Transforming Growth Factor-B (Tgf-B3) Gene Variations in Dual-Purpose Chicken Strains and Its Association with Growth Traits

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Abstract

Studies on TGF-β3 genes suggests that they are crucial for maintaining healthy tissues and development and are located on chicken chromosome 5 which are close to genes that might influence body weight and feed conversion ratio (FCR) in chickens. TGF-β3 gene polymorphs in dual purpose chicken were investigated to understand their association with various chicken growth and morphological traits in three chicken populations. Four hundred and fifty chickens of 150 per strains of Noiler®, FUNAAB Alpha® and Sasso® (150), were reared from day old to 20 weeks of age. Body weight (BW), height at withers (HW), body length (BL), breast girth (BG), and wing length (WL) were measured from 4 weeks to 20 weeks of age at 4 weekly intervals. Genomic DNA was extracted for polymerase chain reaction (PCR) with primers of TGFβ3 gene. The PCR amplicons were sequenced and genotyped using genotype by sequence method, with two SNP primers as two SNP genotypes were identified. The identified SNPs were on exon 3 at positions 7941745 and 7941722 of the TGFβ3 gene which were named 7941745A>C and 7941722A>T, respectively. Chi-square results demonstrated that the population used was under Hardy-Weinberg equilibrium (HWE) for the two SNP genotypes, with 7941745A>C and 7941722A>T having a dominant allele A. Marker-trait association results revealed that 7941745A>C was not significantly associated (P>0.05) with breast girth, body length and wing length, however, there was a significant association (P<0.05) with body weight and height at withers, with strains Sasso and FA showing more association than Noiler. 7941722A>T had no significant association with all the growth traits except body weight (P<0.05) with strains Sasso and FA showing more association. Marker-trait association results suggest that AA and CC genotypes for SNPs 7941745A>C gene could be used as a genetic marker when improving body weight and size of the chicken strains under selection, whereas genotype AA and AT could be used in improving bodyweight only amongst the chicken strains studied. This research, therefore, suggests that the TGF-β3 gene could be a candidate gene that significantly influences the body weight and height at withers of chicken, hence might be used as potential genetic markers in dual purpose chicken breeding.

Keywords: PCR, Transforming Growth Factor-β (TGFβ), Marker Assisted Selection, SNP, body composition.
Running title: Transforming growth factor-β (TGF-β3) gene association with growth traits in chickens

Variations du Gène du Facteur de Croissance Transformant-ß (TGF-ß3) chez des Souches de Poulets à Double Usage

Résumé

Les études sur les gènes TGF-β3 suggèrent qu'ils jouent un rôle crucial dans le maintien des tissus sains et le développement, et sont situés sur le chromosome 5 de la poule, à proximité de gènes susceptibles d'influencer le poids corporel et l'indice de consommation (IC) chez les poulets. Les polymorphismes du gène TGF-β3 chez les poulets à double usage ont été étudiés pour comprendre leur association avec divers traits de croissance et morphologiques dans trois populations de poulets. Quatre cent cinquante poulets (150 par souche) de Noiler®, FUNAAB Alpha® et Sasso® ont été élevés de l'éclosion à 20 semaines d'âge. Le poids corporel (PC), la hauteur au garrot (HG), la longueur du corps (LC), le tour de poitrine (TP) et la longueur de l'aile (LA) ont été mesurés

de 4 à 20 semaines d'âge à intervalles de 4 semaines. L'ADN génomique a été extrait pour une amplification par PCR avec des amorces du gène TGF-ß3. Les amplicons PCR ont été séquencés et génotypés par la méthode de génotypage par séquençage, avec deux amorces SNP identifiées. Les SNPs identifiés se trouvaient sur l'exon 3 aux positions 7941745 et 7941722 du gène TGF-ß3, nommés respectivement 7941745A>C et 7941722A>T. Les résultats du test du chi-carré ont montré que la population étudiée était en équilibre de Hardy-Weinberg (EHW) pour les deux génotypes SNP, avec un allèle dominant A pour 7941745A>C et 7941722A>T. Les analyses d'association marqueur-trait ont révélé que 7941745A>C n'était pas significativement associé (P>0,05) au tour de poitrine, à la longueur du corps et à la longueur de l'aile, mais qu'il y avait une association significative (P<0,05) avec le poids corporel et la hauteur au garrot, les souches Sasso et FA montrant une plus forte association que Noiler. Le SNP 7941722A>T n'avait pas d'association significative avec tous les traits de croissance sauf le poids corporel (P<0,05), les souches Sasso et FA présentant une plus grande association. Les résultats suggèrent que les génotypes AA et CC pour le SNP 7941745A>C pourraient être utilisés comme marqueurs génétiques pour améliorer le poids et la taille des souches de poulets sous sélection, tandis que les génotypes AA et AT pourraient être utilisés pour améliorer uniquement le poids corporel parmi les souches étudiées. Cette recherche suggère donc que le gène TGF-ß3 pourrait être un gène candidat influençant significativement le poids corporel et la hauteur au garrot des poulets, et pourrait ainsi servir de marqueur génétique potentiel dans l'élevage de poulets à double usage.

Mots-clés : PCR, Facteur de Croissance Transformant-ß (TGF-ß), Sélection Assistée par Marqueurs, SNP, composition corporelle.

Introduction

Chickens raised for meat or eggs are bred to grow quickly and produce well. These traits, along with the makeup of the chicken's carcass traits, are influenced by many genes (Anh *et al.*, 2015). Scientists are still figuring out how these genes work together to control growth, which is a complex process involving hormones and the nervous system (Zhang *et al.*, 2008; Okafor *et al*., 2021). Traditionally, improving chicken growth, meat quality, and egg production has been challenging because these traits are influenced by many genes. But, new breeding techniques like marker-assisted selection can help because this method uses specific genes linked to these traits to predict a chicken's overall genetic potential for good performance (Dekkers 2004). These special genes are like signposts pointing to the desirable qualities.

Meanwhile as chicken feed remains a major expense for farmers, making up around 70% of the total cost of production, improving how quickly they grow and their body size is important. This includes traits like body weight, weight gain, body length, and breast width. Scientists can therefore investigate the genes that control these traits to develop better breeding programs for chickens raised for meat and egg production (Sharma, Bottje and Okimoto 2008).

Transforming growth factor beta (TGF-β) gene is a powerful signaling molecule, one of many in a large family. These molecules play a critical role in many essential biological processes like shaping organs, development, and cell specialization (Saxena *et al.*, 2009; Jin et al. 2013). In chickens, there are four different versions of TGF-β, called TGF-β1, TGF-β2, TGF-β3, and TGF-β4 (Roberts and Sporn 2012). TGF-β3 is a special protein, similar to others called cytokines, that helps cells become specialized during development and embryo formation (Sanders and Wride 1997; Jin *et al*. 2013). Different forms (isoforms) of TGF-β3 were found to influence chick embryo development and play a broad role in how cells specialize, multiply, grow, and build structures around them. Studies suggests that the TGF-β3 gene is crucial for maintaining healthy tissues and development (Li *et al*., 2003). This gene is located on chicken chromosome 5 and is close to genes that might influence body weight and feed conversion ratio (FCR) in chickens (Jin *et al*., 2013). Also, candidate gene approach has become a powerful technique for the genetic improvement in chicken breeding programs, and can result in increased

efficiency in detecting the required production performance traits (Anh *et al.*, 2015).

Growth traits are essential parameters in assessing the potential of genetic improvement and development of any livestock breed/strain. Studies have shown that growth traits measurements from body-length, shank-length and chest-girth serve as good indicators of growth (Okoro *et al*., 2005). Also, there is dearth of information on identification of the association of TGFβ3 gene polymorphism commensurate to the growth of chicken strains reared in Nigeria viz Funaab alpha, Sasso and Noiler chicken strains, which are developed as dual-purpose strains. This study is therefore aimed at investigating the variations in the TGFβ3 genes on the three chicken strains and to associate how fast they grow, final body weight, how long their bodies are, their height at withers and wing length to the SNP variation identified. If a connection is found, these variations could potentially be used as markers to identify chickens with the best genes for breeding superior strains of the dual-purpose chickens developed and reared in Nigeria

Materials and Methods

This research was carried out at the Demonstration Farm of the Faculty of Agriculture, University of Port-Harcourt, Choba, Port-Harcourt, Nigeria, where the chicken strains – Funaab Alpha, Noiler and Sasso parents were reared. The eggs collected from the F1 generation of the chicken strains were hatched and raised from day old to 20 weeks of age, to conduct this experiment. The farm is located at longitude and latitude of 4.770N and 6.450E. The annual average temperature is between 25.01 to 27.79ºC and annual average rainfall of 203.03mm (Uko et al. 2016).

Experimental Birds and Procedure

A total of four hundred and fifty 1-day-old chicks of both sexes comprising 150 Noiler, 150 Funaab Alpha and 150 Sasso strains with average of 60 males and 90 females, respectively per strain were all hatched, and reared from day old till 20 weeks of age, from eggs of their parents reared in the farm. The three strains were developed independently of each other by the breeders of the different strain. During the period of this experiment, the birds were fed with commercial diets according to their age. A starter diet (from 0-4 weeks of age) which contained 20% crude protein and 2800 kcal of metabolizable energy (ME) and a grower's diet (from 4-20 weeks) containing 18.5% crude protein and 2920 kcal of ME was given to the birds ad-libitum. Each bird was tagged for proper identification. Routine medication and vaccination were also carried out when required.

Phenotypic Data

Data on growth parameters were collected on a weekly basis while feed intake record was collected daily. Body weight was measured using electronic scale and measuring tape for the body measurements - breast girth, body length, wing length and height at withers. The body measurements were taken from day old to 20 weeks of age as follows:

- Breast girth (cm): This was taken as the circumference of the breast around the deepest region of the breast; measuring tape was used to take the reading.
- Body Length (cm): This was taken as the distance between the last cervical vertebral before the thoracic vertebra and the caudal vertebrae and were done with the use of a measuring tape.
- Wing length (cm): The length of the wing from the end of the wing to the bone that attaches the wing to the body was recorded as the wing length. Measurement was taken using the measuring tape.
- Height at withers (cm): This was taken as the neck base line to the foot and was done with the use of a measuring tape.

Phenotypic Data analysis

The phenotypic data generated was analyzed for base statistics using the statistical model below in a

Completely Randomized Design (CRD) with Breed (B), and sex (S) as fixed effects according to the model.

$Y_{ijk} = \mu + B_i + S_k + e_{ij}$.

Where, Y_{ijk} is the response variable, μ is the population mean and e_{ijk} is the random error. Analysis of variance (ANOVA) using the General Linear Model procedure (SPSS. 2022) was deployed and significant means was separated using Duncan New Multiple Range Test (SPSS, 2022).

Genomic Samples

Genomic samples were collected and prepared according to Okoro *et al.*, 2014.

Polymerase Chain Reaction

Polymerase Chain Reaction (PCR) was done using: 2.5uL of 10xPCR buffer, 1.0uL of 25Mm $MgCl_2$, 1.0uL of 5pMol forward primer and 1.0uL of 5pMol reverse primer, with the TGF-β3 primer used. Amplification of TGF-β3 gene covering intron 3, exon 4, and intron 5 on chromosome 5 was done using a pair of forward primers (5′ TCA GGG CAG GTA GAG GGT GT 3') and reverse (5' GCC ACT GGC AGG ATT CTC AC 3') primers. The primers were designed using primer 3.0 software and the published nucleotide sequence of the Gallus Gallus TGF-β3 gene (NCBI Gene Bank 396438uid). Also added were 1.0 uL DMSO, 2.0 ul of 2.5Mm DNTPs, 0.1 ulTaq 5u/uL, 3.0 ul of 10ng/uL DNA and 13.4 l water. A touch down PCR condition was used which was involved in initial denaturation at 94˚C for 5minutes, 9 Cycles of Denaturation at 94˚C for 15seconds, annealing temperature at 65˚C at 20seconds 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 20seconds and extension at 72˚C for 30 seconds and a final extension at 72˚C for 7 minutes. The PCR amplicons were then taken to International Institute for Tropical Agriculture (IITA) Ibadan Nigeria's Next Generation Sequencing Laboratory for the Sequencing of the amplicons, and subsequent genotyping.

Table 1. Primer sequences used in genotyping for the transforming growth factor β3 gene SNPs identified

SNP Name	Primer	SNP Primer sequence (5′ to 3′)
AF459834.1	Forward	TCAGGGCGGTAGAGGGTGTGCT
	Reverse	GCCACTGGCAGGATTCTCACTCC
	Extension	TCCCCAAAGAACTCTGTA
AF459834.2	Forward	ACGTTGGATGCAGAGTTGTTGC
	Reverse	GGATGGTCTGTGTTCCCGGGCC
	Extension	CTCCACCCATTGGATTA

SNP genotyping

The SNP primers for genotyping the TGF-β3 gene were identified, selected and procured by IITA biotechnology experts with the PCR primer and 1 extension primer each designed for the SNPs identified (Table 1).

Genotyping of the 150 birds was performed using the direct genotyping by sequencing (GBS) method (Campbell, Harmon & Narum 2015), being a method that could simultaneously identify and genotype a large number of SNPs that has been successfully applied to a wide range of biological species

Genomic analysis

Genotype and Allele frequencies

The SNP genotypes and allele frequencies were calculated by simple allele counting method (Chromas 2018). The possible deviations of allele and genotype frequencies from the Hardy–Weinberg equilibrium was examined with Chromas Bioinformatics Software (Chromas 2018) with Pearson's Chi-square test.

Genomic Association analysis

50 birds each from Noiler, FA and Sasso strains respectively with pedigree information of their parents were used for an association study of genetic markers with body weight and morphological traits. Genomic and the phenotypic data generated were subjected to Association analysis based on ANOVA

using General Linear Model Procedure of SPSS software (SPSS 2022), with fixed effect of Strain (L_i), fixed effects of SNP genotype (SG_j), random effect of family (F_k) and random error term e_{ijk} effects according to the model:

$Y_{ijk} = \mu + L_i + SG_j + F_k + e_{ijk}$

Where, Y_{ijk} is the response variable and μ is the population mean. Significant differences between least-squares means of the different genotypes were calculated using Duncan New Multiple Range Test (SPSS 2022). SNPs that deviated from the Hardy-Weinberg equilibrium based on significant Chi-square values were discarded. Also, SNPs with minor allele frequency <1% across all individuals were removed.

Results and Discussion

Phenotypic Results

The mean values of the different growth parameters (Body weight, Height at withers, Body length, Breast girth, and Wing length) of the strains studied at different weeks (4, 8, 12, 16, and 20 weeks) shows that the Sasso strain had significantly higher body weight than the other strains for all the growth traits studied from week 4 to 20 weeks of age (Table 2). Noiler strain was significantly higher in terms of body length, breast girth and wing length than the other strains at 4 weeks of age.

Table 2. Mean body trait parameters of the chicken strains studied

Means with different superscripts ^{abc} within the same row column of a particular parameter is significantly different (P<0.05). SEM = Standard Error of Mean. N=Noiler, S=Sasso, FA=Funaab-Alpha.

The correlation between the body weight and the body parameters of the chicken strains studied shows a significant positive relationship (P<0.001) between them all (Table 3). The correlation coefficient between them is also high (above 0.5) showing a high correlation coefficient between the body weight and all the body parameters measured.

Table 3. Phenotypic correlation between body weight and body parameters measured

Genomic Results

Amplified nucleotide sequence analysis

The amplified nucleotide sequence analysis shows the amplified PCR products of the TGFβ3 gene in the three strains of chicken studied. (Figure 1). The amplicon size of 294 bp was generated during the amplification.

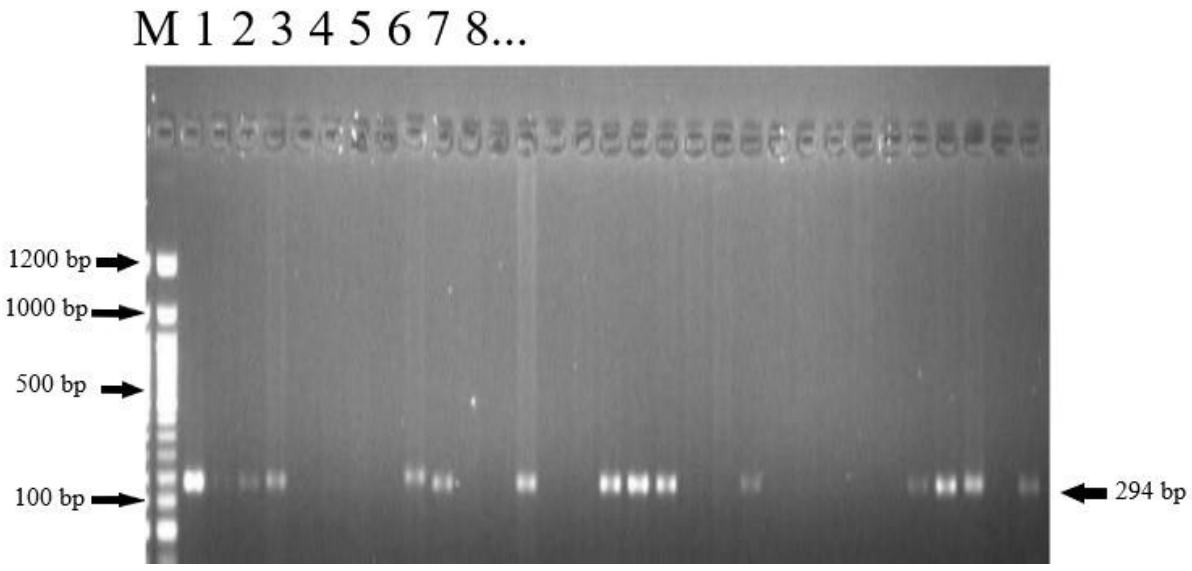

Figure 1. TGFβ3 gene fragments amplicon. M, DL 1200 DNA marker (1200bp, 1000bp, 500bp, and 100bp, respectively); Lanes 1- 8, fragments of TGFβ3 gene amplified.

Genotype and Allele frequencies of SNP genotypes identified.

Two SNP genotypes were identified. The identified SNPs were on exon 3 at positions 7941745 and 7941722 of the TGFβ3 gene which were named 7941745A>C and 7941722A>T respectively. Chi-square results demonstrated that the population used was under Hardy-Weinberg equilibrium (HWE) for the two SNP genotypes, with 7941745A>C and 7941722A>T having a dominant allele A (Table 3). Meanwhile, for the genotypic frequencies, the homozygous dominant genotype AA and the homozygous recessive genotypes CC and TT had high (50% and 86%) and low (7% and 1%) values respectively. The heterozygous genotypes AC and AT however, showed medium frequencies of 43% and 14% respectively. The values of the PIC, HWE, He and AE shows that the 7941745A>C has higher occurrence than 7941722A>T (Table 4).

Table 4. SNP genotypes and allele frequencies of the TGFβ3 gene studied

Position in Genome	SNPs	Allelic Frequencies		Genotypic Frequencies			PIC	HWE (Chi square)	He	AE
7941745	7941745A>C	A	C	AA	CA	CC				
		0.71	0.29	0.5	0.43	0.07	0.32	0.72	0.41	1.69
7941722	7941722A>T	A	T	AA	TA	TT				
		0.93	0.07	0.86	0.14	0.01	0.12	0.11	0.13	1.15

SNPs = Single Nucleotide Polymorphisms, PIC = Polymorphic Information Content, HWE = Hardy-Weinberg Equilibrium, He = Heterozygosity, AE = Effective no of Alleles.

The polymorphic information content (PIC), which is a measure of the informativeness of a genetic marker, shows that a higher PIC value indicates that the marker is more informative for distinguishing between different genotypes. In this case, both SNPs have relatively medium PIC values (0.32 for 7941745A>C and 0.12 for 7941722A>T) showing medium similarity. The Hardy-Weinberg equilibrium (HWE), is the condition that describes the relationship between allele and genotype frequencies in a population. If the genotypic frequencies are in HWE, it suggests that the population is not undergoing any evolutionary or selective pressures. In this case, both SNPs are in HWE, as indicated by the values of 0.7 and 0.11 which are not significant Chi-square values ($P<0.05$). The expected heterozygosity (He), which is a measure of the genetic diversity within a population shows that both SNPs have relatively high He values (0.41 and 0.13, respectively), implying a medium to low genetic diversity. The allelic expression (AE), which is a measure of the relative expression levels of each allele, shows that AE values of 1.69 for 7941745A>C and 1.15 for 7941722A>T, indicating that one allele is expressed more than the others in each SNP, as evidenced in this study.

Genomic Association of 7941745A>C SNP genotypes (AA, CA) and 7941722A>T SNP genotypes (AA, TA) to the growth traits of the chicken populations studied.

The 7941745A>C SNP genotypes shows that recessive homozygous SNP genotype CC had genotypic frequency of less than 1%, and therefore was not considered in the association study. However, the AA and CA SNP genotypes were significantly associated for body weight and height at withers (Table 5). Amongst the AA SNP genotypes, the Sasso strains were significantly higher than the rest, while the CA genotypes were significant amongst FA and N strains, although not different statistically.

Table 5. Association of the polymorphism of TGFβ3 gene with growth traits of chicken strains studied

SNPs	Genotype	Strain	BW (g) Mean±SEM	HW (cm) Mean±SEM	BL (cm) Mean±SEM	BG (cm) Mean±SEM	WL (cm) Mean±SEM
7941745A>C	AA	N	1477.0±131.60^{b}	11.55±0.70^{ab}	9.35±0.47	10.95±0.51	8.50±0.36
		FA	1554.0±83.23^{b}	10.86±0.44^{ab}	8.86±0.30	10.16±0.32	7.94±0.22
		S	2030.0±186.10^{a}	13.40±0.99^{a}	11.20±0.66	10.80±0.73	8.80±0.50
	CA	N	1232.1±107.45^{b}	9.77±0.57^{b}	8.47±0.38	9.67±0.42	7.40±0.29
		FA	1375.0±93.05^{b}	11.88±0.49^{a}	9.28±0.33	8.98±0.36	7.82±0.25
7941722A>T	AA	N	1330.0±100.84^{b}	10.48±0.48	8.82±0.33	10.18±0.34	7.84±0.27
		FA	1611.0±92.05^{a}	11.1±0.44	8.93±0.30	10.23±0.31	7.95±0.25
		S	1324.0±130.18^{b}	11.53±0.62	9.17±0.43	8.70±0.44	7.83±0.35
	TA	S	1778.0±159.44^{a}	13.15±0.76	10.41±0.53	10.32±0.54	8.33±0.43

Means with different superscripts ^{abc} within the same column of a particular SNP is significantly different (P<0.05). N = Noiler, FA = Funaab Alpha, S = Sasso, BW = Body weight, HW = Height at Withers, BL = Body length, BG = Body girth, WL = Wing length.

Meanwhile, the height at withers parameter was significantly associated amongst all the strains for AA genotype, while amongst the CA genotypes, the FA strains were significantly higher than the N strains.

Also, amongst the 7941722A>T SNP genotypes, only two were identified – AA and TA (Table 5), with body weight being the only trait associated. All the strains were amongst the genotypes segregated under AA, with FA being significantly more associated than the others. However, only S strain was segregated amongst the TA SNP genotypes being significantly higher amongst all the other strains in both AA and TA genotypes.

These results were similar to the findings of Li *et al.* (2003) and Amirinia *et al*. (2011) who reported significant association with growth traits of chickens. Hence selecting those genotypes for future breeding for high growth rate in dual purpose chicken will achieve rapid progress in their growth for both meat and egg production. This is in line with the general ideas that the process of identifying the QTL responsible for the economic important traits in chicken will facilitate poultry breeding programs as molecular genetic information is an easier and faster required means to enhance genetic improvement amongst animal species (Huang *et al.*, 2009; Amirinia *et al*., 2011; Campbell *et al.*, 2015). Also (Jin *et al.,* 2013) had reported that the candidate gene approach is a very powerful method, needed to investigate genomic associations of gene polymorphisms with economically important traits in farm animals.

Growth and body composition and its final expression is the result of interaction among genetic, nutritional and environmental factors, which are a comprehensive reflection of development of various parts of the chicken body (Huang *et al*., 2009). The current research findings will help the dual-purpose chicken breeders because this study of *TGF-ß3* gene as a potential candidate gene of QTL that is useful in the selection of chicken, will help for both in improving meat and egg production. Our findings shows that TGFß3 SNP has significant (P < 0.05) effect on body weight

and height at withers at 20 weeks of age which is also in line with the findings of (Li *et al*., 2003; Amirinia *et al*., 2011; Jin *et al.*, 2013) who reported that SNP in T*GF-ß3* gene significantly ($P < 0.1$) affected body weight of broilers at 6 weeks.

Conclusion

Marker Assisted Selection can be an ideal option to improve selection in dual purpose chicken production. The results from the current study indicated that a SNP marker in the *TGFß3* gene was associated with body composition traits (body weight and height at withers) in chickens growing to point of lay, and are therefore, a potential marker for molecular MAS programs in commercial dual purpose chicken lines, which is suitable for rural chicken farmers that needs sustainable meat and egg production in the long run.

Authors' contributions

OVMO, OUE and ABO conceptualized and designed this study, **CNJ** collected the data for this study, and wrote the initial draft of this manuscript; **OVMO and ABO** conducted the statistical

analyses, collaborated in interpretation of the results, and finalized the manuscript. **OVMO, AB** and **OUE** developed the original hypotheses, designed the experiments, collaborated in interpretation of the results. **CNJ, OUE, AB** and O**VMO** read and approved the finalized manuscript.

Conflict of Interest

We certify that there is no conflict of interest with any financial or otherwise dealings with any organization regarding the materials discussed in the manuscript. Also, the authors do not have any conflict of interest in this article.

Compliance with Ethical Standards - none

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Data Availability

Data will be made available on reasonable request.

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