rejduch *et al*. (2010) reported that expression of *IGF-2* gene declined with age. The results also confirmed

the findings of Sun *et al*. (2014) who observed that age had significant effect on the expression of the *IGF-I* gene in sheep. The expression of the *IGF-I* gene in Hu sheep showed a significant difference among the two-day-old, one month-old and three-month-old stages (Sun *et al*., 2014). Huang and Xie (2009) also showed that IGF-I expression differed between the six-month-old stage and the four, three, two, one month-old, and two-day-old stages.

Table 2: Least Squares Means and Standard Errors of Ct of IGF-1 and IGF-2 according to age of Balami Sheep Breed

Factors	IGF-1 (Ct)	IGF-2 (Ct)
Overall means	23.10±0.68[a]	25.33±0.68[a]
Balami (15)	21.96±1.14[b]	23.38±1.14[b]
Age (years)	*	*
3 (9)	25.64±1.46[a]	28.01±1.46[a]
5 (9)	20.18±1.93[b]	23.51±1.93[b]
6 (9)	25.28±1.93[a]	27.15±1.93[a]
7 (9)	24.13±1.95[a]	25.61±1.95[a]
8 (9)	19.89±0.97[b]	22.04±0.97[b]

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.

CONCLUSION

The expression levels of IGF-1 and IGF-2 varied with age. It showed significant (P<0.05) fluctuations that corresponded to growth phases. The knowledge of IGF-1 gene expression can be valuable for selecting fast-growing animals and enhancing production traits through marker-assisted selection.

REFERENCES

Abbaya, H Y., Dauda, A. (2018). Morphormetric Differentiation of Yankasa Sheep in Maiduguri, North-Eastern Nigeria. *Journal of Genetics and Genetic Engineering*, 2 (3): 1-6.

Adejoro, F. A. and Salako, A. E. (2012). Morphobiometric Characterization of the Balami Sheep of Nigeria. *African Journal of General Agriculture*, 8(4):169-173.

Akpa, G. N., Abbaya, H. Y. and Saley, M. E. (2017) comparative evaluation of the influence of species, age and sex on carcass characteristics of camels, cattle, sheep and goats in Sahel environment. *Animal Research International*, 14(1): 2588 – 2597.

Arora, R. and Bhatia, S. (2006). Genetic diversity of magra sheep from India using microsatellite analysis. *Asian Australasian Journal Animal Science,* 19(7): 938-942.

Ben-Hamouda, M. and Megdiche, S. (2021). The Effect of Age and Location Pattern on the Morphometry of Purebred Redhead Barbary Ewes Reared under Arid Climate. *Iranian Journal of Applied Animal Science*, 11 (3): 539-545.

Birteeb, P. T., Olusola, S. P., Yakubu, A., Adeke, M. A. and Ozoje, M. O. (2012). Multivariate characteristics of the phenotypic traits of Djallonke and Sahel sheep in Northern Ghana. *Tropical Animal Health and Production*, 10: 12-14.

Dauda, A., Okon, B., Ibom, L. A. and Abbaya, H. Y. (2018). Phenotypic Characterization of Balami Breed of Sheep in Maiduguri, North-Eastern Nigeria *Nigerian Journal of Animal Science,* 20 (2): 26-32.

Fayeye, T.R. and Fatai, A.I. (2017). Morphometric Characterisation of Uda, Balami and West African Dwarf Sheep. *Wayamba Journal of Animal Science,* P1553 - P1560.

Fowden, A. L. (1995). Developmental regulation of insulin-like growth factors in the fetal sheep. *Reproduction, Fertility and Development*, 7(3): 471-483.

Gluckman, P. D., Morel, P. C., Ambler, G. R., Breier, B. H., Blair, H. T., and McCutcheon, S. N. (1992). Elevating maternal insulin-like growth factor-I by dietary protein supplementation prevents maternal nutritional effects on postnatal growth. *Domestic Animal Endocrinology*, 9(2): 103-108.

Jiang, Y.L, Fan, X.Z., Xiao, L.R., Xiang, R.L., Hu, X.X., Du, L.X. and Wu, C.X. (2002). Association of T-A mutation in the promoter region of myostatin gene with birth weight in Yorkshire pigs. *Asian-Austria Journal of Animal Science*, 15:1543-1545.

Lan, X.Y., Pan, C.Y., Chen, H., Lei, C.Z., Hua, L.S., Yang, X.B., Qiu, G.Y., Zhang, R.F. and Lun, Y.Z. (2007). *Ddel* polymorphism in coding region of goat POU1F1 gene and its association with production traits. *Asian Australian Journal of Animal Science,* 20 (9): 1342-1348.

Mohammed, A., Raji, A.O. and Igwebuike. J.U. (2018). Genetic Variability of Nigerian Sheep Breeds at the Insulin-Like GrowthFactor-1 (IGF-1) Gene Locus. *Nigeria Journal of Animal Production*, 45 (1): 51-60.

Peeters, R., Kox, G., and van Isterdael, J. (1996). Environmental and maternal effects on early postnatal growth of lambs of different genotypes. *Small Ruminant Research,* 19 (1): 45-53.

Sambrook, J., Peter, M., Michael R.G. and Howard, H. (2012). *Molecular Cloning*: *A Laboratory Manual* (Fourth Edition). Cold Spring Harbour Laboratory Press, New York.

Schwerine, M.T., Brockman, G., Vanselow, J. and Seyfert, H.M. (1995). Perspectives of molecular genome analysis in livestock improvement- an overview. *Animal Resource and Development,* 42: 14-26.

Silveira, C., Ferreira, A. M., Sponchiado, M., Gomes, C. M., Viau, P., Leao, B. C., and Pugliesi, G. (2020). Age-related changes in the ovarian function and fertility of ewes. *Theriogenology,* 147: 14-21.

Teixeira, D. L., Miranda-de la Lama, G. C., Villarroel, M., and Maria, G. A. (2021). Effects of age, sex, and live weight on the behavioural and physiological responses of sheep during pre-slaughter handling. *Applied Animal Behaviour Science*, 234, 105175.

Yunusa, A.J., Salako, A.E. and Oladejo, O.A. (2013). Principal component analysis of the morphostructure of Uda and Balami sheep of Nigeria. *International Research Journal of Agricultural Sciences,* 1(3): 45-51.