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Association of IGF 1 gene polymorphisms with some morphometric traits of Nigerian indigenous sheep breeds

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Abstract

The relationship between IGF 1 gene polymorphisms and some morphometric traits of the Balami, Uda and Yankasa sheep breeds of Nigeria was investigated. Blood samples and morphometric measurements were obtained from 150 sheep (50 for each of the three breeds) at the Maiduguri Livestock market and abattoir while DNA was extracted at the Biotechnology laboratory of the University of Maiduguri. Evaluation of results revealed 2 alleles (A and B) and 3 genotypes (AA, AB and BB) for all the breeds. The Uda and Yankasa breeds had higher frequency of allele B (0.64 and 0.56, respectively) while Balami had higher allele frequency for A (0.61). Balami and Yankasa had high heterozygosity for IGF-1 gene while Uda had high homozygosity for B. For Balami sheep, the genotype BB had higher (P < 0.05) body weight and heart girth (56 kg and 79 cm, respectively) than AB for body weight (45.80 kg) and BB for heart girth (69.67 cm). Conversely, genotype AA had higher body length (94.33 cm) than AB (73.80 cm). IGF 1 gene polymorphisms did not significantly (P < 0.05) affect most morphometric traits of the Yankasa and Uda sheep except height at withers where the genotype AB had higher (P < 0.05) height at withers (71.77 cm) than AA (53.50 cm) for Yankasa sheep. Thus, the three breeds were found to be 100% polymorphic at the regulatory region of IGF-1 locus and this gene may be used as a marker for some morphometric traits in Nigerian indigenous sheep.

Keywords: body weight, heart girth, height at withers, breeds, sheep

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Introduction

The population of sheep in Nigeria is about 8 13.2 million and majority of them are found in the Northern region of the country (Bourn et. al., 1986). There are four major breeds of sheep in Nigeria with the Balami, Uda and Yankasa breeds being wide spread in the Northern region of the country and the WAD in the South. Sheep have characteristic features that make them survive by enabling them to tolerate the peculiarities of their environment (humid or arid). For example, they have long legs to walk long distances in search of food and water in arid environments (Yunusa et. al., 2013).

The growth of any organism is under the influence of factors such as genotype, hormones and nutrition in a complex interaction. Sheep that grows fast; breeds early, produce more lambs and reach market weight early, ensuring quicker returns to the farmer (Akinyemi and Salako, 2012). In order to maximise production in animal breeding, selection for improvement is a very important strategy. However, the design of such improvement programmes requires genetic parameter estimates which are necessary to predict genetic gains. Rashidi et. al. (2008) observed that, selection must be based on genetic merit instead of phenotype for genetic progress because factors such as age, sex and, type and year of birth influence their estimation.

Insulin-like growth factor I (somatomedin C) is a candidate gene located in the euchromatin region of chromosome 3. Because of its involvement in the expression of complex traits, the association of this gene polymorphisms with economically important traits in farm animals have been investigated (Rothschild and Soller, 1997). Growth, skeletal and immune function traits and their association with IGF 1 gene have been examined using this approach in sheep (Adam and Maddox, 1993; He et. al., 2003; Bahrami et. al., 2013). Insulin-like growth factor I gene plays an important role in childhood growth and stimulates skeletal growth, cell differentiation and metabolism in adults. IGF-1 also functions in the regulation of cell growth and development especially in nerve cells, skeletal muscle, cartilage, bone, liver, kidney, nerves skin, hematopoietic cell and lungs as well as cellular DNA synthesis (Sakowaski et. al., 2009; Sattler, 2013). Thus, IGF-1 gene is considered to be a candidate gene for predicting growth and meat quality traits in animal genetic improvement schemes (Machado et. al., 2003; Andrade et. al., 2008). Several reproductive traits (duration of pregnancy, twin ovulations and pre-implantation embryo development) have also been associated with IGF-1 gene in cattle (Sirotkin et. al., 2003; Echternkamp et. al., 2000; Velazquez et. al., 2005). IGF-1 might be a strong candidate gene for reproductive traits as well.

Some methods such as Restricted Fragment Length Polymorphism (RFLP), Amplified fragment length polymorphisms (AFLP), Random Amplification of Polymorphic DNA (RAPD) and Simple Sequence Repeat (SSR) are used to study the genome or phylogenetics of animals. In addition, genetic maps of any animal being studied can be created using some of these methods. Genomic response to selection may also be measured in livestock using genetic markers. Thus, selected and non-selected livestock may be differentiated by presence of different alleles due to a distorted segregation at the genetic markers (Maheswaran, 2014).
The gene for Insulin-like Growth Factor 1 (IGF1) may play important roles in the growth of multiple tissues, including mammalian muscle. Therefore, it has been proposed as a candidate gene for growth traits in farm animals as it could be used in Marker-Assisted-Selection (Honarvar et. al., 2012). IGF-I intron-2 polymorphisms was found to correlate positively with the twinning rate (Kim et. al., 2009). The association of IGF-I gene polymorphisms (5'-flanking region) with birth and weaning weight was reported by Li et. al. (2004). However, Curi et. al. (2004) found no such association.

The aim of this study was to identify IGF 1 gene polymorphisms by PCR-RFLP and evaluate the association between these polymorphisms and morphometric traits of some Nigerian indigenous sheep breeds.

Materials and Methods

The blood samples and morphometric traits for the study were obtained at the Maiduguri livestock market and blood samples were analysed at the Biotechnology Centre, University of Maiduguri. Maiduguri, the Borno State capital is located on Latitude 11°52N and Longitude 13°14E of the Equator. It occupies an area of about 3,000km^2, and is the largest town in North-Eastern Nigeria. The weather condition of the area, for most part of the year, is hot and dry with short raining season in the months of June to September. The soil of the study area is generally sandy-loam.

Maiduguri lies in the Sahel ecological zone, characterized by few trees and vast grassland. The grasses are seasonal and disappears during the dry season. The agricultural activities in the area include arable farming, livestock farming, fishing and hunting. Other major activities in the area, include, trading and weaving of caps.

Blood samples for DNA extraction, were collected from a total of one hundred and fifty (150) adult sheep through the jugular vein, using needle and syringe (5 ml) and preserved in EDTA bottles. Fifty (50) each of Balami, Uda and Yankasa sheep, were randomly sampled. The samples were conveyed to the Biotechnology Centre, University of Maiduguri inside a cooler with ice pack.

Standard kit (Promega, UK) was employed in extracting genomic DNA from the sheep blood samples. Polymerase Chain Reaction was carried out on 50 µl volume containing approximately 1.0 µl of 0.2 µM each of primer (Table 1). 10 µl of 5X GoTaq colourless buffer, 5 µl of 25 Mmol/$MgCl_2$, 1.0 µl of 10 µM each of dNTPs, 2 µl of 50 µM/µl GoTaq polymerase (Promega USA) and 29.25 µl nuclease free water. PCR condition were as follows; initial denaturation of 95^{0}C for 5 minutes, followed by 35 cycles of denaturation at 95^{0}C for 60 seconds, annealing between 55 and 60^{0}C for 30 seconds, extension at 72^{0}C for 60 seconds, with final extension at 72^{0}C for 5 minutes on Master cycler (Eppendorf AG.2233, Hamburg Germany).

RLFP analysis was performed by incubating a mixture of 5 µL of regulatory region of PCR product, 16.3 µL of distilled water, 2.5 µL of enzymes buffer and 1.2 µL of *Hae II/BSP 143 II* (New England Biolabs, Berverly, M.A. USA) at 37^{0}C for 3 hours inside a water bath, allowing the restriction enzymes to cut, at its restriction sites. A 1 % agarose was prepared by weighing 1g of agarose powder and dissolved in 100 ml of 1X TAE buffer then swirled to ensure thorough mixing. The dissolved agarose is microwaved for about 3-4 minutes and allowed to cool to about 60 ^{0}C. Ethidium bromide (10 µL) was added and mixed avoiding bubbles. The gel cassette was assembled and the gel, poured in to the gel trough. The gel comb was inserted and left to set for about 30 minutes. 1X TAE buffer was poured into the electrophoresis tank till it fills up. The gel comb was removed carefully from the solidified agarose, and the side rubber seal dissembled.

The set agarose gel was transferred to the gel tank, ensuring the buffer floods at least 2 mm above the gel. The comb grooves were labeled according to the samples, to identify the lines on the gel photograph. Loading dye (2 µL) and 5 µL of PCR amplicons transferred on to a spread sheet of parafilm M then mixed using 10 µl pipette and the mixture transferred to the gel well. The gel tank was closed and power cords connected. Power pack was switched on and the gel allowed to run for 30 minutes at 120 V. The progress of the gel was monitored by reference to the marker dye. The resulting amplified bands were visualized with UV transilluminator (CSL-MICRODUC System Cleaver Scientific UK) fitted with camera (Canon power short GI2 UK).

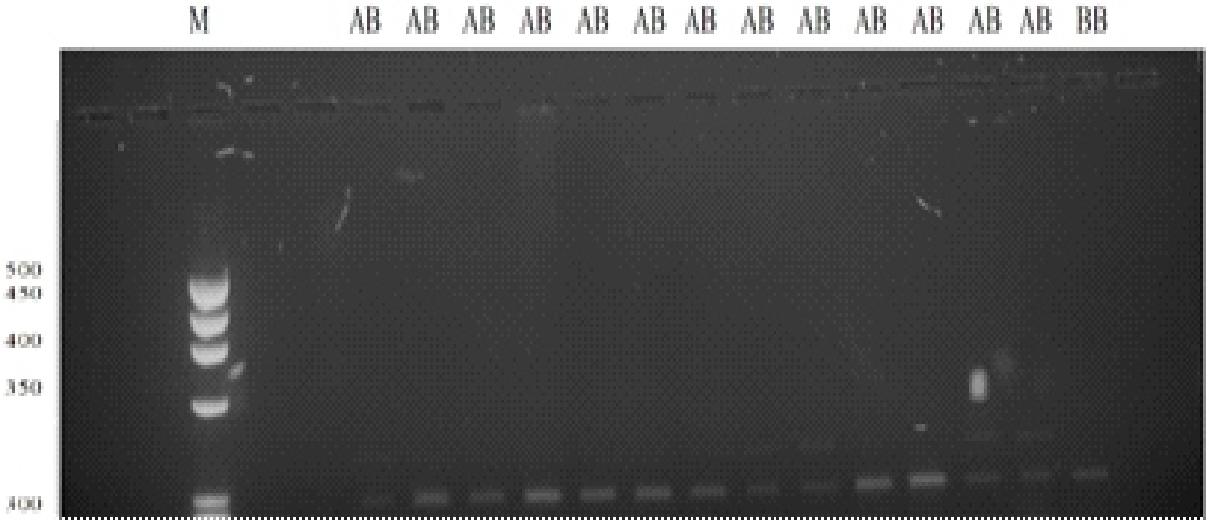

Plate 1. Yankasa sheep

Plate 2. Genetic Profile for Uda Sheep

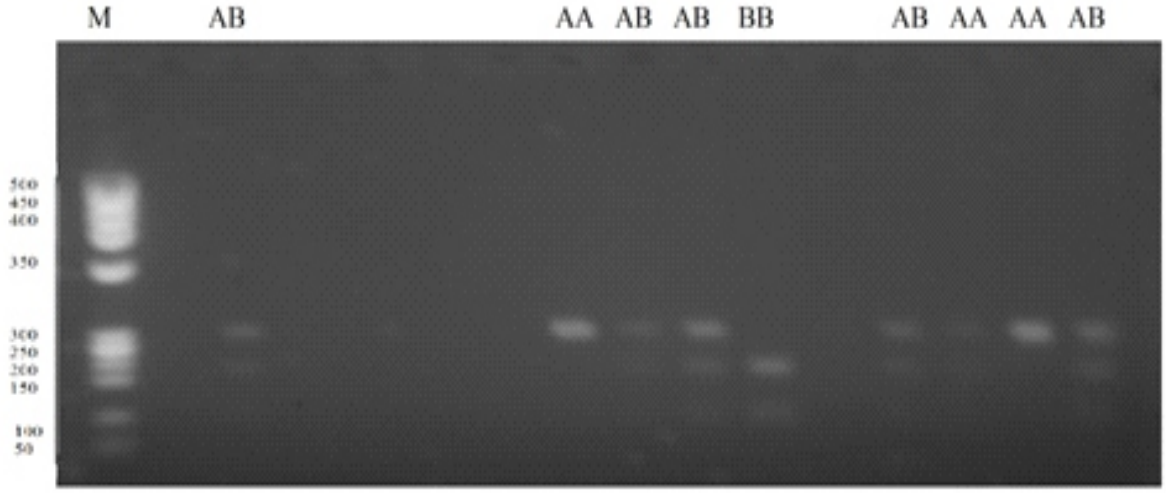

Plate 3. Genetic Profile for Balami Sheep

Table 1: Primers used to amplify sheep genomic DNA for IGF -1 gene analysis.

Primer	Product size AT (^{0}C)	Bp
5′ regulatory region F: TGAGGGAGCCAATTACAAAGC R: CCGGGCATGAAGACACACACAT	55	294
Exon 1 F: TCACTGTCACTGCTAAATTCAG R:CTTCAAGAAATCACAAAAGLAGCAC	60	228

The morphometric traits were measured thus:
Body Weight (BW): The weight of sheep was taken using a hanging weighing balance and the reading taken when the animal had stopped moving.
Height at withers (HW): This was measured as the distance from the ground to the points of the withers with a measuring stick graduated in centimeters.
Body length (BL): This was measured as the distance from the occipital protuberance to the base of the tail. It was measured with a measuring tape (cm).
Heart girth (HG): This was measured as the narrowest circumference immediately posterior to the front legs with a measuring tape.
The procedure used for the analysis of the association between IGF-1 polymorphisms and morphometric traits was the General Linear Model of the statistical package SPSS, with the following model:

$Y_{ij} = \mu + G_i + \varepsilon_{ij}$

where Y_{ij} = dependent variable; μ = general mean; Gi = effect of the i-th genotype of IGF1 (AA, AB, BB) and εij = random error. Significant means were compared by the Least Significant Difference method in the same statistical package.

Results and Discussion

The genotype and allele frequencies of the three sheep breeds are presented in Table 2. Evaluation of results revealed 2 alleles and 3 genotypes for all the breeds (Plates 1, 2 and 3). The alleles were A and B and genotypes AA, AB and BB. Thus, the three breeds were found to be 100% polymorphic with two different alleles in the 5'regulatory region of IGF-1 locus and monomorphic in exon 1 for the population. However, Hajihosseinlo et. al. (2013) reported polymorphisms of IGF 1 gene at exon 1 in Makui sheep and Kim et. al. (2009) in intron 2. This inconsistency may be due to breed differences. The Uda and Yankasa had high frequency of allele B (0.64 and 0.56, respectively) while Balami had high allele frequency for A (0.61). Bahrami et. al. (2013) observed high allele frequency for A in a study on Mehraban sheep though the authors used the SSCP variant of IGF-1. The frequencies of the genotypes are: Balami; AA (0.33), AB (0.56) and BB (O.11); Uda; AA (O.21), AB (0.29), and BB (0.50) and Yankasa; AA (0.21), AB (0.46) and BB (0.33). The allelic frequencies are Balami A (0.61) and B (0.39), Uda A (0.36) and B (0.64) and Yankasa A (0.44) and B (0.56). Balami and Yankasa had similar patterns of genotype distribution while that of Uda was different. Balami and Yankasa had high heterozygosity for IGF-1 gene while Uda had high homozygosity for B. He et. al. (2012) and Bahrami et.. al. (2013) also observed three genotypes (AA, AB and BB) in Mehraban sheep in Hamedan Province, Iran and Chinese sheep (small tail Han, Hu, Texel and Dorset ewes). However, the genotype frequencies observed by He et. al. (2012) and Bahrami et. al. (2013) were different from those recorded in this study as their results indicated higher homozygosity for AA. This may be due to difference in breeds. Genetic variation describes naturally occurring genetic differences among individuals of the same species. This variation permits flexibility and survival of a population in the face of changing environmental circumstances. Consequently, genetic variation is often considered an advantage, as it prepares the breed or species for the unexpected.

Table 2: Genotype and Allele Frequencies of IGF -1 in Nigerian Sheep

		Genotype Frequency			Allele Frequency	
Breed	**Locus**	**AA**	**AB**	**BB**	**A**	**B**
Balami	IGF -1	0.33	0.56	0.11	0.61	0.39
Uda	IGF -1	0.21	0.29	0.50	0.36	0.64
Yankasa	IGF -1	0.21	0.46	0.33	0.44	0.56

The Effects of IGF 1 gene polymorphisms on morphometric traits of the Balami sheep are presented on Table 3. IGF 1 gene polymorphisms significantly ($P < 0.05$) affected most morphometric traits of the Balami sheep except height at withers. The genotype BB had higher ($P < 0.05$) body weight and heart girth (56 kg and 79 cm, respectively) than AB for body weight (45.80 kg) and BB for heart girth (69.67 cm). Conversely, genotype AA had higher body length (94.33 cm) than AB (73.80 cm). It is not surprising that genotype BB with a higher heart girth measurement also had a higher body weight. This may be due to the fact that heart girth is a measure of condition and higher heart girth measurements usually translates to a higher body weight. The result also showed that all genotypes did not differ in height due to non-significant genotype effect on height at withers.

Table 3: Effects of IGF 1 gene polymorphisms on morphometric traits of the Balami sheep

		Genotype		
Morphometric traits	**Overall mean**	**AA**	**AB**	**BB**
Body Length (cm)	82.00 ± 6.21	94.33 ± 5.97 ^{a}	73.80 ± 4.62 ^{b}	86.00± 10.34 ^{ab}
Heart girth (cm)	74.00 ± 6.20	69.67 ± 7.39 ^{b}	75.60 ± 5.72 ^{ab}	79.00 ± 12.79 ^{a}
Height at withers (cm)	77.22 ± 5.38	78.00 ± 4.35	75.40 ± 3.37	84.00 ± 7.54
Body weight (kg)	49.33 ± 7.81	53.00 ± 8.24 ^{ab}	45.80 ± 6.30 ^{b}	56.00 ± 14.23 ^{a}

Means within rows with different superscripts are significantly different ($P<0.05$)

The Effects of IGF 1 gene polymorphisms on morphometric traits of the Yankasa sheep are presented on Table 4. IGF 1 gene polymorphisms did not significantly ($P < 0.05$) affect most morphometric traits of the Yankasa sheep except height at withers. The genotype AB had higher ($P < 0.05$) height at withers (71.77 cm) than AA (53.50 cm). This might indicate that the AB genotypes were taller than the others. However, the three genotypes AA, AB and BB did not differ significantly ($P > 0.05$) for body length, heart girth and body weight.

Table 4: Effects of IGF 1 gene polymorphisms on morphometric traits of the Yankasa sheep

		Genotype		
Morphometric traits	**Overall mean**	**AA**	**AB**	**BB**
Body Length (cm)	69.68 ± 5.37	58.00 ± 6.62	70.94 ± 2.27	70.00 ± 3.82
Heart girth (cm)	66.96 ± 5.33	55.50 ± 7.56	68.53 ±2.59	66.33 ± 4.37
Height at withers (cm)	68.72 ± 4.93	53.50 ± 7.66 ^{b}	71.77 ± 2.62 ^{a}	65.17 ± 4.42 ^{ab}
Body weight (kg)	42.44 ± 4.05	38.00 ± 6.89	43.47 ± 2.36	41.00 ± 3.98

Means within rows with different superscripts are significantly different ($P < 0.05$)

The Effects of IGF 1 gene polymorphisms on morphometric traits of the Uda sheep are presented on Table 5. IGF 1 gene polymorphisms did not significantly ($P < 0.05$) affect any morphometric traits of the Uda sheep. The three genotypes AA, AB and BB did not differ significantly ($P > 0.05$) for body length, heart girth, height at withers and body weight. Nazari et. al. (2016) also reported non-significant effect of IGF 1 gene polymorphisms on early growth traits of Iranian Zandi sheep. Similarly, Hajihosseinlo et. al. (2013) earlier reported non-significant effect of IGF 1 gene polymorphisms on height at withers of Makui sheep in Iran. The authors however observed significantly ($P < 0.05$) higher body length measurements for BB genotype compared to AA and AB.

Table 5: Effects of IGF 1 gene polymorphisms on morphometric traits of the Uda sheep

Morphometric traits	Overall mean	Genotype AA	AB	BB
Body Length (cm)	71.35 ± 7.56	74.00 ±10.47	71.95 ± 9.06	70.00 ± 6.85
Heart girth (cm)	61.78 ± 6.42	63.33 ± 9.28	63.75 ± 8.45	60.00 ± 6.07
Height at withers (cm)	67.51 ± 8.01	72.00 ± 9.62	67.75 ± 8.51	65.57 ± 6.43
Body weight (kg)	41.93 ± 5.00	44.60 ± 6.21	43.50 ± 5.38	39.86 ± 4.07

Means within rows with different superscripts are significantly different (P<0.05)

Conclusion

The IGF 1 gene was 100% polymorphic in Balami, Yankasa and Uda sheep breeds. Significant association was established between IGF 1 genotype and some morphometric traits in Balami and Yankasa sheep. This might indicate that it could be used as a genetic marker for such traits.

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