

Association between chicken growth hormone gene and growth performance of chickens raised in South-south Nigeria

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ABSTRACT

The study assessed the single nucleotide polymorphism of chicken growth hormone and its association with growth performance in chicken raised in South-South Nigeria. A total of 91 chickens that comprised of 56 Sasso, 23 Shika brown and 12 Shika brown x Sasso crosses were used for the growth data and 24 samples that comprised of 8 samples from each breed were sequenced for this study. Growth data were collected for a period of 12 weeks. Blood samples were also collected for DNA extraction, Polymerase Chain Reaction and DNA sequencing. Growth Hormone Gene polymorphism and its effect on growth data were analysed using SAS (2001). DNA sequences were aligned and Single Nucleotide Polymorphism (mutation) (G761A) in Exon 1 was detected using Mega 6.0. The result showed no breed and mutation effect on growth data at Week 1. However, body weight varied from 79.60g in Sasso to 73.20g in Shika brown. Also, the mutant genotype GA had higher numerical mean body weight (85.25 ± 5.49 g), mean body length (8.50 ± 0.46 cm) and mean wing length (8.50 ± 0.61 cm) than the normal GG genotype. For the body length, the highest numerical value was observed in the mutant genotype (GA) in Sasso (9.00 ± 0.65 cm). However, the normal genotype GG in Sasso gave the highest numerical breast girth (10.67 ± 0.27 cm). The results presented in the study may explain the variation in the performance of the chicken breeds studied. This information is very useful in developing a breeding programme for the Nigerian indigenous chicken.

Keywords: Polymorphism, growth hormone gene, growth performance, chicken

INTRODUCTION

The importance of poultry especially chicken to man cannot be over emphasized as it has over the years provided man with companionship, meat, eggs and feathers. According to FAO (2006) livestock production is very important to the socio-economic development of the economy of Nigeria because it contributes about 9-10% of agricultural Gross Domestic Product (GDP). UNDP (2006) explained that the population of chicken in Nigeria is estimated at 150.682 million. Out of this figure, 25% of the chicken are commercially reared, 15% are semi-commercially reared, while 65% are reared at the backyard (UNDP, 2006). Over the years, the Nigerian indigenous chicken breeds have undergone

improvement to improve their growth performance at the phenotypic level (Adebamboet *al.*, 1999; Ibe, 1993) but only a few improvement studies have been done at the molecular (gene) level (Agaviezoret *al.*, 2012). In chicken improvement studies, some candidate genes including chicken growth hormone gene have been examined to see their effect of growth performance in chicken (Wang *et al.*, 2000). Chicken Growth Hormone (cGH) is one of the most important genes that can affect chicken performance traits because of its important function in growth and metabolism (Wang *et al.*, 2000). The cGH contains four exons and five introns with an overall length of 4.1kb (Kansaku *et al.*, 2008). Vignal *et al.* (2002) reviewed on single nucleotide polymorphism

(SNP) and other types of molecular markers which can be used for improvement programmes in chicken. Yan *et al.* (2003) also worked on the association between single nucleotide polymorphisms of the chicken growth hormone gene and chicken growth and carcass traits. This study therefore assessed single nucleotide polymorphism of chicken growth hormone and its association with growth performance in Nigerian improved local chicken (Shika brown), an exotic chicken (Sasso) and their cross.

MATERIALS AND METHODS

The study was done at the University of Port Harcourt Demonstration Farm, Choba; Animal Science Department Laboratory of the University of Port Harcourt, Port Harcourt and International Institute of Tropical Agriculture (IITA) Biotechnology Laboratory, Ibadan, Nigeria. The parent stock of Sasso and Shika brown which were donated by African Chicken Genetic Gains-Nigeria (ACGG-NG) project were artificially inseminated to get F1 crosses of Sasso (Sasso male vs Sasso Female) and Shika brown (Shika brown vs Shika brown) and also to get their cross (Shika brown male vs Sasso female). The eggs laid were collected and well labelled over a week and hatched to produce a total of 91 chickens that comprised 56 Sasso, 23 Shika brown and 12 Shika brown x Sasso crosses. The chicks were housed on deep litter and fed *ad libitum* with a starter diet (from 0 - 4 weeks of age) containing 20% crude protein and 2800 kcal of metabolisable energy and a growers diet (from 4 to 12 weeks) containing 18.5% crude protein and 2920 kcal of metabolisable energy for a period of 12 weeks.

Growth data were collected on a weekly basis. These included: Breast girth (the circumference of the chest), Body weight (entire weight of the animal), Keel length

(the length from the sternum axially along the midline of the sternum), Height at withers (from the top to the shoulder to the feet), Thigh length (length of the lower lap), Length of wings (the point of attachment of the wings to the body to the end of the wings). The breast girth, keel length, height at withers, length of wings were measured using a measuring tape while the body weight was measured using an electronic scale.

Blood collection for DNA extraction

3ml of blood was collected from a total of 24 chickens (8 samples from each of the 3 breeds studied) from the wing vein in a 5ml EDTA bottle that was gently mixed with the EDTA in the bottle to prevent coagulation and kept at -20°C until DNA extraction. DNA was extracted using ZymoBead DNA extraction kit following the manufacturer's instruction. The quality of DNA was assessed using a Nanodrop spectrophotometer and gel electrophoresis using 1.5% agarose gel.

Polymerase Chain Reaction Cocktail mix

The DNA was subjected to the following cocktail mix and condition for the 25µl. Polymerase Chain Reaction: 2.5ul of 10x PCR buffer, 1.0ul of 25Mm MgCl₂, 1.0ul of 5pMol forward primer and 1.0ul of 5pMol reverse primer (Table 1). Also, DMSO was 1.0 ul, 2.5Mm DNTPs was 2.0 ul, Taq 5u/ul was 0.1 ul, 10ng/ul DNA was 3.0 ul and water was 13.4 ul. A touch down PCR condition was used which involved initial denaturation at 94°C for 5 minutes, 9 Cycles of Denaturation at 94°C for 15 seconds, Annealing temperature at 65°C at 20 seconds and extension at 72°C for 30 seconds. This was followed by 35 cycles of Denaturation at 94°C for 15 seconds, Annealing temperature at 55°C at 20 seconds and extension at 72°C for 30 seconds and a final extension at 72°C for 7 minutes.

Table 1.Primer for Chicken Growth Hormone

Name	Sequence	Gene	Size	Annealing temp
cGH-F	5'- TCCCAGGCTGCGTTTTGTTACTC-3'	Chicken Growth	429	65°C
cGH-R	5'- ACGGGGGTGAGCCAGGACTG - 3'	Hormones		

DNA Sequencing and Data analysis

Polymerase Chain Reaction (PCR) products were sent for sequencing at the International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria. DNA sequences were aligned using Mega 6.0 to determine mutation (Single Nucleotide Polymorphism). A Single Nucleotide Polymorphism (SNP) of G704A in the Exon 1 region of the chicken growth hormone was detected. The association between this SNP and growth data was done by comparing the performance of individual chickens with the normal amino acid with those having the mutant amino acid. The effect of this mutation on growth data was analysed using SAS (2001) with significant differences separated.

RESULTS

Table 2 shows the variation on growth traits across the three breeds of chicken studied at week 1. No breed effect was observed. However, body weight varied from 79.60g in Sasso to 73.20g in Shika brown. Also, body length varied from 8.60cm in Shika brown to 8.25cm in Shika brown x Sasso. Values of breast girth ranged from 9.80cm in Sasso to 8.00cm in Shika brown x Sasso. Wing length also ranged from 8.80cm in Shika brown to 7.60cm and Sasso. Thigh length ranged from 4.00cm in Sasso x Shika brown to 3.60cm in Sasso and Shika brown. For keel length, the values ranged from 2.74cm in Shika brown x Sasso to 2.00cm in Shika brown.

Table 2. Variation in growth traits across breeds at Week 1

Breed	Body weight (g)	Body length (cm)	Breast girth (cm)	Wing length (cm)	Thigh length (cm)	Keel length (cm)
Sasso	79.60±3.94	8.40±0.40	9.80±0.58	7.60±0.51	3.60±0.24	2.03±0.21
Shika brown	73.20±5.73	8.60±0.51	9.20±0.20	8.80±0.58	3.60±0.24	2.00±0.83
Shika brown x Sasso	78.25±6.14	8.25±0.25	9.00±0.00	8.00±0.70	4.00±0.00	2.75±0.94

Table 3 shows the effect of the mutation of G>A (Guanine changing to Adenine) on growth parameters collected at week 1. There was no significant ($p>0.05$) effect of this change on growth parameters. However, the mutant genotype GA had higher mean body weight (85.25±5.49g), mean body

length (8.50±0.46cm) as well as mean wing length (8.50±0.61cm) than the normal GG genotype. Also, the normal genotype had higher mean breast girth 9.62±0.16cm, thigh length (3.76±0.17cm) and keel length (1.83±0.54cm).

Table 3. Effect of G >A mutation (Single Nucleotide Polymorphism) on growth parameters at week 1

Genotype	Body weight (g)	Body length (cm)	Breast girth (cm)	Wing length (cm)	Thigh length (cm)	Keel length (cm)
GG (Normal)	73.29±3.72	8.37±0.31	9.62±0.16	7.71±0.41	3.76±0.17	1.83±0.54
GA (Mutant)	85.25±5.49	8.50±0.46	8.75±0.23	8.50±0.61	3.75±0.26	1.00±0.79

Table 4 shows the effect of the interaction between breed and G>A mutation on growth parameters. Only breast girth and keel length were significantly ($p<0.05$) affected by this

interaction. The highest variation in body weight occurred in the mutant genotype (GA) in Shika brown x Sasso (86.500±7.77g). For the body length, the

highest value was observed in the mutant genotype as well (GA) in Sasso ($9.00\pm 0.65\text{cm}$). However, the normal genotype GG in Sasso gave the highest breast girth ($10.67\pm 0.27\text{cm}$). For wing length, the highest value was noticed in the mutant genotype in Shika brown x Sasso with a value of $9.00\pm 0.86\text{cm}$. Furthermore, the normal and mutant genotypes in Shika

brown x Sasso gave same and highest value of thigh length ($4.00\pm 0.36\text{cm}$). For keel length, the normal genotype (GG) in Shika brown x Sasso gave the highest value ($3.50\pm 1.12\text{cm}$). There was no mutant genotype found in Shika brown so values were not recorded for all the parameters considered in this study.

Table 4.Effect of breed and G > A mutation (Single Nucleotide Polymorphism) on growth parameters

Breed	Genotype	Body weight(g)	Body length (cm)	Breast girth (cm)	Wing length (cm)	Thigh length (cm)	Keel length (cm)
Sasso	GG (Normal)	76.67±6.34	8.00±0.53	10.67±0.27 ^a	7.33±0.71	3.67±0.30	0.00±0.00 ^b
	GA (Mutant)	84.00±7.77	9.00±0.65	8.50±0.33 ^b	8.00±0.87	3.50±0.36	0.00±0.00 ^b
Shika brown	GG (Normal)	73.20±4.91	8.60±0.41	9.20±0.21 ^b	8.80±0.55	3.60±0.23	2.00±0.71 ^{ab}
	GA (Mutant)	0.00±0.00	0.00±0.00	0.00±0.00 ^c	0.00±0.00	0.00±0.00	0.00±0.00 ^b
Shika brown x Sasso x	GG (Normal)	70.00±7.77	8.50±0.65	9.00±0.33 ^b	7.00±0.87	4.00±0.36	3.50±1.12 ^a
	GA (Mutant)	86.500±7.77	8.00±0.65	9.00±0.33 ^b	9.00±0.86	4.00±0.36	2.00±1.12 ^{ab}

a, b, c: Means in the same column with different superscripts are significantly ($p < 0.05$) different

DISCUSSION

The effect of growth hormone on growth parameters in chicken has been assessed by some authors (Nie *et al.*, 2005; Muin and Lamatauw 2013; Nguyen *et al.*, 2015). Breed did not significantly affect growth data at week 1. Furthermore, Sasso had higher body weight. More so, there was no significant difference between the association of GG and GA alleles and all growth traits considered. However, when this association was assessed across breeds, a significant effect was observed for breast girth and keel length. The cross between Sasso and Shika brown were significantly higher with this association than other breeds. This result is in line with those reported by other authors. Nie *et al.* (2005) reported that the Single Nucleotide Polymorphism within a specific region of the growth hormone gene (G1705A) maintained a significant association with almost all growth traits in an F2 reciprocal cross between two Chinese chicken breeds. Body weight varied in the SNP studied across breeds though not significant ($p>0.05$). This is in accordance with the works of Muin and Lamatauw (2013) who observed that the G>A SNP showed a positive effect on the body weight. They concluded that allele G exhibited a generally positive effect on chicken growth and was completely dominant in all breeds studied. This indicated that chicken growth hormone (cGH) gene is associated with body weight and some other growth parameters. Ghormade *et al.* (2012) in their work on chicken growth hormone gene polymorphism and its associations with growth traits observed that the different genotypes revealed significant differences for adult body weight at 20 weeks and 40 weeks. Genotype AB was superior to AA and BB for adult body weight at 20 weeks and 40 weeks and that the significant differences between genotypic least squares means among all the 3 breeds indicated the effect of growth hormone gene locus on growth traits.

In conclusion, there was no significant difference between the association of GG and GA mutation on all growth traits considered. However, when this association was assessed across breeds, a significant effect was observed for breast girth and keel length. The cross between Sasso and Shika brown were significantly higher with this association than other breeds. These results confirm the findings of other authors who suggested that chicken growth hormone might be used as a marker in marker assisted selection in chicken improvement programmes..

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