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# Polymorphism of pituitary specific transcription factor-1 (PIT-1) gene at exon 6 and its association with zoometric traits of FUNAAB Alpha chickens

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**ABSTRACT.** Pituitary specific transcription factor 1 (PIT-1) gene plays a critical role in the regulation of growth and development of muscle in chicken. This study was conducted to determine polymorphism of PIT-1 gene in FUNAAB Alpha chickens and its association with body weight and body morphometric traits (body length, thigh length, keel length wing span and wing length). Genomic DNA was extracted from ninety-seven (97) FUNAAB Alpha chickens at eight (8) weeks of age. Genotyping was done using PCR-RFLP technique and growth parameters were also recorded. Results revealed alleles A and B with genotypes AA, AB and BB which were significantly ($p < 0.05$) associated with body weight and body morphometric traits of chickens. BB genotype had the highest frequency of 0.52 compared with AA and AB genotypes which had 0.32 and 0.16, respectively. Particularly, the PIT-1 genotypes (AA, AB and BB) were significantly ($p < 0.05$) associated with body morphometric traits at 6$^{th}$ week. Also the interaction effect of PIT-1 genotypes and sex showed a significant ($p < 0.05$) association with body weight at 4$^{th}$ and 8$^{th}$ week, in which the BB males showed superior performance over the females. Our study concluded that PIT-1 gene at exon 6 was polymorphic and could be explored as biomarker for improvement of growth performance in FUNAAB Alpha chicken.

**Keywords:** genotype; PCR-RFLP; body measurement; DNA; poultry; growth.

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## Introduction

The poultry sector provides employment for more than 70% of Nigerians, either directly or indirectly. An estimated 300 thousand tons of poultry meat are produced annually. With a net worth of USD 1.7 billion annually, poultry is the most commercialized agricultural sector (Food and Agriculture Organization [FAO], 2019). Between 20 and 51 percent of poultry-keeping households' revenue comes from activities related to their livestock. Through provision of meat and eggs to the population, poultry industry has contributed to food security and improve nutrition. The annual consumption of poultry meat per capita is 1.8 kilogram (FAO, 2019). According to Udeh and Ighobesuo (2023), local chicken constitutes 80% of poultry production in Sub-Saharan Africa and Nigeria has an estimated population of 180 million local chickens.

FUNAAB Alpha chicken, is a tropical adapted slow-growing broiler chicken. The chicken is an improved Nigerian indigenous breed of chicken developed in 2018 at the Federal University of Agriculture, Abeokuta, Ogun State, Department of Animal Breeding and Genetics (Ojoawo et al., 2021). It was developed to meet the growing demand for animal protein in a rapidly increasing population in Nigeria.

Several genes regulate growth, and pituitary specific transcription factor -1 (PIT-1 or POU class) is the most important (Bhattacharya et al., 2012). According to Jin et al. (2018), the PIT-1 gene belongs to the transcription regulator family. PIT-1 gene plays a crucial role in the formation of the anterior pituitary (Zhao et al., 2004); it also functions as a transcription factor for genes that affect growth in chickens, including prolactin, growth hormone, and transforming growth factor β (Bhattacharya et al., 2012). Studies by Nie et al. (2008) and Retnosari et al. (2020) revealed that there are 6 exons and 7 exons of PIT-1 gene in mammals and birds, respectively. This PIT-1 gene is regarded as a candidate gene for production performance because of its diverse range of bioactivities and essential regulatory role (Nie et al., 2008). Generally, various studies have associated PIT-1 gene with growth, carcass and fat related traits in pigs (Yu et al., 1995; Brunsch et al., 2002;

Song et al., 2005.); growth and carcass in cattle (Zhao et al., 2004; Xue et al., 2006). Numerous species have been found to carry the PIT-1 cDNA and the sequence of chicken cDNA has been investigated by Tanaka et al. (1999). Zhao et al. (2004), studied polymorphism at exon 6 and associated it with body morphometric in dairy cattle and early-age body weight in beef cattle. The exon 6 of PIT-1 gene have been associated with body weight of chicken at 8 weeks of age (Nie et al., 2008). Similarly, previous studies by Rodbari et al. (2011) and Bello et al. (2020), have successfully detected PIT-1 gene polymorphism and their associations with body weight in different chicken populations. It is obvious that this genetic marker has much contribution to body weight and body morphometric, Therefore, understanding the relationship between PIT-1 gene polymorphism and zoometric traits in FUNAAB Alpha chicken will further provide genetic basis for their body morphometry thereby enhancing targeted breeding programmes aimed at improving the productivity of FUNAAB Alpha chickens.

## Materials and methods

### Experimental animals and procedure

The experiment was carried out at the Federal University of Agriculture, Abeokuta, Poultry Breeding Unit. Two hundred and ten FUNAAB Alpha day-old chicks purchased from the hatchery unit of the Federal University of Agriculture, Abeokuta were used for the study. Every experimental chick was wing-tagged and reared under the same management conditions. Commercial diet (broiler starter at 0–4 weeks and broiler finisher at 5–8 weeks) was given to the chickens *ad-libitum*. There was also unlimited access to clean cool water.

Body weight of chickens and body morphometric traits (body length, thigh length, keel length wing span and wing length) were recorded fortnightly until the birds were 8 weeks old. Ninety-seven chickens were randomly selected from the two hundred and ten chickens for blood collection which was carried out under aseptic conditions and the blood was transferred to an FTA card which was kept for further analysis.

Genomic DNA extraction was carried out with solution-based JENA Bioscience Animal tissue DNA Preparation Kit following manufacturer's instructions. A NanoDrop Lite Spectrophotometer was used to verify the quantity and purity of the DNA, and only high-quality DNA samples were utilized for further analysis. For the amplification, the following particular primers set with a fragment size of 483 bp were employed:

(F: TGGGAAGAACAGTTTATGGC; R: TGGCTAGCTTGTAAGGGAATC) (Nie et al., 2008). PCR reaction was carried out with Jena Bioscience Red Load Taq Master(5x). Each PCR reaction mixture consisted of 5 µL mastermix, 1 µL (10 pmol) each of PIT-1 primers (forward and reverse), 5 µL template DNA. To achieve a total reaction volume of 25 µL, 13 µL of nuclease free was added. An Applied Biosystem 2720 Thermocycler (USA) was used to perform PCR amplification. The mixture was first denatured for two minutes at 94°C. It then underwent thirty cycles of denaturation for thirty seconds at 94°C, annealing for thirty seconds at 52.4°C, and then, extension was done for thirty seconds at 72°C. Finally, it underwent a final extension for two minutes at 72°C. The amplified PCR products were stained with meastrosafe and evaluated on a 1% agarose gel to verify the presence of desired gene fragment.

BspHI restriction enzyme was used in the restriction analysis of the PCR products according to manufacturers' recommended conditions for 15 minutes. The total reaction volume contained 10 µL of PCR product, 1.5 µL of 10x buffer (Tris-borate) and 0.1 µL BspHI Restriction Enzyme (New England Biolab, USA) and 3.4 µL nuclease free water. The reaction mixture was incubated at 37°C for 15 min. and enzyme inactivated at 80°C for 20 min. The digested products were resolved on a 2% (wt/vol) agarose gel dissolved in 0.5X Tris-borate buffer, stained with meastrosafe stain and visualized under blue light transilluminator (New England BioGroup, USA). The molecular size ladder was used to estimate the molecular sizes of the digests and amplicon.

### Statement of animal rights

The approval for this research was granted by the Animal Care and Use Committee of the Federal University of Agriculture, Abeokuta, Ogun state, Nigeria.

### Statistical analysis

The direct gene counting method was used to estimate genotype and allele frequencies. Using the GenAlex 6.5 software tool, the genotype and allele frequencies were examined for Hardy-Weinberg Equilibrium (HWE). The effects of sex and genotypes of PIT-1 on bodyweight and body zoometric traits were analysed using the

general linear model procedure of the Statistical Analysis System program (SAS version 9.1). Tukey HSD was used to separate means that were significant ($p < 0.05$).

The following model was utilized to estimate the effect of sex and PIT-I polymorphism on body morphometric traits and body weight:

$Y_{ijk} = \mu + G_i + S_j + (GS)_{ij} + \varepsilon_{ijk}$

Where:

$Y_{ijk}$ = the observed value of the dependent variable

$\mu$ = overall population mean

$G_i$ = fixed effect of the $i^{th}$ PIT-1 polymorphic variant (AA, AB, BB)

$S_j$ = fixed effect of the $j^{th}$ sex (j = male, female)

$(GS)_{ij}$ = interaction effect of $i^{th}$ PIT-1 polymorphic variant and $j^{th}$ sex

$\varepsilon_{ijk}$ = random residual error

To verify that the observed genotype frequencies agree with Hardy-Weinberg equilibrium, a Chi-squared test was performed. This is to check if the PIT-1 locus of chickens' population used in this study is under selection influence.

## Results and discussion

### PCR-RFLP analysis of PIT-1 (exon 6)

The digestion of PIT-1 / BspH1 was polymorphic, displaying two alleles (A and B) with three distinct genotypes (AA, AB, and BB) (Figure 1). The A allele produced a fragment size of 455 bp, whereas the B allele produced a fragment size of 236 bp. The A and B allele frequencies were 0.36 and 0.64, respectively. Additionally, AA, AB, and BB had genotype frequencies of 0.32, 0.16, and 0.52, respectively. The chi-Square test (27.40) was significant for Hardy-Weinberg equilibrium. In this study, PIT-1 gene (exon 6) was polymorphic and the polymorphism were related to growth traits of FUNAAB Alpha chickens. The three genotypes AA, AB and BB identified from the polymorphism, agreed with the reports of Rodbari et al. (2011) who reported three genotypes for PIT-1 gene/Taq1 and Nie et al. (2008) who reported genotypes CC, CT and TT. However, our findings contradicted the result of Bello et al. (2020) who reportedly found homozygous AA and heterozygous AB genotypes from the samples of Fulani and Yoruba ecotypes used in their study. Allele B was more prevalent than A allele in the population of chicken used for the study. The frequency of BB genotype which was higher than that of AA and AB. These observed frequencies contradicted the report of Bello et al. (2020) who obtained 0.71 and 0.29 frequencies for AA and AB genotypes, respectively. It is also contrary to the findings of RODBARI et al. (2011) who obtained genotypic frequencies of AA (0.61); AB (0.32) and BB (0.07). The Hardy-Weinberg equilibrium tested with chi-square showed that the population was not at equilibrium ($p < 0.05$). This may be due to high selection programme in which the FUNAAB Alpha chicken have been subjected to for meat production.

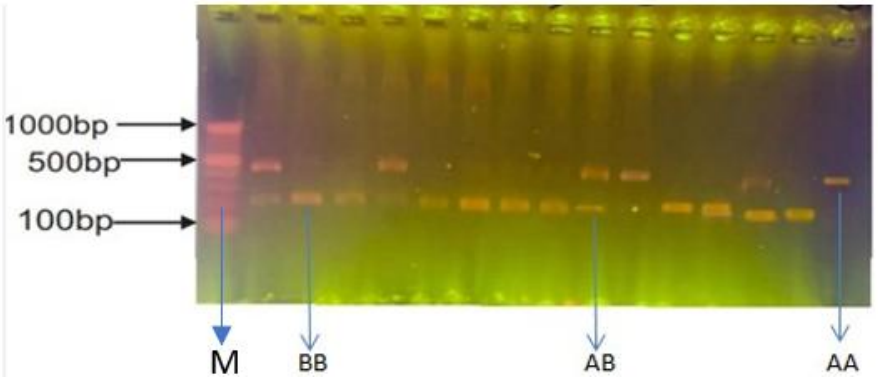


**Figure 1.** PCR-RFLP pattern for PIT 1 gene with BspH1 digestion. M-Molecular marker of 100 bp.

## Relationship between PIT-1 genotypes and growth traits

Due to its genetic location on chromosome 1, which is quite close to the QTL region regulating chicken growth and development, the PIT-1 gene is considered a significant candidate gene (Bello et al., 2020). Musa et al. (2016) reported that the PIT-1 gene affects the growth rate and carcass characteristics. Rodbari et al. (2011); Bhattachharya et al. (2012) and Ahmadi et al. (2019) have associated PIT-1 gene to body weight in different chicken. The PIT-1 genotypes significantly ($p < 0.05$) affected wing length, wing span and body length at 6$^{th}$ week of age (Table 1). Our study shows that the BB genotype was significantly higher than AA genotype in the parameters examined. The result of this study (Table 1) showed that body weight was not significantly ($p < 0.05$) affected by PIT-1 gene in any of the weeks recorded. This is contrary to the report of Nie et al. (2008) who observed association between exon 6 of PIT-1 gene and body weight at week 8. The result of this study also disagreed with the report of Jiang et al. (2004) who found significant association of PIT-1 with body weight at week 8. These differences in the reports could be as a result breeds of chickens used in these experiments and possibly, the level of selection in the population under consideration. The report by Rodbari et al. (2011), which is also at variance with the report of this study, showed that the BB genotype of PIT-1 gene was significantly associated with body weight at sixth week of age. Apart from the fact that the breeds of chicken could be the reason for the differences observed in various experiment, it could also be due to the restriction enzyme used, given that restriction enzymes are known to be specific to restriction. To the best of our knowledge, there is not much information published in literature about the relationship between PIT-1 gene polymorphism and parameters such as wing length, wing span and body length in chickens, the results of this study revealed that BB genotypes had significantly higher values for wing length, wing span and body length compared to AA genotype at 6 weeks. In effect, PIT-1 gene (exon 6) could be used as a genetic marker for selection for these traits in FUNAAB Alpha chicken at 6$^{th}$ week.

**Table 1.** Effect of PIT-1 genotype on body weight and body morphometric traits.

| Week | Parameters | Pit-1 | Genotypes | |
|---|---|---|---|---|
| | | AA | AB | BB |
| | BW (gm) | 112.11±5.70 | 122.54±6.42 | 119.92±3.92 |
| | WS (cm) | 17.43±0.51 | 17.35±0.57 | 17.83±0.35 |
| 2 | WL (cm) | 7.13±0.30 | 6.01±0.34 | 7.02±0.20 |
| | TL (cm) | 5.12±0.21 | 4.84±0.23 | 5.05±0.14 |
| | BL (cm) | 9.38±0.20 | 9.26±0.23 | 9.37±0.14 |
| | KL (cm) | 3.86±0.13 | 3.82±0.15 | 3.97±0.09 |
| | BW (gm) | 349.42±20.54 | 392.22±23.16 | 406.12±14.11 |
| | WS (cm) | 27.35±0.48 | 28.42±0.55 | 28.51±0.33 |
| 4 | WL (cm) | 11.80±0.25 | 12.17±0.29 | 12.41±0.17 |
| | TL (cm) | 8.53±0.37 | 7.88±0.42 | 8.81±0.25 |
| | BL (cm) | 13.20±0.37 | 13.20±0.42 | 13.44±0.25 |
| | KL (cm) | 6,02±0.17 | 6.10±0.19 | 6.32±0.12 |
| | BW (gm) | 695.12±4.14 | 807.77±47.05 | 782.15±28.73 |
| | WS (cm) | 29.30±0.58$^{b}$ | 30.64±0.65$^{ab}$ | 31.15±0.40$^{a}$ |
| 6 | WL (cm) | 12.80±0.32$^{b}$ | 13.48±0.37$^{ab}$ | 13.97±0.22$^{a}$ |
| | TL (cm) | 9.03±0.31 | 8.43±0.35 | 17.37±0.35 |
| | BL (cm) | 15.68±0.50$^{b}$ | 17.09±0.57$^{ab}$ | 17.37±0.35$^{a}$ |
| | KL (cm) | 7.62±0.16 | 7.73±0.19 | 7.74±0.11 |
| | BW (gm) | 1036.18±59.21 | 1118.75±65.74 | 1202. ±39.67 |
| | WS (cm) | 39.76±0.58 | 40.76±0.64 | 40.75±0.39 |
| 8 | WL (cm) | 18.02±0.37 | 17.91±0.41 | 17.90±0.25 |
| | TL (cm) | 12.07±0.44 | 11.43±0.49 | 11.89±0.30 |
| | BL (cm) | 18.86±0.62 | 20.53±0.68 | 20.29±0.41 |
| | KL (cm) | 8.68±0.29 | 9.07±0.32 | 8.80±0.19 |

$^{ab}$; mean along the same row with different superscript are significantly different ($p < 0.05$); BW- body weight; WS – wing span; WL – wing length; TL – thigh length; BL – body length; Kl – keel length.

The effect of sex on body weight, wing length and wing span became evident at week 8 (Table 2), where males had significant ($p < 0.05$) higher values for body weight, wing length and wing span, respectively, than females. The is in consonance with the results obtained by Abe (2022) and Amao (2022) on body weight, who reported the male chicken having higher body weight than their female counterpart. The findings of this study

highlight the impact of sexual dimorphism in chickens emphasizing the importance of considering sex differences in breeding and selection programmes to optimize growth traits.

**Table 2.** Effect of sex on body weight and body morphometric traits.

| | | Sex | |
|---|---|---|---|
| Week | Parameters | F | M |
| 2 | BW (gm) | 116.71±4.43 | 119.68±4.47 |
| | WS (cm) | 17.71±0.40 | 17.37±0.40 |
| | WL (cm) | 6.92±0.23 | 6.57±0.23 |
| | TL (cm) | 4.93±0.16 | 5.07±0.16 |
| | BL (cm) | 9.54±0.16 | 9.26±0.16 |
| | KL (cm) | 3.89±0.10 | 3.88±0.10 |
| 4 | BW (gm) | 365.99±16.01 | 399.19±16.07 |
| | WS (cm) | 27.93±0.38 | 28.25±0.38 |
| | WL (cm) | 12.14±0.20 | 12.12±0.20 |
| | TL (cm) | 8.10±0.30 | 8.72±0.29 |
| | BL (cm) | 13.03±0.29 | 13.53±0.29 |
| | KL (cm) | 6.04±0.13 | 6.26±0.13 |
| 6 | BW (gm) | 734.15±32.85 | 789.21±32.15 |
| | WS (cm) | 30.04±0.46 | 30.09±0.45 |
| | WL (cm) | 13.28±0.26 | 13.55±0.25 |
| | TL (cm) | 8.56±0.25 | 9.11±0.24 |
| | BL (cm) | 16.71±0.40 | 16.71±0.39 |
| | KL (cm) | 7.63±0.13 | 7.77±0.13 |
| 8 | BW (gm) | 1053.41±44.77$^{b}$ | 1185.57±46.62$^{a}$ |
| | WS (cm) | 39.10±0.44$^{b}$ | 41.75±0.46$^{a}$ |
| | WL (cm) | 17.48±0.28$^{b}$ | 18.41±0.29$^{a}$ |
| | TL (cm) | 11.48±0.33 | 12.12±0.35 |
| | BL (cm) | 19.47±0.47 | 20.35±0.48 |
| | KL (cm) | 8.58±0.22 | 9.12±0.23 |

$^{ab}$; mean along the same row with different superscript are significantly different ($p < 0.05$); BW- body weight; WS – wing span; WL – wing length; TL – thigh length; BL – body length; Kl – keel length.

The interaction between the PIT-1 genotypes and sex of FUNAAB Alpha chicken (Table 3) showed significant results at week 4, 6 and 8. At week 4, the value obtained for body weight of BB male was significantly ($p < 0.05$) higher than AA female. At week 6, BB males had higher values for wing length than value obtained for AA females. Also, values recorded for week 8, clearly showed that BB male was higher ($p < 0.05$) than AA female. To our knowledge, interaction effect of PIT-1 genotypes and sex has not been reported in literature. Hence, there is paucity of previously published work to compare with our findings. However, the interaction revealed that the BB male had better performance, especially for body weight. Biological information obtained from this study, will help improve breeding programmes for FUNAAB Alpha chicken and will subsequently aid development of the breed for better meat production.

**Table 3.** Effect of interaction between PIT-1 genotype and sex on body weight and body morphometric traits.

| | | RFLP Genotype | | | | | |
|---|---|---|---|---|---|---|---|
| Week | Parameters | AA×F | AA×M | AB×F | AB×M | BB×F | BB×M |
| 2 | BW (gm) | 102.03±7.51 | 122.19±8.56 | 128.44±9.58 | 116.65±8.56 | 119.65±8.56 | 120.19±5.77 |
| | WS (cm) | 17.12±0.67 | 17.75±0.76 | 18.09±0.86 | 16.62±0.76 | 17.93±0.47 | 17.74±0.52 |
| | WL (cm) | 7.55±0.39 | 6.70±0.45 | 6.19±0.50 | 6.02±0.45 | 7.03±0.28 | 7.01±0.30 |
| | TL (cm) | 5.12±0.27 | 5.13±0.31 | 4.76±0.35 | 4.91±0.31 | 4.93±0.19 | 5.18±0.21 |
| | BL (cm) | 9.58±0.26 | 9.18±0.30 | 9.48±0.34 | 9.04±0.30 | 9.57±0.19 | 9.57±0.20 |
| | KL (cm) | 3.81±0.18 | 3.92±0.20 | 3.90±0.22 | 3.74±0.20 | 3.97±0.12 | 3.96±0.14 |
| 4 | BW (gm) | 297.66±27.09$^{b}$ | 401.20±30.89$^{ab}$ | 405.50±34,53$^{ab}$ | 378.94±30.89$^{ab}$ | 394.82±19.54$^{ab}$ | 417.42±20.37$^{a}$ |
| | WS (cm) | 27.00±0.64 | 27.70±0.73 | 28.44±0.81 | 28.40±0.73 | 28.36±0.46 | 28.65±0.48 |
| | WL (cm) | 12.00±0.33 | 11.60±0.38 | 11.94±0.43 | 12.40±0.38 | 12.48±0.24 | 12.35±0.25 |
| | TL (cm) | 8.12±0.49 | 8.95±0.56 | 7.25±0.62 | 8.50±0.56 | 8.92±0.32 | 8.70±0.37 |
| | BL (cm) | 12.69±0.49 | 13.70±0.56 | 13.00±0.62 | 13.40±0.56 | 13.40±0.35 | 13.48±0.37 |
| | KL (cm) | 5.85±0.22 | 6.20±0.26 | 6.00±0.29 | 6.20±0.26 | 6.28±0.16 | 6.40±0.17 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 6 | BW (gm) | 629.83±57.27 | 760.41±59.82 | 798.48±70.14 | 817.06±62.74 | 774.15±38.91 | 790.16±42.30 |
| | WS (cm) | 28.33±0.80 | 30.27±0.83 | 30.88±0.97 | 30.40±0.87 | 30.9±0.54 | 31.40±0.59 |
| | WL (cm) | 12.29±0.45$^{b}$ | 13.32±0.47$^{ab}$ | 13.75±0.55$^{ab}$ | 13.20±0.49$^{ab}$ | 13.81±0.30$^{ab}$ | 14.14±0.33$^{a}$ |
| | TL (cm) | 8.33±0.43 | 9.72±0.49 | 8.31±0.53 | 8.31±0.53 | 9.05±0.29 | 9.06±0.32 |
| | BL (cm) | 16.00±0.69 | 15.36±0.72 | 17.13±0.84 | 17.13±0.84 | 17.02±0.47 | 17.73±0.51 |
| | KL (cm) | 7.38±0.23 | 7.86±0.24 | 7.81±0.28 | 7.81±0.28 | 7.69±0.15 | 7.80±0.17 |
| 8 | BW (gm) | 911.46±74.08$^{b}$ | 1160.88±92.39$^{ab}$ | 1135.66±99.00$^{ab}$ | 1101.84±87.65$^{ab}$ | 1113.09±54.36$^{ab}$ | 1290.97±57.80$^{a}$ |
| | WS (cm) | 37.86±0.73$^{b}$ | 41.67±0.91$^{a}$ | 36.31±0.96$^{ab}$ | 42.20±0.86$^{a}$ | 40.12±0.53$^{ab}$ | 41.39±0.57$^{ab}$ |
| | WL (cm) | 17.54±0.46 | 18.50±0.57 | 17.13±0.61 | 18.70±0.54 | 17.77±0.34 | 18.02±0.36 |
| | TL (cm) | 11.75±0.55 | 12.39±0.69 | 10.94±0.73 | 11.95±0.65 | 11.75±0.40 | 12.02±0.43 |
| | BL (cm) | 18.50±0.77 | 19.22±0.96 | 19.81±1.02 | 21.25±0.91 | 20.10±0.56 | 20.49±0.60 |
| | KL (cm) | 8.09±0.36 | 9.28±0.45 | 8.94±0.47 | 9.20±0.42 | 8.17±0.26 | 8.89±0.28 |

$^{ab}$; mean along the same row with different superscript are significantly different ($p < 0.05$). BW- body weight; WS – wing span; WL – wing length; TL – thigh length; BL – body length; Kl – keel length.

## Conclusion

The observed variants (AA, AB and BB ) of PIT-1 gene (exon 6) were found to be associated with early development of wing length, wing span and body length of FUNAAB Alpha chicken studied. The positive relationship between genotype BB and these parameters at week 6 is an indication that the PIT-1(exon 6) is a potential bio-marker for early development and selection in FUNAAB Alpha chicken especially for wing length, wing span and body length.

## Data availability

Please be advised that the data used in the research will be made available through the following link upon request. (https://drive.google.com/file/d/1Nu3_oNsb_lIjtrRQWasr-YbxSYuXU-ek/view?usp=drive_link).

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