
EXPRESSION PATTERNS OF OVINE *IGF-1* GENE IN HEART OF SOME NIGERIAN SHEEP BREEDS

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ABSTRACT

Insulin-like growth factor 1 (IGF-1) is encoded by the IGF-1 gene as it is a gene considered to be a candidate marker associated with growth traits in livestock. Tissue IGF-1 concentrations at an early age may be useful indicators of growth rate potential and help in selection of fast growing animals. However, there is dearth of information on gene expression patterns as it affects phenotypic traits in Nigerian sheep. Tissue samples were collected from organ (heart) of the three breeds (Balami, Uda and Yankasa) and five (5) sample from each sex. Messenger RNA was isolated treated with RNase-free DNase RNA converted to cDNA. qPCR protocol were performed using first strand reverse transcription of extracted Messenger RNA into cDNA. cDNA were then used as the template for the qPCR reactions using the SYBR green. The objectives of the study is to determine mRNA expression of ovine Igf-1 gene in heart of some Nigerian sheep breeds. The study showed that the growth-related gene was relatively expressed in heart tissue of the adult sheep. The fold change indicates IGF-1 gene is up regulated in the heart of Uda followed by Balami and Yankasa. This study provides insight into the differential abundance of the gene in heart tissues amongst the three sheep breeds. Uda expresses IGF-1 gene better than Balami and Yankasa breed, these may indicate better performance in Uda breed. Farmers can decide to select Uda for further improvement in their population.

Key words; Expression, Insulin-like growth factor 1 (IGF-1), Ovine and mRNA (messenger Ribonucleic Acid).

INTRODUCTION

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, Brockman, Vanselow and Seyfert, 1995; Lan *et al.*, 2007). In quantitative genetics, there are a number of single genes associated with growth, development and function, which were studied as excellent candidates for linkage relationships with quantitative traits of economic importance. Few studies have been carried out on IGF-1 and its relationship with growth and development traits of animals (Lan *et al.*, 2007). Insulin-like growth factor 1 (IGF-1) is encoded by the *IGF-1* gene (IGF1) as it is a gene considered to be a candidate marker associated with growth traits in livestock (Thue and Buchanan, 2002; Zhang, Zhang, Luo, Yue, Gao, and Jia 2008; Wu-Jun *et al.*, 2010). It has non-suppressible insulin-like activity and is a primary mediator of the effects of growth hormone (Salmon and Daughaday, 1957). The ovine *IGF-1* gene was mapped to chromosome 3 with identification number 443318 (NCBI, 2015). Higher IGF-1 concentrations would have a positive effect on foetal growth and birth weight in sheep (Sun *et al.*, 2014). Variation in IGF-1 levels also correlated with live weight gain (Huang and Xie, 2009).

Tissue IGF-1 concentrations at an early age may be useful indicators of growth rate potential and help in selection of fast growing animals and other useful production traits. However, there is dearth of information on gene expression patterns and molecular evolution of ovine *IGF-1* as it affects phenotypic traits in Nigerian sheep breeds. There is therefore a need to undertake molecular genetic analysis of this gene and show their relationship with growth traits in these breeds of farm animals. This information is required for Marker-Assisted Selection (MAS) and breeding for their future genetic improvement. The objectives of the study is to determine mRNA expression of Ovine *Igf-1* gene in the heart of Some Nigerian Sheep Breeds.

MATERIALS AND METHODS

The samples for the study were collected from the Maiduguri abattoir and some households in Maiduguri and analysed at the Biotechnology Centre, University of Maiduguri and African Bioscience Laboratory, Ibadan, Oyo State. Tissue samples collected from an organ (heart) per animal from the three breeds (Balami, Uda and Yankasa) and five (5) samples from each sex, i.e. $3 \times 5 \times 2 = 30$. Tissue samples were preserved in RNALater solution before analysis. Total RNA was isolated using Geneaid (Presto™ DNA/RNA/Protein Extraction Kit) according to the method of Sambrook, Peter, Michael, and Howard (2012) and preserved at -20°C before laboratory 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). The extracted RNA was converted to cDNA using the FIREScript RT cDNA Synthesis KIT. The synthesized cDNA was amplified using the Biorad My iQ Real Time PCR machine®. A two-step Quantitative Polymerase Chain Reaction (qPCR) protocol were 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 $2^{-\Delta\Delta\text{CT}}$ method.

RESULTS AND DISCUSSION

Table 1 showed the Ct values for heart tissue in Nigerian sheep breeds. *IGF-1* gene was expressed in all the heart of Balami Uda and Yankasa sheep breeds. Insulin-like growth factors 1 across breed, in sheep displayed the highest levels of expression in heart. The highest and the lowest expression of *IGF-1* gene in the heart tissue was observed in Uda (20.89) and in Yankasa sheep breeds (24.29) respectively. The fold change indicates *IGF-1* is up regulated in Uda heart followed by Balami then Yankasa. The expression of the *IGF-1* gene in the heart of sheep plays a crucial role in various physiological processes, including cardiac development, growth, and remodeling. *IGF-1* is a potent growth factor that regulates cell survival, proliferation, differentiation, and metabolism. A fold change value greater than one indicates an increase in the measured variable, while a value less than one indicates a decrease. The fold change indicates *IGF-1* is up regulated in Yankasa heart followed by Balami then Uda. The expression level of *IGF-1* in the heart tissues of Uda breed was significantly higher than that of Balami and Yankasa breed (<0.05).

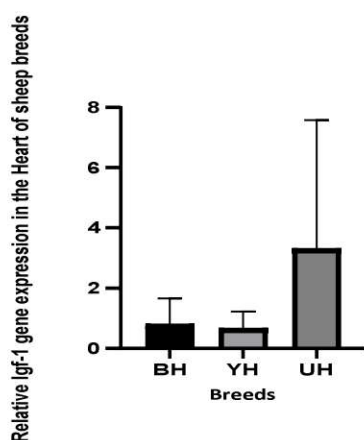
IGF-1 functions to increase amino acid utilization during protein synthesis and inhibits protein degradation, and thus, accordingly, promotes the proliferation of bone and muscle cells. The magnitude of the fold change describes the extent of the change. A higher absolute fold change value indicates a more significant change. Significant difference of expression profiles were observed between the Uda and Balami sheep. *IGF-1* expression levels in the heart was significantly higher in the Uda compared with Balami and Yankasa sheep ($P<0.01$). There was no difference between the expression of *IGF-1* in Balami and Yankasa breeds ($P>0.05$). This is in similar with the findings by Sun *et al.* (2014), who showed that there was significant difference in the expression of *IGF*-in Chinese sheep.

Table 2. Relative Quantification of IGF-1 Gene Expression in Heart Tissue

Breed	IGF-1 Ct	GAPDH Ct	SD IGF-1	SD GAPDH	SE	Δ Ct IGF-1	Avg. Δ Ct IGF-1	$2^{-\Delta\Delta Ct}$ IGF-1	SE
B1H1						1.43			
B2H2						-4.32			
B4H4						1.81			
B5H5						-0.63			
B6H6	23.19	23.68	3.78	2.95	1.96	-0.74	-0.49	1.40	0.20
Y1H1						-4.74			
Y2H2						-2.09			
Y3H3						0.74			
Y4H4						-0.16			
Y5H5						1.15			
Y6H6	24.29	24.88	5.27	3.33	2.54	1.62	-0.09	1.06	0.25
U1H1						-0.98			
U2H2						-4.89			
U3H3						-6.47			
U4H4						-1.41			
U5H5						-2.69			
U6H6	20.89	24.13	5.55	4.13	2.82	-3.02	-2.66	6.32	0.28

GAPDH Ct: Glyceraldehyde-3-Phosphate Dehydrogenase Circle Threshold, IGF1 Ct: Insulin-Like Growth Factor-1 Circle Threshold, SD: Standard Deviation, SE: Standard Error, BH: Balami Heart, YH: Yankasa Heart and UH: Uda Heart.

The ovine mRNA *IGF-1* gene was highly expressed in Uda, moderately expressed in the heart of Balami and weakly expressed in Yankasa (Figure 1). This is similar to the observations made by Gu, Zhang, Li and Li (2009); Xu (2002); Huang and Xie (2009) and Sun *et al.* (2014) who revealed that the *IGF-1* gene is expressed in heart tissue of sheep.

**Figure 1:** Relative Expression of IGF-1 in the heart of some Nigerian sheep

CONCLUSION

This study showed that the growth-related genes was relatively expressed in the heart of all the three breeds of sheep. The study provides insight into the differential abundance of genes in heart tissue amongst the three sheep breeds. Overall, gene expression in sheep plays a crucial role in their growth and development. Studying the gene expression patterns of *IGF-1* in tissues of sheep provides valuable insights into the roles of *IGF-1* in growth and development. Understanding gene expression can help scientists improve sheep breeding programs, enhance production efficiency and develop

strategies and management. Uda breed expresses *IGF-1* gene better than Balami and Yankasa breed, these may indicate better performance in Uda breed. Farmers can decide to select Uda breed for further improvement in their population. Therefore, complementary experiments and understanding of its mechanisms can be used to improve animal performance using this gene's diversity of expression.

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