ALLELIC POLYMORPHISM OF INSULIN-LIKE GROWTH FACTOR-1 GENE AND ITS ASSOCIATION WITH GROWTH TRAITS IN IMPROVED NIGERIAN INDIGENOUS LAYING CHICKENS

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Abstract

The relationship of insulin-like growth factor-1 (IGF-1) polymorphism with growth traits was investigated in improved Nigerian indigenous laying chickens reared in derived savanna zone of Nigeria. One-hundred and thirty-three birds were used for the study and comprises of 4 different genotypes. Genomic DNA was extracted from obtained bloods and the polymorphism of IGF-I gene was detected by PCR-RFLP-PstI. Both allelic and genotypic frequencies were calculated and Statistical analysis of data was done using LSD (least significant difference) method as applied in the General Linear Model (GLM) procedure (SAS 9.1). Genotyping of birds revealed two alleles A (0.54), C (0.46) and three genotypes AA (41), AC (60), CC (32). AC genotype has the highest frequency of 53% while for AA and CC the frequencies are 30% and 17% among selected repeated backcrossed chickens. Analyzing different genotypes reveals non- significant effects on growth traits of the chickens involved. Based on the results of this study, the application of IGF-1 locus in Marker Assisted Selection programs can be advised.

Keywords: *Insulin-like Growth Factors (IGF1), Improved Nigerian chickens, growth traits, gene polymorphism, Hardy-Weinberg equilibrium*

Introduction

Nigeria is endowed with an impressive array of indigenous and locally adapted livestock which have significant roles in socio-economic life of the rural and semi urban populace. These livestock are reservoir of untapped valuable rare genes and alleles which are linked to their adaptability and survival. Nigerian indigenous chickens are functionally valuable because they play vital roles in human nutrition and contain genetic materials which may have been lost in the improved gene pool (Oyewola, Akinyemi, Osaiyuwu, Fijabi, Shobanke, Nwokorie & Ewuola, 2018). They possess relic traits or genetic variants that are either absent in modern improved chicken stocks. Selection of Nigerian indigenous for improved growth and higher mature weight is imperative. Traditional approach of selection which is based on phenotype is time consuming and very difficult to achieve (Musa, Orunmuyi, Akpa, Olutunmogun, Muhammed & Adedibu, 2015). The use of molecular marker which is a powerful tool in animal breeding for genetic improvement to define the genotype and predict the performance of animal very early life is important.

The candidate gene approach has been used as a powerful method for evaluating the effect of contributing genes which are involved in the expression of quantitative variations between individuals in the population (Kwon & Goate 2000; Nagaraja, Aggrey, Yao, Zadworny, Fairfull, & Kuhnlein, 2000). Candidate genes that are extensively studied in chickens are those of the growth hormone axis, because of the wide range of biochemical and physiological processes that they regulate. Abbasi, and Kazemi, (2013)

described Insulin-Like Growth Factors (*IGF*) to consist of a family of polypeptide hormones structurally linked with insulin with multiple metabolic and anabolic functions. The *IGFs* have been shown to regulate body and muscle growth in chickens (Duclos, 1998). Several studies have shown that circulating *IGF-I* affects growth rate in poultry (Goddard, Wilkie & Dunn, 1988; Scanes, Dunnington, Buonomo, Donoghue & Siegel 1989; Ballard, Johnson, Owens, Francis & Upton, 1990).Insulin like growth factor (*IGF1*) is associated with several phenotypic features in chicken including production and reproduction traits. *IGFI* gene could be linked to the quantitative trait loci (QTL) that control egg production, egg quality, body weight and reproductive traits in chicken (Kim, Seo & Ko, 2004; Li, Zhu, Chen, Song, Shu & Han, 2010; Tang, Sun, Ou, Zhang, Xu & Zhang, 2010).

The aim of this study was to evaluate the *IGFI* gene polymorphism and its relationship with growth traits (body weight, body length, keel length, thigh length and wing length) in Nigerian improved laying chickens.

Materials and Methods

Experimental Site

The experiment was carried out at the Poultry Unit of Teaching and Research Farm, Ladoke Akintola University of Technology, Ogbomoso, Oyo state, Department of Animal Breeding and Genetics, Biotechnology Laboratory and Central Biotechnology Laboratory, Federal University of Agriculture, Abeokuta, Ogun State. Nigeria.

Blood Sample Collection

Blood samples were obtained randomly from 133 chickens from 4 different genotypes through the wings vein using 2ml syringe and transferred to EDTA bottles to serve as anti-coagulating agent, after which the samples were stored in the laboratory at -20°C.

DNA Extraction

Genomic DNA was extracted from the blood, using the Qiagen DNA extraction kits following manufacturer protocol.

DNA Quantification

The purity and concentration of the extracted DNA was carried out using Nano-drop spectrophotometer as follow: 1 ml of DNA was place in the Nano-drop machine with the help of micro-pipette after which it was measured by Nano-drop machine and the concentration was noted while DNA samples of low contraction were worked upon before analysis.

Establishment of a PCR-RFLP Assay

Working dilutions of extracted DNA were prepared for each individual at a concentration of 50 ng/µg. Primers forward (5'GACTATACAGAAAGAACCAC-3') and reverse (5'-TATCACTCAAGTGGCTCAAGT-3') were used for the polymerase chain reaction (PCR) amplification (Nagaraja *et al.*, 2000). Each cycle was consisted of initial denaturation at 94°C for 5 minutes, 94°C for 4 minutes, 45 sec at 60°C, 60 sec at 72°C and final extension at 72°C for 10 minutes. The PCR products were run in an agarose gel electrophoresis to verify the result. The PCR product or amplicon were digested with a restriction enzyme, *Pst1* and digested products were electrophoresed for 1 h at 80 V on a 2.5% agarose gel as shown in Plate 1.

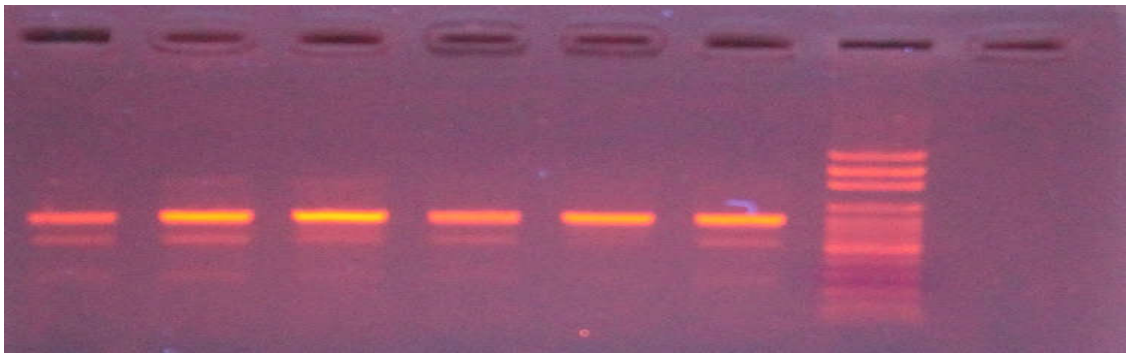

Plate 1: Insulin –like growth factor (*IGF-1*) gene optimization on agarose gel

Individual PCR-RFLP fragment sizes for each gene were determined by visualizing the banding pattern under ultraviolet light as shown in Plate 2.

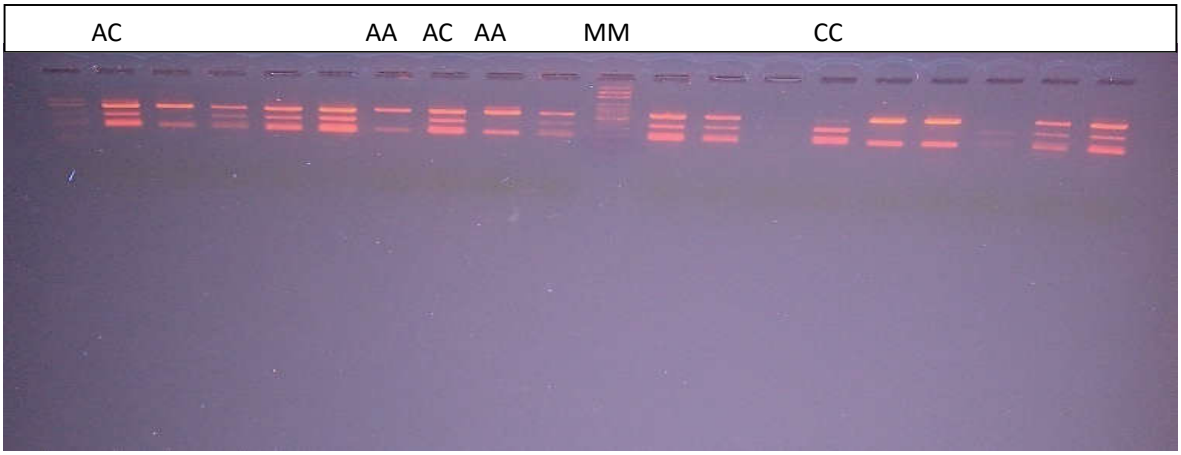

Plate 2: PCR- RFLP pattern for Insulin – like growth factor (*IGF-1)* gene polymorphism

The growth traits under study were measured thus;

Body weight (BW): This was measured with the use of an electronic kitchen scale with maximum capacity of 5kg/11lb.

Breast girth (BG): This was measured as the circumference of the breast around the deepest region of the breast. A tape rule calibrated in centimeters was used to take the measurement (cm).

Keel length (KL):- This was taken as the length of the sternum.

Body length (BL):- It was measured as the distance between the base of the neck and the cloaca.

Shank length (SL): This was taken as length in cm of the shank from the hock joint to the spur of either leg.

Wing length (WL): This was measured as length in cm between tips of right and left wings after both are stretched out in full. A tape rule was used to take the measurement (cm) by the procedure described by FAO, (2012) and Amao (2020).

Genotype and Allele Frequencies

Genotype and allele frequencies were calculated according to the formula described by Nei & Kumar (2000) using *IGF-1* genotyping data. Genotype frequency was calculated with the following formula:

$$xi = \frac{\sum_{i=1}^{n}(nii)}{N}$$

Allele frequency was calculated with the following formula:

$$xii = \frac{(2nii + \sum_{j=1}^{\cdot}(nij)}{(2N)}$$

Where:

xii = Genotype frequency
xi = Allele frequency
nii = Number of samples with ii genotype
nij = Number of samples with ij genotype
N = Total number of samples

Statistical Analysis

The *IGF-1* polymorphism and growth traits data were analyzed using the General Linear Model procedure of SAS (2009) while significance between genotypes was assessed by Duncan multiple range test of SAS (2009). The following model was used:

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

Y_{ijk} = Observation on growth traits

μ = Overall mean

G_i = Fixed effect of the genotypes (1, 2, 3, 4)

e_{ij} = the random error common to measurement in each bird and assume to be normally and independently distributed with a mean of zero and variance δ^2

Results and Discussion

Table 1: The allele and genotype frequencies of the *IGF1* gene polymorphism

	Allele Frequencies		Genotype Frequencies		
Locus	**A**	**C**	**AA**	**AC**	**CC**
IGF-1 gene	0.54	0.46	0.30	0.53	0.17

IGF-1 = Insulin – like growth factor gene

Table 1 shows the allele and genotype frequencies of the *IGF1* gene polymorphism. The result indicates that two types of allele frequencies were identified as A of 0.54 of the population and C of 0.46 of the population. The frequency of A allele was higher than that of C while the genotypic frequencies of 30 %, 53 % and 17 % were observed for genotype AA, AC and CC.

The population of the birds used, the chi-squared for the genotypic frequency was estimated and it was observed that there was no significant effects which indicated that the frequency of the genotype does not varied from expectation of Hardy-Weinberg equilibrium and this was in accordance with the previous findings of Wheto, Ilori, Akano, Adebambo, Ojoawo, Durosaro, Sanda & Adebambo (2016) and Abbasi & Kazemi (2013) reported that chi-squared test on the genotype frequencies shows no deviation from Hardy-Weinberg equilibrium in the Mazandaran native fowls population.

The three genotypes found namely AA, AC and CC which show fragments at different base pair (bp) using *Pst1* for PCR-RFLP with allelic frequency A and C in the present result indicated that allele A was higher than that of C and genotypic values of AC was highest followed by AA and least value recorded for CC. The observed frequency was in accordance with the findings of Wheto *et al.* (2016); Babayi, Seyedabadi, Gorbani and Zarghami, (2014).

They observed that frequency of genotype (AA) homozygote individual was lower than the frequency of homozygote individual with AC. Ghelghachi, Seyedabadi and Lak, (2013) reported higher allele A than that of C in Arian broilers while Mehdi, Hamidreza, Abolfazl and Nosratollah (2014) found that the allele A was higher than B in Azarbaijan native chicken.

The genotypic frequencies observed presently was also similar to the findings of Ali, Javed, Ali, Akram, Dawood and Saleem,

(2016) for Desi chicken, Mariandayan, Solihin, Sulandari and Sumantri,(2013) for Indonesian native chicken and Ghelghachi *et al.* (2013) for Arian broilers. These authors reported that heterozygote individual (AB) was superior in value than either AA or BB homozygote individual frequencies. However, the observation of Jafari, Pakdel and Esmailkhanian, (2015) contradicted the present findings who noted that genotypic frequency of AA homozygote individual had more value than that of AB heterozygote individual and BB homozygote individual respectively for Isfahan native fowls with highest genotypic frequency for homozygote individual (BB) than homozygote individual AA and heterozygote individual (AB) for Mazandaran native fowls.

The least square means and the standard errors of genotype and *IGF-1* gene polymorphism interaction on the growth traits of repeated reciprocal backcrossed chickens' population is presented in Table 2

Table 2: Least square means and standard errors of genotype x *IGF-1* gene polymorphism interaction on growth traits of chickens' population

Genotype	*IGF-1*	BDW	BL	CH	KL	SHL	TL
RIFE	AA	1309.00 ± 20.99	21.55 ± 1.11	22.53 ± 2.34	15.90 ± 1.91	9.88 ± 0.45	14.35 ± 0.78
	AC	1337.00 ± 56.54	21.84 ± 1.01	22.64 ± 1.89	17.30 ± 1.34	9.83 ± 0.89	14.45 ± 0.67
	CC	1360.00 ± 45.22	23.37 ± 1.13	22.63 ± 1.56	18.12 ± 1.67	9.86 ± 0.99	14.55 ± 0.35
RIFF	AA	950.25 ± 12.89	20.80 ± 0.56	22.90 ± 2.13	15.10 ± 1.67	9.00 ± 0.83	15.95 ± 0.51
	AC	941.25 ± 11.89	20.78 ± 0.67	22.74 ± 1.90	15.07 ± 1.34	8.91 ± 0.45	15.87 ± 0.89
	CC	940.00 ± 11.11	20.77 ± 1.04	22.78 ± 1.89	15.08 ± 1.67	8.92 ± 0.56	15.90 ± 0.59
RINF	AA	1251.25 ± 11.99	23.81 ± 1.23	25.81 ± 2.10	17.37 ± 1.78	10.46 ± 0.67	18.55 ± 0.56
	AC	1285.11 ± 21.90	23.79 ± 1.45	25.78 ± 1.89	17.47 ± 1.22	10.45 ± 0.89	18.68 ± 0.34
	CC	1253.00 ± 23.12	23.90 ± 1.68	25.90 ± 2.05	17.20 ± 1.45	10.42 ± 0.99	18.45 ± 0.67
RINN	AA	1860.00 ± 13.88	28.55 ± 1.56	26.60 ± 2.13	18.00 ± 1.90	11.00 ± 0.78	18.00 ± 1.04
	AC	1675.00 ± 23.33	28.37 ± 1.44	26.40 ± 2.56	17.95 ± 1.67	11.03 ± 0.67	18.54 ± 0.90
	CC	1695.00 ± 23.45	28.11 ± 1.67	26.18 ± 1.67	17.96 ± 1.89	11.02 ± 0.56	18.50 ± 0.89

BDW = body weight, BDL = body length, CH = chest girth, KL = keel length, SHL = shank length, TL = thigh length, *IGF-1* = Insulin – like growth factor gene, AA = homozygote dominant gene, AC = heterozygote gene, CC = homozygote recessive gene, RIFE = Reciprocal Improved Fulani Ecotype, RIFF = Reciprocal Improved frizzle feather, RINF = Reciprocal Improved Normal feather, RINN = Reciprocal Improved Naked neck.

Interestingly, there were no significant (P>0.05) effects between the genotype and *IGF-1* gene polymorphism. Although, the polymorphism of *IGF-1*gene polymorphism shows that it is a potential candidate gene associated with growth and body composition (Whetto *et al*., 2016; Bhattachanya, Chatterjee, Dushyanth, Paswan, Shukia & Shanmugam, 2015), egg production (Aminafshar & Fathi, 2012) and egg quality traits (Li *et al.*, 2010) but the non-significant effects of *IGF-1* gene polymorphism on the growth traits observed in the present study was in accordance with findings of Wheto *et al*. (2016), Ali *et al.* (2016) and Ghelighachi *et al.* (2013). These authors on their different studies with *IGF-1* gene polymorphism on chickens reported that *IGF-1* gene polymorphism had no significant effects on either growth or carcass traits of chickens. Babayi *et al.* (2014) found no significant differences on *IGF-1*gene polymorphism with some growth traits in West-Azerbaijan native chicken in Iran.

Reports of Mazurowski, Frieske, Kokoszyñski, Mroczkowski, Bernacki and Wilkanowska, (2015) on *GH* polymorphism of body weight and selected body dimension in Peckin and Mallard ducks found non-significant effects of *GH* gene polymorphism on the body weight and other selected body conformation measured. Musa, Orunmuyi, Akpa, Olutunmogun and Afolabi-Balogun, (2016) found no significant variation association of *IGF-1* gene polymorphism on body weight of Nigerian indigenous chickens that corroborated with this current findings.

The result was also in line with the findings of Moe, Shimogiri, Kawabe, Nishibori, Okamoto, Hashiguchi and Maeda (2009) who claimed that non-significant difference existed in Asian native chicken populations and gene expression using insulin-like growth factor 1. Promwaree, Laopaobeon, Vongpralub, Phasuk, Kunhareang, Booknum and Duangjinda (2013) reported non-significant effect of insulin-like growth factor 1 gene polymorphism association with growth traits in Thai synthetic chickens. Also, Mehdi, Hamidreza, Abolfazl and Nosratollah (2014) found non-significant effect of insulin-like growth factor 1 gene polymorphism associations with some growth traits in West-Azerbaijan native chicken using PCR-RFLP techniques.

Conclusion

The results obtained in this study indicate that the population analyzed deviated from Hardy-Weinberg equilibrium. Allele A in the *IGF-1* gene of improved Nigerian laying chickens is the predominant allele and *IGF-1* gene polymorphism did not indicated any significant relationship with the growth traits of the improved Nigerian indigenous laying chickens.

Recommendation

Insulin growth factor 1 (*IGF-1*) is a potential candidate gene that expressed relationship with growth traits in improved Nigerian indigenous laying chickens.

References

Abbasi, H. A & Kazemi, M. M. (2013).Detection of polymorphism at the insulin like growth factor-I gene in Mazandaran native chicken using polymerase chain reaction restriction fragment length polymorphism method. *American Journal of Animal and Veterinary Sciences*, 6 (2): 80 – 83.

Ali, A., Javed, K., Ali, A., Akram, M., Dawood, M. & Saleem, A. H. (2016). Polymorphism of insulin-like growth factor-1 gene and its association with growth rate in design chicken of Pakistan. *The Journal of Animal and Plant Sciences*, 26 (3): 858-861.

Amao, S.R. (2020). Growth performance traits of meat-type chicken progenies from a broiler line sire and Nigerian indigenous chickens' dams reared in southern guinea savanna condition of Nigeria. *Discovery*, 56 (289): 66-73.

Aminafshar, M, & Fathi, R. (2012). Single nucleotide polymorphisms in intron 1 of growth hormone gene and it's association with economic important traits in Iranian Fars native fowl. *Annals of Biological Research,* 3: 4028-4032.

Babayi, M. S., Seyedabadi, H., Gorbani, A. & Zarghami, N. (2014). IGF-I Gene polymorphism and its associations with some growth traits in West-Azerbaijan native chicken using PCR-RFLP techniques. *Bulletin of Environment, Pharmacology and Life Sciences*, 3(6): 29-32.

Ballard, F., Johnson, J.R.J., Owens, P. C., Francis, G. L. & Upton, F. M. (1990). Chicken insulin-like growth factor-I: Amino acid sequence, radioimmunoassay and plasma levels between strains and during growth. *Gen. Comp. Endocrinol.,* 79: 459-468.

Bhattacharya, K., Chatterjee, R. N., Dushyanth, K., Paswan, C., Shukia, R. & Shanmugam, M. (2015). Polymorphism and expression of insulin-like growth factor 1 (*IGF1*) gene and its association with growth traits in chicken. *British Poultry Science,* 56 (4): 398-407.

Duclos, M. J. (1998). Regulation of chicken muscle growth by insulin-like growth factors-I Trends in comparative endocrinology and neurobiology. *Ann. N. Y. Acad. Sci.,* 839: 166-171.

FAO. (2012). Phenotypic characterization of animal genetic resources. FAO Animal Production and Health Guidelines No.11. Rome.

Ghelghachi, A. A., Seyedabadi, H. & Lak, A (2013). Association of growth hormone gene polymorphism with growth fatness traits in Arian broilers. *International Journal of Biosciences*, 3(12): 216 – 220.

Goddard, C., Wilkie, R. S. & Dunn, I. C. (1988). The relationship between insulin-like growth factor-1, growth hormone, thyroid hormones and insulin in chickens selected for growth. *Domest. Anim. Endocrinol.,* 5: 165-176.

Jafari, A., Pakdel, A. & Esmailkhanian, S. (2015). Growth hormone gene polymorphism in two Iranian native fowls. *Poult. Sci. J.,* 3(1): 99-104.

Kim, M. H., Seo, D. S. & Ko, Y. (2004). Relationship between egg productivity and insulin-like growth factor-I genotypes in Korean native Ogol chickens. *Poultry Science*, 83:1203–1208.

Kwon, J. M. & Goate, A. M. (2000). The candidate gene approach. *Alcohol Research and Health*, 24: 164–168.

Li, H., Zhu, W., Chen, K., Song, W., Shu, J. & Han, W. (2010). Effect of the polymorphism of *GHR* gene and *IGF-I* gene on egg quality in Wenchang chicken. *Research Journal of Poultry Science*, 3:19–22.

Mariandayani, H. N., Solihin, D. D., Sulandari, S. & Sumantri, C. (2013). Polymorphisms of Insuline-like growth factor-I (*IGF-1)* and Pituitary Positive Transcription Factor-1 (*Pit-1*) genes and their effect on growth traits in Indonesian native chickens. *Proceedings of International Conference of the 4th Green Technology*, Faculty of Science and Technology Islamic of University State Maulana Malik Ibrahim Malang, 75-85.

Mazurowski, A., Frieske, A., Kokoszyñski, D., Mroczkowski, S., Bernacki, Z. & Wilkanowska, A. (2015). Examination of growth hormone (*GH*) gene polymorphism and its association with body weight and selected body dimensions in ducks. *Folia Biologica* (Kraków) 63: 43-50.

Mehdi, S. B., Hamidreza, S., Abolfazl, G. & Nosratollah, Z. (2014). *IGF-I* gene polymorphism and its associations with some growth traits in West-Azerbaijan native chicken using PCR-RFLP techniques. *Bull. Env. Pharmacol. Life Sci.,* 3(6):29-32.

Moe, H. H., Shimogiri, T., Kawabe, K., Nishibori, M., Okamoto, S., Hashiguchi, T. & Maeda, Y. (2009). Genotypic frequency in Asian native chicken population and gene expression using insulin-like growth factor I (*IGF1*) gene promoter polymorphism. *Journal of Poultry Science,* 46:1-5.

Musa, A. A., Orunmuyi, M., Akpa, G. N., Olutunmogun, A. K., Muhammed, H. & Adedibu, H. (2015). Diallel analysis for body weight involving three genotypes of Nigerian indigenous chickens. *South African Journal of Animal Science*, 45(2): 188-197.

Musa, A. A., Orunmuyi, M., Akpa, G. N., Olutunmogun, A. K. & Afolabi-Balogun, N. B. (2016). Effects of insulin-like growth factor 1 *(IGF-1*) polymorphism on bodyweight of Nigerian indigenous chickens. *African Journal of Agricultural Research*, 1(14):1194-1198.

Nagaraja, S. C., Aggrey, S. E., Yao, J., Zadworny, D., Fairfull, R. W. & Kuhnlein, U. (2000). Trait association of a genetic marker near the *IGFI* gene in egg-laying chickens. *Journal of Heredity*, 91, 150–156.

Nei, M. & Kumar, S. (2000). *Molecular evolution and phylogenetics*, UK: Oxford University Press, pp 333.

Oyewola, K. A., Akinyemi, M. O., Osaiyuwu, O. H., Fijabi, O. E., Shobanke, I. A., Nwokorie, G. I. & Ewuola, K. M. (2018). Association between *IGF-1* gene polymorphisms and body weight in Nigeria locally adapted turkeys. *Nigerian Journal of Genetics*, 32 (1): 207-211

Promwaree, N., Laopaobeon, B., Vongpralub, T., Phasuk, Y., Kunhareang, S., Booknum, W. & Duangjinda, M. (2013). Insulin-like growth factor I gene polymorphism associated with growth and carcass traits in Thai synthetic chickens. *Genetic Molecular Resources,* 12(4): 4332-4341.

SAS (2009). Statistical analysis system, multiple incorporation. Users Guide Statistical version. Cary, NC, USA.

Scanes, C.G., Dunnington, E. A., Buonomo, F.C., Donoghue, D. J. & Siegel, P. B. (1989). Plasma concentrations of insulin like growth factors *IGF-I and IGF-II* in dwarf and normal chickens of high and low weight selected lines. *Growth Dev. Aging.*, 53: 151-157.

Tang, S., Sun, D., Ou, J., Zhang, Y., Xu, G. & Zhang, Y. (2010). Evaluation of the *IGFs (IGF1 and IGF2)* genes as candidates for growth, body measurement, carcass, and reproduction traits in Beijing You and Silkie chickens. *Animal Biotechnology*, 21: 104–113.

Wheto, M., Ilori, R. M., Akano, K., Adebambo, O. A., Ojoawo, H. O., Durosaro, S. O., Sanda, A. J. & Adebambo, A. O. (2016). Effect of *IGF1* gene polymorphism on carcass traits in improved Nigerian indigenous chicken. Proceedings of the 41th Conference of Nigerian Society for Animal Production, 20-24 March, 2016. Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. 6-9.