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Single nucleotide polymorphism in the insulin-like growth factor-1 gene and its effects on growth traits in Yankasa sheep

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Target Audience: Animal Breeders, Farmers, Animal Physiologist and Scientists.

Abstract

A study was conducted to determine single nucleotide polymorphism of the IGF-1 gene and its effect on some growth traits in Yankasa breed of Sheep. Random samples of 100 sheep (50 males and 50 females) were selected for the molecular study and the phenotypic evaluation. Animals were measured for growth traits namely: birth weight, average daily gain, weaning weight, weights at 6, 8 and 12 months, chest girth and height at withers. Blood samples were collected from the animal's neck region through the jugular veins into 0.5ml EDTA vacutainer tubes and transferred to the laboratory for DNA extraction. Total DNA extraction was made with ZR-96 Genomic DNA miniprep. Frequency of alleles were calculated according to Hardy-Weinberg's equation and also subjected to Chi-Square analysis to test for Mendelian inheritance ratio for band. Genotypes, growth traits, sex and interaction were determined through statistical analysis. Both genotypic and allelic calculated χ^2 values (9.07 and 16.94) for Mendelian inherited ratio were greater than the tabulated values of 5.99 and 3.84 at 5% level of significance. All growth traits (birth weight, weaning weight, average daily gain, weight at 6 months, weight at 12 months, height at withers and chest girth) with the exception of body weight (Kg) at 8 months showed significant ($P<0.05$) variations. With the exception of average daily gain (g/day), which was non-significant ($P>0.05$) across the sexes, all other growth characteristics differed significantly ($P<0.05$) across sexes with the highest traits in the male compared to the females. Observed trend showed significant ($P<0.05$) interactions among the measured traits with the exception of average daily gain. Male Sheep with AA and AB genotype were similar in performance across all traits (birth weight, average daily gain, weaning weight, weight at 6 months, weight at 8 months, weight at 12 months and height at withers) with the exception of chest girth (cm). It can be concluded that Yankasa sheep with AA genotype had significant higher propensity for growth than those with genotype AB and BB.Yankasa sheep with AA genotype could be used for genetic improvement programs targeted to growth traits in Nigeria. It is recommended that polymorphism of the IGF-I gene may be a potential molecular marker for growth traits in Yankasa sheep.

Keywords: Yankasa sheep, Single nucleotide polymorphism, Insulin-like growth factor, Growth traits.

Description of problem

The economic importance of Sheep in developing nations cannot be over-emphasized. Sheep with their small body size, high productive capacity and rapid growth rates are ideally suited to production by resource-poor smallholders. The population of Sheep in Nigeria is currently estimated at 33.9

million making up 3.1% of the world's total (1). The Yankasa is a meat breed in north and north central Nigeria. The Yankasa is a medium-sized breed of Sheep, bred and selected for fecundity, high birth weight, rapid growth rate and good performance of offspring.

The use of polymorphic genes as a detectable molecular marker is a promising alternative to the conventional methods of traits selection once these genes are proven to be associated with traits of interest in animals (2).

The main objective of the application of molecular biology techniques to animal genetic improvement programs currently consists in identifying, mapping, and analyzing polymorphisms of the genes involved in the main metabolic pathways that are related to animal growth and distribution of nutrients to the different tissues (3). Recently, investigators and breeders focus on Marker-Assisted Selection (MAS) and genome analysis. The GH gene pathway contains various interdependent genes, such as GH, insulin-like growth factor1 (IGF1), pituitary specific transcription factor1 (PIT1), growth hormone releasing hormone (GHRH), somatostatin growth hormone releasing hormone receptor (GHRHR), growth hormone receptor (GHR), and others (4). For growth traits, GH, GHR, insulin-like growth factor I (IGF-I), leptin (LEP), caprine-pituitary-specific transcription factor-1 (POU1F1), caprine myostatin (MSTN), and bone morphogenetic protein (BMP) genes are necessary for bone formation, birth weight, weaning weight, body condition, and muscle growth (5). Insulin-like Growth Factor-1 (IGF-1) gene is also considered to be a factor that regulates growth, differentiation and the maintenance of differentiated function in numerous tissues and in specific cell types of mammals through binding to a family of specific membrane-associated glycoprotein receptors (6). The predicted sequence of amino acid of IGF-I peptide differs from the human, bovine, and porcine IGF-Is at a single amino acid (at position 66, alanine is substituted for proline) and differs from rat and mouse IGF-Is at positions 4 and 5, respectively.

Polymorphic traits are used as a selection criterion in species with longer generation intervals and traits of great economic importance (7). Keys to the emergence of genomics were advances in DNA marker technology. These advances have resulted in a wealth of genetic markers including allozymes, mtDNA, RFLPs, RAPDs, AFLPs, microsatellites SNPs, and ESTs with potentially widespread utility in a variety of livestock endeavors. A marker with high polymorphism is easier to identify which also means better selection performance (8). With markers, the generation interval could be reduced or halved while doubling the genetic gain and maintaining the same increase in accuracy of selection (9). The principles, potential power, advantages and disadvantages, requirements and application of single nucleotide polymorphism in assessments of genetic variability and inbreeding, parentage assignment, species and strain identification, hybridization, and marker-assisted identification of quantitative trait loci (QTL) through the construction of genetic linkage maps. Therefore, breeding for optimal growth traits and higher gains are the main considerations in goat and Sheep breeding programs. Most genetic variation is represented by single nucleotide polymorphisms and many of them are believed to cause phenotypic differences between individuals.

The objective of this study was to search for SNP in the IGF-I gene in Yankasa Sheep and to determine growth traits of Yankasa Sheep. This is intended to be the first step of a more in-depth study of the IGF-1 gene of the Yankasa Sheep breed in order to establish a

breeding program based on marker assisted selection.

Materials and Methods

Experimental site

The research was carried out at the Small Ruminant Research Programme of National Animal Production Research Institute (NAPRI) Shika, Zaria, Kaduna State, Nigeria. Shika lies between latitude 11^0 12'N, longitude 7^0 33'E and at an altitude of 640m above sea level. The area falls within the Northern Guinea Savannah having an average annual rainfall of 1100mm which starts from late April or early May to mid-October, followed by a dry period (which is divided into early and late dry periods). The early dry period is characterized by cold period and lasts from November to February. The mean temperature is about 24.4^{o}C (14.5-39.5^{o}C) with the lower humidity of 21% and 72% occurring during the early dry and wet seasons respectively (10).

Experimental Animals and their management

The animals were kept separately at the Experimental Unit of NAPRI and reared under semi-intensive system. The breed was randomly selected among the stock at NAPRI. The ewes that were selected for the study were between two and three years. All the sheep were maintained on concentrate diets. Supplementary feeding was provided to the advance pregnant and lactating ewes and young lambs. The sheep were housed during night in sheds covered with asbestos sheets with open sides during winter and in open corrals made by chain link fencing during summer months. Space of about 1.5 sq. meters per sheep was provided. The sheep were fed concentrate supplements (3% of body weight) in the morning at 8:00 am. The concentrates were compounded using cotton seed cake, ground maize grain or maize offals, bone meal, vitamin and mineral premix and salt to make a diet of 18% crude protein (CP) for weaners and 15% CP for yearlings and other adult sheep. Gamba grass (*Andropogon gayanus*) hay was also provided after the concentrates. Nursing ewes and their lambs were kept intensively up to weaning at 90 days while the other sheep in the flock were daily released to graze on improved pastures of *Digitariasmutsii; Bracharia decumbens;* Gamba grass; *Cynodon dactylon and Hyperenia rufa*, between 8:00 am and 4:00 pm. Drinking water was provided *ad libitum*. More feed were allocated to pregnant ewes during the last two month of pregnancy corresponding to 3% of body weight. Routine medication consisted of anti-helminthic, drenching (deworming) which was carried out every month for the suckling lambs and every 45 to 60 days for the weaned and adult sheep.

Selection of sheep for blood sampling

Blood samples (6ml) were collected through jugular venipuncture in the morning (7.00am) from 140 randomly selected ewes (n=70) and rams (n=70) of Yankasa sheep within the age range of 2-3 years. The blood samples were placed in ethylenediamine tetra-acetic acid (EDTA) tubes to prevent coagulation and were transported in ice-pack to the African Biosciences Laboratory, Ibadan, Nigeria.

Genomic DNA extraction

The blood samples were transferred to eppendorf tubes and frozen at -40˚C for storage. The DNA extraction procedure was performed at room temperature (15-30˚C).

(a) Lysis step

50uL of whole blood sample was placed in the eppendorf tube. Add 200uL of genomic lysis buffer (mercaptoethanol added) to make a 4:1 volume of genomic lysis buffer to the sample. Samples were vortexed for 4-6 seconds, then kept at room temperature for 5-10 minutes. The mixture was transferred to a spin column

in a collection tube and centrifuged at 10,000g for one minute, then the collection tube was discarded with the flow.

(b) Wash step

The spin column was transferred to a new collection tube. 200uL of DNA was added to pre-wash buffer to the spin column and centrifuged at 10,000g for one minute. 500uL of g-DNA wash was added to the buffer in the spin column and was centrifuge at 10,000g for one minute**.**

c) Elution step

The spin column was transferred to a clean micro centrifuge tube. 50uL DNA Elution buffer was added to the spin column. The samples were incubated at room temperature for 2-5minutes and centrifuge at top speed (16,000g) for 30seconds to elute the DNA and stored at -200^{o}C for future use.

Gel check

One percent (1%) agarose gel was prepared by placing 1g of agarose powder in a conical flask, make up to 100ml with Tris buffer and allowed to dissolve using a microwave for 2-3minutes. The gel was allowed to cool by running water at the base of the flask for a few minutes until it is no longer hot to touch (ensure the gel does not solidify while cooling). 5uL of ethidium bromide was added to the flask. Gel was poured into the tray and insert combs and the gel was allowed to solidify for approximately 20minutes. Samples were prepared by mixing 5UL of the extracted DNA sample with 1uL of the loading dye. 6uL of sample/loading dye was loaded into a well (one sample per well). The gel was run at 100volts for 50minutes and the gel was placed in the UV transilluminator to visualize.

The allele and genotype frequency was estimated by direct gene counting method as described by (11) and was used to score IGF-1 bands based on the separation of electrophoretic migration as follows;

- A high faster band was designated as AA homozygote.
- The presence of a single slower band was designated as BB homozygote.
- Any of a mixture midway between fast and slow bands was designated as AB heterozygotes.

This analysis was carried out at African Biosciences Laboratory, Ibadan.

Primer sequencing results

Primers were designed according to the mRNA sequence of *Ovis aries* IGF1 gene (GenBank No. NM_001009774) and two EST sequences of *Ovis aries* IGF1 gene (GenBank No. X69472 and X17229). Primer sequence, amplified region, annealing temperature and product size are as listed in Table 1. Three genotypes with two alleles were detected by the primer used.

Body weight measurement

The body weight of lambs at birth was recorded within 24 hrs of lambing. The lambs were subsequently weighed at three months of age and thereafter six, eight and twelve months of age in the morning before they had any access to water.

Weaning weight: Measured as the weight of the dam when its offspring was separated

Average daily gain in the body weight of individual animal was calculated by using the following formula below:

$$\textbf{ADG} = \frac{W_2 - W_1}{t_2 - t_1}$$

Where: W_2= Final body weight (kg)

W_1= Initial body weight (kg)

t_2 = Age of the animal at the end of period (days)

t_1 = Age of the animal at the beginning of period (days)

Table 1 :The sequence, amplified region, annealing temperature and product size of the primer for Sheep IGF-1 gene analysis

Primer	Primer Sequence(5' - 3')	Amplified Region	Annealing Temp (^{0}C)	Product Size (bp)
P1	R: CATATTTTTCTGCATAACTTGAACCT F: TGAGGGGAGCCAATTACAAAGC	5'regulatory region	55	294

Body linear Measurements

Height at wither: Measured by taking the measurements of the circumference of the chest behind the forelegs.

Chest girth: Measured as a distance from the surface of the platform to the withers of the animal.

Data collection

As stated above, the Yankasa Sheep was measured for growth traits (birth weight, weight at 3, 6 and 9 months, average daily gain, weaning weight, chest girth and height at withers) each of the experimental Sheep was scored according to its band for the SNPs either as fast (AA), midway (AB) or slow (BB).

Statistical analysis

On the basis of identified genotypes of Yankasa Sheep, the frequency of alleles was calculated according to Hardy-Weinberg's equation (7). This equation is based on the binomial expansion of $(p+q)^2=1$ which gives $p^2+2pq+q^2=1$

Where; p = Dominant allele

q = Recessive allele

The traits of interest were collected and analyzed using the General Linear Model (GLM) procedure of the SAS program (12), the following statistical model was used.

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

Where:

Y_{ij}= growth traits,

μ = the overall mean,

G_i= the fixed effect of the i^{th} genotype for IGF-1,

e_{ij}=the random residual error.

Mendelian inheritance ratio for band (genotype) inheritance was tested using chi-square test. The Chi-square test is denoted by:

$$\chi^2 = \frac{\sum(O-E)^2}{E}$$

Where, O= Observed frequency of the allele

E= Expected frequency of the allele

Results and Discussion

Table 2 shows the frequency distribution of genotype and alleles observed for IGF-1 polymorphism in Yankasa sheep.

The AA genotype had the highest frequency with a value of 0.58 followed by AB (0.28) and BB (0.14). Allelic frequency observed were 0.72 for the faster polymorph (A) and 0.28 for the heavier but slower polymorph (B).

The Chi Square distribution table of IGF-1 gene polymorphism in the Sheep population is shown in Table 3. Both genotypic and allelic calculated χ^2 values (9.07 and 16.94) for Mendelian inherited ratio were greater than the tabulated values of 5.99 and 3.84 at 5% level of significance.

Observed allelic frequency of 0.72 and 0.28 for the A and B polymorphs of IGF-1 gene were similar to the values (0.71 and 0.29) reported in Zel sheep population and (0.76 and 0.24) in Mehraben sheep (13 and 14). While this differed from the values of (0.89 and 0.11) reported in Iranian Baluchi sheep (15). This difference in the frequency of the polymorphs of IGF-1 gene variants may be due to different genetic selection programs being used in different countries, or because some of the IGF-1 gene variants are breed-specific, or simply because insufficient sheep were studied

to fully reveal the variation that exists in sheep. These genetic differences need further clarification by investigating more sheep samples and breeds, from different areas or countries. There existed conspicuous absence of other polymorphic variants such as the C and G alleles reported in literature (16 and 17). The frequency of Variant B polymorphs of IGF-1 gene was low in Yankasa sheep (28.0%). This might suggest that variant B may negatively associate with a trait or traits that have been selected against historically.

The frequency of A variant in this study was low compared to the frequency of 0.89 for A variant reported by (15) in Iranian Baluchi sheep. This was subsequently confirmed by identification of three intron-3'UTR haplotypes (A-a, A-b and A-c) associated with A in the New Zealand Merino sheep. Therefore, it is conceivable that the growth traits association previously seen with the Merino and Romney sheep is the result of a different intron A- 3'UTR haplotype than that present in the Suffolk population and/or the effect is moderated by some other genetic influence or environment effects.

Genotypic frequencies obtained were consistent with those observed and reported in different sheep breeds (13, 14 and 15), while reports of non-deviation from Hardy-Weinberg equilibrium reported by these authors were at variance with what was obtained in this study. The principle of HWE states that the genetic variation in a population will remain constant from one generation to the next in the absence of disturbing factors. This could be due to the sheep having been "selected" and being derived from related rams and thus the frequency of the IGF-1 gene variants may be different between the sires and dams of these lambs. It may also be the result of some other effect that changes association between IGF-1 gene and assessments of some body composition traits in the Yankasa sheep genotype frequency. These variations may be due to several factors such as breed and genotype and environmental interactions along with impact of selection and mating as the animals genotyped were from an experimental stock. However the proclivity of the A allele and its hybrid genotype in this and other study have shown that this fast moving band of IGF-1 gene may be superior as a target candidate gene for selection and breeding of sheep in Nigeria since it has been estimated that up to 60% of the variance in IGF-1 serum level has a genetic basis (18).

Table 4 shows the differences in growth traits among the sexes. With the exception of average daily gain (g/day), which was non-significant ($P>0.05$) across the sexes, all other growth characteristics (birth weight, weaning weight, weight at 6 months, weight at 8 months, weight at 12 months, height at withers and chest girth differed significantly ($P<0.05$) across sexes with the highest traits in the male compared to the females.

Sex as a highly significant source of variation observed in the present investigation was supported by various authors in both mutton and wool breeds of sheep (19). The observed differences in studied traits between the male and female of Yankasa sheep were consistent with the reports of (20) in Yankasa sheep using animals from the same stock. Birth weight ranges of 2.08-2.80 Kg obtained were within the range of 2.64-3.02Kg obtained by these authors. Similarly, males were heavier, taller and broader than the females according to (20) which is similar to what was obtained in this study. This might partly be due to the pre- and pre-weaning advantage in male as compared to female lambs (20). These differences between male and female lambs were similar to those reported in beef cattle (21; 20 and 22). In those reports, steers were heavier, taller and bigger in girth; more muscled but with less fat compared to heifers both at weaning and post-weaning ages.

However, sex of lambs did not affect

average daily gain in this study contrary to the results from other investigations (23; 24; 25). Some authors also attributed the difference in body weight gains of male and female lambs with advancement of age to the increasing differences in endocrine system and that endocrine functions are often sexually dimorphic, different in males and females. He explained further that programming of sexual dimorphism begins with embryonic expression of the sex-determining gene (SRY) in males and secretion of Mullerian-inhibiting hormone (anti-Mu¨llerian hormone, MIH), which prevents development of internal reproductive tracts of females. The expression of sexual dimorphism in terms of overall body weight gain (considering the entire population) was possibly masked by genetic potentials for fast growth expressed by males.

Table 5 shows the interaction between polymorphic forms of IGF-1 and Sex on growth traits in Yankasa Sheep. Observed trend showed significant ($P<0.05$) interactions among the measured traits with the exception of average daily gain. Male Sheep with AA and AB genotype were similar in performance across all traits (birth weight, average daily gain, weaning weight, weight at 6 months, weight at 8 months, weight at 12 months and height at withers) with the exception of chest girth (cm) where AA was significantly ($P<0.05$) higher than AB. Both AA and AB males had higher growth characteristics than BB males with the exception of weight at 8 months where all genotypes were similar for the males. Body weight at 12 months showed the AA female having higher value than BB male. Females of the AA and AB genotype had higher growth characteristics both nominally and significantly ($P<0.05$) than the BB genotype for all other measured traits (birth weight, average daily gain, weaning weight, weight at 6 months, weight at 8 months, weight at 12 months and height at withers).

It has been reported that IGF-1 serum level is influenced by many factors, such as nutritional status, liver function, and serum levels of sex steroids and insulin, the secretion of this peptide is mainly regulated by growth hormone (25). This pre-supposes that there exist interaction between sex (through sex steroids) and IGF-1 gene and its circulating genotypes. Findings in this study has confirmed this assertion due to the significant ($P<0.05$) differences obtained for the interaction between genotype and sex on growth traits measured with the exception of average daily gain. The superiority of the AA and AB genotype over the BB genotype across sexes in the Yankasa Sheep testifies to the superiority of the fast moving A allele over the slow moving B allele.

Values obtained across the levels of interaction were similar to those obtained by (20) in the morphometric measures of Yankasa Sheep.

Literature reviews have shown that results vary in association between *IGF-I* gene polymorphism and growth traits among different breeds. Linkage disequilibrium of the *IGF-I* gene with QTLs could be the main reason of the inconsistent results. Therefore, quantitative traits are regulated by the large number of genes and also are affected by the interaction of these genes, so it is usual to observe the different effect of a candidate gene associated with a particular trait in a population.

Conclusion and Applications

1. The Yankasa sheep with A.A. genotype had higher propensity for growth than those with genotype AB and BB. Therefore, Yankasa sheep with AA genotype should be used for genetic improvement programs targeted to growth traits in Nigeria.
2. The current results indicated that polymorphism of the IGF-I gene is a potential molecular marker for growth traits in Yankasa sheep.

Table 2: Frequency distribution of genotype and alleles observed for IGF-1 polymorphism in Yankasa sheep.

Genotypes	AA	AB	BB	Alleles	A	B
Frequency	0.58	0.28	0.14	Frequency	0.72	0.28

Table 3: Chi square distribution table of IGF-1 gene polymorphism in the sheep population

Genotypes	No of Animals	Expected	Observed	***Calc.*** χ^2	Tab. χ^2	P<0.05
AA	140	70	81.2	9.07	5.99	*
AB		35	39.2			
BB		35	19.6			
Alleles						
A		75	100.8	16.94	3.84	*
B		25	39.2			

Calc. = Calculated value. Tab. = Tabulated value

Table 4: Sexual dimorphism in growth traits of studied sheep

	Male	Female	SEM	LOS
Birth weight (Kg)	2.80 ^{a}	2.08 ^{b}	0.31	*
Weaning weight (Kg)	13.29 ^{a}	10.19 ^{b}	1.47	*
Average Daily Gain (g/day)	0.11	0.11	0.01	NS
Weight at 6 months (Kg)	23.29 ^{a}	18.73 ^{b}	2.04	*
Weight at 8 months (Kg)	30.78 ^{a}	27.22 ^{b}	1.21	*
Weight at 12 months (Kg)	33.99 ^{a}	28.77 ^{b}	2.23	*
Height at withers (cm)	59.4 ^{a}	52.82 ^{b}	3.86	*
Chest Girth (cm)	69.46 ^{a}	64.06 ^{b}	2.01	*

^{ab} means across rows differsignificantly (P<0.05); NS: Nosignificant.

Table 5: Interaction between polymorphic forms of IGF 1 and sex on growth traits in Yankasa sheep

	AA		AB		BB		SEM	LOS
Traits	Male	Female	Male	Female	Male	Female		
Birth weight (Kg)	2.75^{a}	2.14^{b}	2.70^{a}	2.08^{b}	2.32^{b}	2.01^{b}	0.18	*
Weaning weight (Kg)	13.63^{a}	11.25^{b}	13.98^{a}	11.81^{b}	12.15^{b}	12.11^{b}	0.65	*
AverageDaily Gain (g/day)	0.10	0.10	0.11	0.11	0.11	0.11	0.01	NS
Weight at 6 months (Kg)	23.96^{a}	21.26^{b}	22.91^{a}	20.35^{b}	22.75^{a}	19.27^{b}	1.23	*
Weight at 8 months (Kg)	29.77^{a}	27.42^{b}	28.83^{a}	26.54^{bc}	28.84^{a}	25.35^{c}	0.93	*
Weight at 12 months (Kg)	32.05^{a}	28.78^{a}	31.18^{a}	27.16^{b}	29.39^{b}	26.90^{c}	1.29	*
Height at withers (cm)	62.98^{a}	58.05^{b}	63.27^{a}	53.55^{c}	55.31^{bc}	53.71^{c}	2.23	*
Chest Girth (cm)	71.63^{a}	67.46^{bc}	69.08^{b}	65.60^{c}	65.71^{c}	62.59^{c}	1.16	*

^{abc} means across rows differ significantly (P<0.05); NS: No significant

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