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Myostatin Gene Polymorphisms and their Relationships with Growth Parameters of Broiler Chickens

[1]Adewole, R. A., [2]Adedeji, T. A., [3]Emiola, I. A., [4,6]Adebambo, A. O. and [5]Fayeye, T.R.
[1]Department of Animal Science, P.M.B. 1601, Al-Hikmah University, Ilorin, Nigeria.
[2]Department of Animal Production and Health, P.M.B. 4000, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.
[3]Department of Animal Nutrition and Biotechnology, P.M.B. 4000, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.
[4]Department of Animal Breeding and Genetics, P.M.B. 2240, Federal University of Agriculture, Abeokuta, Nigeria.
[5]Department of Animal Production, P.M.B. 1515, University of Ilorin, Nigeria.
[6]ACUTIG Laboratory Nigeria Limited, Abeokuta.

***Corresponding Author:** radewole@alhikmah.edu.ng or adewoleadeolaa@gmail.com*
***Phone Number:** +2349036546762*

Abstract

This experiment was designed to investigate the relationship between Myostatin (MSTN) gene polymorphisms and growth parameters in Ross 308, Cobb 500, Arbor Acres (AA) and Arbor Acres Plus (AAP). Two-hundred-day-old chicks were raised under similar conditions. Body weight (BDW), chest girth (CG) and thigh length (TL) were measured at day-old, six and twelve weeks in the randomly selected birds. At 12th week, blood samples were collected via brachial vein of forty birds for DNA extraction. Amplicons were visualized on 1% agarose gel. The amplified products of exon 1 were sequenced and Single Nucleotide Polymorphisms (SNPs) were identified using codon code aligner. The result revealed larger SNPs with Ross 308 exhibiting the highest number (eleven SNPs) followed by AA with six, AAP and Cobb 500 with five each. Significant (P<0.05) effects of SNPs on growth parameters were observed across strains. Adenine at 65A>G in AA and guanine at 64A>G in AAP were linked to higher BDW. In Cobb 500, guanine at 55A>G was associated with superior BDW and CG while Ross 308 showed the most extensive polymorphism with Adenine influencing higher BDW almost at all the loci. These findings confirmed the role of MSTN gene polymorphisms in modulating growth performance in broilers. Thus, Ross 308 is considered the most suitable strain due to its greater frequency of beneficial Myostatin alleles, which are linked to improved growth characteristics and a higher growth rate.

Key words: Myostatin, polymorphism, growth parameter, single nucleotide polymorphism, broiler, allele

Introduction

Broiler chickens are mainstay of global food security, providing cheap source of high-quality protein (FAO, 2022). The poultry industry drives economic growth, generating job opportunities and provision of income especially in under developed countries (World Bank, 2021). In Nigeria, broiler chicken production plays a pivotal role in the agricultural sector, meeting the excellent

protein demands of a rapidly growing and increasingly urbanized population (National Bureau of Statistics, 2023). However, the sector struggles with numerous challenges, including limited access to superior genetic stock, high-quality feed and adequate veterinary services, all of which hinder optimal production efficiency (Adene and Oguntade, 2006). A fundamental factor influencing broiler production efficiency is genetics, as broiler strains differ in their growth potential, feed conversion efficiency and muscle yield (Havenstein *et al*., 2003).

The continuous demand for enhanced growth rates and improved carcass quality especially in broiler chickens calls for urgent attention. However, achieving these goals remains challenging due to the complex genetic factors that regulate muscle development and growth in broiler chickens. *Myostatin* gene, being a critical regulator of muscle development, there is little knowledge about polymorphisms within the *Myostatin* gene which can provide insights into variations in muscle growth among broiler chickens. Most existing studies have been conducted in controlled environments outside Nigeria, creating a gap in understanding how *Myostatin* gene variations influence broiler performance under local production conditions.

This study focuses on four prominent commercial broiler strains namely; Ross 308, Cobb 500, Arbor Acres Plus, and Arbor Acres. Each of these strains has distinct growth characteristics. Ross 308 is known for rapid growth rates, making it ideal for markets demanding and quick production cycles (Aviagen, 2023). Cobb 500 is favoured for its high breast meat yield, aligning with consumer preferences for lean protein (Cobb-Vantress, 2023). Arbor Acres Plus and Arbor Acres, with the former representing an advanced version, are valued for their adaptability to diverse production environments and overall growth performance (Hubbard Breeders, 2023). Despite these advances in strain selection, variations in growth rate, muscle development and feed efficiency persist within and among these broiler strains. The genetic constitution of growth performance in broiler chickens are highly complex, involving multiple genes and regulatory pathways (Schmidt *et al*., 2009). Among these (growth hormone, insulin-like growth factor etc.) the *Myostatin* (*MSTN*) gene has emerged as a key player in muscle development and growth regulation (Lee, 2004). *Myostatin,* a member of the Transforming Growth Factor-Beta (TGF-β) superfamily, functions as a negative regulator of skeletal muscle growth by inhibiting muscle cell proliferation and differentiation (Chen *et al.,* 2021). Variations in the *MSTN* gene particularly single nucleotide polymorphisms (SNPs) can lead to alterations in *MSTN* expression, influencing muscle mass and overall growth performance (Seo *et al*., 2018). These genetic variations may account for observed differences in body weight, chest girth and thigh length among broiler strains. The *MSTN* gene exerts its effects by binding to receptors on muscle cells, triggering signaling pathways that suppress muscle hypertrophy (Lee, 2004).

Specific SNPs in the exon 1 region of the gene have been linked to variations in *MSTN* protein function, making this exon a focal point for genetic studies (Zhang *et al*., 2019). Research has shown that certain SNPs within Exon 1 are associated with increased muscle mass and improved feed conversion efficiency, although inconsistencies exist due to differences in experimental design, environmental influences, and broiler strains studied (Smith and Jones, 2020). Therefore, understanding how *MSTN* polymorphisms influence growth traits could aid in developing targeted breeding strategies to enhance broiler performance and productivity (Schmidt *et al*., 2009).

Materials and Methods

Experimental Site

The experiment was conducted at the Poultry Unit of the Teaching and Research Farm of the Department of Animal Science, Al-Hikmah University, Igbaja Campus, Kwara State located approximately 56kms north-east of Ilorin and north-north-east of Ajase-Ipo, on

Latitude 8º23'60"N and Longitude 4º53'60"E at an altitude of 394.97 meters and 1295.83 feet above sea level (Google, 2023). The molecular analysis was carried out at Inqaba Biotech West Africa Limited, Ibadan, Oyo State located on latitude 7º23'47"N and longitude 3º55'0"E (Google, 2023).

Experimental Animals, Feeds and Management

Data were generated from two-hundred-day-old chicks of mixed sex comprising of fifty chicks per strain of broiler namely; Arbor Acre, Cobb 500, Ross 308 and Arbor Acre Plus. Birds were raised in a deep litter system which was divided into five cells containing forty birds per cell and thereafter the birds were weighed, leg banded individually and later wing tagged at 4 weeks for easy identification. Prior to the arrival of the day-old chicks, the brooder pen was washed with Lysol® and fumigated with formaldehyde, lime and potassium permanganate. The dimension of each brooder pen was 10m × 10m. Wood-shaving was spread on the floor of each cell and heat source was placed at the centre of each cell to provide warmth for the chicks. At the arrival of the chicks, they were given multi vitamin drugs which served as an anti-stress. The chicks were brooded for two weeks. Adequate heat, ventilation, medication and feeding were provided. The chicks were duly vaccinated and antibiotics was administered as required. The litter was changed at five days intervals to prevent accumulation of ammonia gas. All chicks remained on deep-litter for 12 weeks. The birds had *ad libitum* access to commercial feed and water throughout the experiment. All birds were placed on convectional broiler starter crumble containing 21.0% Crude Protein and 2900kcal/kg Metabolizable Energy for four weeks and finisher pellet containing 17.0% Crude Protein and 3000kcal/kg Metabolizable Energy till the end of the experiment.

Growth Parameters and Sampling

Morphometric parameters of the selected forty birds measured include, Body weight (BDW), Chest Girth (CG) and Thigh Length (TL) at day-old, 6 and 12 weeks of age.

Body Weight (g): This was recorded by measuring individual bird at day old, 6 and 12 weeks with a sensitive digital scale in grammes.

Chest Girth (cm): This was determined as the circumference of the breast around the deepest region of the breast using a measuring tape in centimetres.

Thigh Length (cm): This was measured as the length of the longest bone (femur) from the pelvic bone to the hock joint using a measuring tape in centimetres.

Molecular Analysis Procedure

At 12th week of age, 1ml of blood was collected from 10 selected birds from each of Arbor Acre, Arbor Acre Plus, Cobb 500 and Ross 308 broiler birds. Blood was collected with syringe via brachial vein into EDTA bottles as described by Ayorinde *et al.* (2001) and Sewalem *et al.* (2002) for molecular analysis.

Extraction of DNA

DNA was extracted from whole blood sample collected with the use of commercially available Quick-DNA^{TM} Miniprep Kit manufactured by Zymo Research Epigenetics Company, USA following the manufacturer's procedure.

DNA Quantification

Extracted gDNA was quantified for purity and concentration using Nanodrop spectrometer. The integrity of the DNA was checked using gel electrophoresis by running Iμl of each gDNA sample on 1% Agarose gel at 120V for 20 minutes.

Primer

The *Myostatin* exon 1 gene was amplified using the designed forward and reverse primer sequences as used by Tanjung *et al.* (2019).

- Forward: 5′-ATGCAAAAGCTAGCAGTCTATG-3′
- Reverse: 5′-ACTCCGTAGGCATTGTGATAAT-3′

Polymerase Chain Reaction (PCR)

The extracted DNA was amplified using Polymerase Chain Reaction (PCR). The amplification reaction was carried out in PCR tube using programmable thermocycler (Mastercycler pro by Eppendorf). Polymerase Chain Reaction amplification was performed in a volume of 25 µl mixture containing 2 µl of DNA template, 1 µl of each forward and reverse primer and 12 µl of master mix and 9 µl of sterilized distilled water.

Quantification of PCR Products

The concentration of the purified PCR product was determined using UV spectrophotometry or fluorescent quantification.

DNA Electrophoresis

Electrophoresis was carried out on 2 µl sample from PCR amplicon at 100 volts for 45 minutes in 1 % agarose gel in 1 × TAE buffer containing 1.0 µl stain of ethidium bromide. After electrophoresis, the gel was taken into gel documentation machine to view the bands using Ultra-violet illumination. The PCR products (amplicons) was visualized using Agarose gel electrophoresis for the *Myostatin* gene with concentrations of 2.0%.

Sequencing of DNA

Polymerase Chain Reaction (PCR) products of the DNA fragment was sequenced unidirectionally with forward primer using the Big Dye version 3.1 sequencing. The sequence data was collected automatically on the ABI PRISM 3100 Genetic Analyser (Applied Biosystems).

Editing and Analysis of Sequence

The sequence was aligned, trimmed and edited using Bioedit® software. The edited sequence was blasted (BLASTN) (http://www.ncbi.nlm.nih.gov/BLASTN/) against other sequences in the GenBank to ascertain the similarity and identity with other chicken sequences of *Myostatin* gene in the database. The edited sequences were further aligned using CLUSTALW for further analyses.

SNPs Identification

Codon Code Aligner was used for the detection of the Single Nucleotide Polymorphisms (SNPs) present in the chicken populations' sequences which were associated with reference sequence using CLUSTALW for alignment and DnaSP to confirm the SNPs.

Association Analysis

The relationship between SNP markers and body weight, chest girth and thigh length were determined using a single-factor multivariate ANOVA. Means were separated using the Duncan Multiple Range Test procedure of SAS 9.4 (2023) software.

The association model used is as follows:

$Y_i = \mu + G_i + \varepsilon_{ij}$

Where;

μ = Population mean;

G_i = Fixed effect of the i^{th} SNPs (1, 2…n)

ε_{ij}= Residual error.

Results

The single nucleotide polymorphisms (SNPs) identified in exon 1 of *Myostatin* gene in Arbor Acre (AA) are presented in Table 1. Six SNPs were identified in the selected AA strain comprising of five transition and one transversion mutations respectively in which the nucleotide changes were between purine/purine, pyrimidine/purine and pyrimidine/pyrimidine. Polymorphism 56A>G (singleton), 65A>G (parsimony), 200C>G (singleton) and 329C>T (parsimony) were specific to different loci in AA.

The SNPs identified in exon 1 of *Myostatin* gene in Arbor Acre Plus (AAP) are presented in Table 2. Five SNPs were identified in the selected AAP strain comprising of three transition and two transversion mutations respectively in which the allele changes were between purine/purine, pyrimidine/purine and pyrimidine/pyrimidine. Polymorphism 55A>G (singleton), 64A>G (parsimony), 199C>G (singleton) and 328T>C (singleton) were found on AAP loci.

The single nucleotide polymorphisms identified in exon 1 of *Myostatin* gene in Cobb 500 are presented in Table 3. Five SNPs were identified in the selected Cobb 500 strain comprising of three transition and two transversion mutations respectively in which the nucleotide changes were between purine/ purine, pyrimidine/ purine and pyrimidine/ pyrimidine. Polymorphism 55A>G (parsimony), 64A>G (singleton), 199C>G (singleton) and 328T>C (singleton) were found on Cobb 500 loci.

The SNPs identified in exon 1 of *Myostatin* gene of Ross 308 are presented in Table 4. Eleven SNPs were identified in the selected Ross strain comprising of seven transition and four transversion mutations respectively in which the nucleotide changes were between purine/purine, pyrimidine/purine and pyrimidine/pyrimidine. Polymorphism 3A>G (parsimony), 49A>G (parsimony), 50C>A (parsimony) 56A>G (singleton) 66A>G (parsimony) 201C>G (singleton) and 330T>C (singleton) were found in Ross loci.

The effects of single nucleotide polymorphisms exon 1 on growth parameters of Arbor Acre broiler chickens are presented in Table 5. There was a significant SNPs effect on body weight (BDW), chest girth (CG) and thigh length (TL) across ages except at day-old in locus 65A>G. At locus 65A>G, the proportion of both Adenine and Guanine were almost the same for all the growth parameters measured at day-old. At week 6, Adenine had higher effect on BDW while Guanine had higher effect on both CG and TL. Adenine had higher mean values for all the growth parameters at week 12. At location 329C>T, Thymine had higher proportion on all the parameters measured on the chicken at day-old and week 12. However, at week 6, Cytosine was higher in both CG and TL while Thymine was higher only BDW.

The effects of single nucleotide polymorphisms exon 1 on growth parameters of Arbor Acre Plus broiler chickens are presented in table 6. There was a significant SNPs effect on all the growth parameters measured at day-old and 6th week respectively. At position 64A>G, Guanine had higher proportion of BDW and TL at day-old and BDW, CG and TL at 6 weeks. The proportion of both Adenine and Guanine were the same for all the growth parameters measured at week 12.

The effects of single nucleotide polymorphisms exon 1 on growth parameters of Cobb 500 broiler chickens are revealed in Table 7. There was a significant SNPs effect on all the growth parameters measured at both week 6 and 12. At location 55A>G, Adenine and Guanine were the same proportion for all the growth parameters measured at day-old. Guanine was higher in BDW and CG while Adenine was higher in TL at week 6. Guanine was observed with higher level for all the parameters at week 12.

The effects of single nucleotide polymorphisms exon 1 on growth parameters of Ross chickens are shown in Table 8. There was a significant SNPs effect on the growth parameters across ages except CG at day-old, TL at week 12, BDW at week 6 of 3A>G, 49A>G, 50A>C and 66A>G respectively. At locus 3A>G, the A allele conferred higher BDW and TL at day-old. Adenine associated better with BDW and CG while Guanine associated better with TL at week 6. At week 12, higher BDW was recorded with G allele while Adenine conferred higher CG. Generally, position 49A>G, higher body parameters were observed with birds having A allele at all ages with the exception of CG at week 12. Higher body parameters at position 50A>C in all ages generally occurred in birds with C allele except TL at day-old and CG at week 12. Adenine allele birds at SNP 66A>G possessed higher BDW, CG and TL at all ages except TL at day-old and BDW at 12 weeks.

Table 1: Polymorphism identified in exon 1 of *myostatin* gene in Arbor acre broiler chicken

Genotype	Polymorphism (SNPs)	SNPs Type	Mutation Type
AE3F	56A>G	Singleton	Transition
AB2F	65A>G	Parsimony	Transition
AC3M	65A>G	Parsimony	Transition
AE3F	200C>G	Singleton	Transversion
AB2F	329C>T	Parsimony	Transition
AC3M	329C>T	Parsimony	Transition

A = Adenine, **G** = Guanine, **C** = Cytosine and **T** = Thymine.

Table 2: Polymorphism identified in exon 1 of *myostatin* gene in Arbor acre plus broiler chicken

Genotype	Polymorphism (SNPs)	SNPs Type	Mutation Type
APC2M	55A>G	Singleton	Transition
APB8M	64A>G	Parsimony	Transition
APB9F	64A>G	Parsimony	Transition
APC2M	199C>G	Singleton	Transversion
APC2M	328T>C	Singleton	Transversion

A = Adenine, **G** = Guanine, **C** = Cytosine and **T** = Thymine.

Table 3: Polymorphism identified in exon 1 of *myostatin* gene in Cobb 500 broiler chicken

Genotype	Polymorphism (SNPs)	SNPs Type	Mutation Type
CA7M	55A>G	Parsimony	Transition
CB9M	55A>G	Parsimony	Transition
CA7M	64G>A	Singleton	Transition
CA7M	199C>G	Singleton	Transversion
CA7M	328T>C	Singleton	Transversion

A = Adenine, **G** = Guanine, **C** = Cytosine and **T** = Thymine.

Table 4: Polymorphism identified in exon 1 of *myostatin* gene in Ross 308 broiler chicken

Genotype	Polymorphism (SNPs)	SNPs Type	Mutation Type
RC2F	3A>G	Parsimony	Transition
RC9M	3A>G	Parsimony	Transition
RB3M	49G>A	Parsimony	Transition
RC2F	49G>A	Parsimony	Transition
RC9M	50C>A	Parsimony	Transversion
RE9F	50C>A	Parsimony	Transversion
RC2F	56A>G	Singleton	Transition
RC9M	66A>G	Parsimony	Transition
RD3F	66A>G	Parsimony	Transition
RC2F	201C>G	Singleton	Transversion
RC2F	330T>C	Singleton	Transversion

A = Adenine, **G** = Guanine, **C** = Cytosine and **T** = Thymine.

Table 5: Effects of single nucleotide polymorphisms exon 1 of *MSTN* gene on growth parameters of Arbor acre broiler chickens

SNPS	AGE	ALLELE	BDW (g)	CG (cm)	TL (cm)
65A>G	Day-old	A	36.67±1.76	12.00±0.58	4.60±0.33
		G	36.00±1.00	12.01±0.01	4.00±0.01
	Week 6	A	1858.00±42.72^{a}	35.33±2.33^{b}	18.33±2.40^{b}
		G	1751.00±37.00^{b}	36.00±4.00^{a}	19.50±0.50^{a}
	Week 12	A	5223.33±513.63^{a}	56.33±2.33^{a}	26.07±1.76^{a}
		G	4925.50±675.50^{b}	49.50±2.50^{b}	24.50±0.50^{b}
329C>T	Day-old	C	35.00±1.00^{b}	11.00±0.01^{b}	4.00±0.01^{b}
		T	36.67±1.76^{a}	12.00±0.58^{a}	5.67±0.33^{a}
	Week 6	C	1751.00±37.00^{b}	36.00±4.00^{a}	19.50±0.50^{a}
		T	1858.00±142.72^{a}	35.33±2.33^{b}	18.33±2.40^{b}
	Week 12	C	4925.50±677.50^{b}	49.50±2.50^{b}	24.50±0.50^{b}
		T	5223.33±513.63^{a}	56.33±2.33^{a}	26.67±1.76^{a}

^{ab} = Means occupying the same column with each age having different superscripts are significantly different (P< 0.05). **SNPs** = Single Nucleotide Polymorphisms, **BDW** = Body Weight, **CG** = Chest Girth, **TL** = Thigh Length, **A** = Adenine, **G** = Guanine, **C** = Cytosine and **T** = Thymine.

Table 6: Effects of single nucleotide polymorphisms exon 1 of *MSTN* gene on growth parameters of Arbor acre plus broiler chickens

SNPs	AGE	ALLELE	BDW (g)	CG (cm)	TL (cm)
64A>G	Day-old	A	36.67±1.76^{b}	11.00±0.58^{a}	3.67±0.33^{b}
		G	40.00±2.00^{a}	10.50±0.50^{b}	4.00±0.01^{a}
	Week 6	A	1776.67±76.70^{b}	33.33±0.67^{b}	16.33±0.33^{b}
		G	2110.00±30.00^{a}	37.50±0.50^{a}	21.50±1.50^{a}
	Week 12	A	5188.00±35.06	51.67±3.84	24.67±0.88
		G	5150.00±50.00	51.00±0.01	24.50±0.50

^{ab} = Means occupying the same column with each age having different superscripts are significantly different (P< 0.05). **SNPs** = Single Nucleotide Polymorphisms, **BDW** = Body Weight, **CG** = Chest Girth, **TL** = Thigh Length, **A** = Adenine and **G** = Guanine.

Table 7: Effects of single nucleotide polymorphisms exon 1 of *MSTN* gene on growth parameters of Cobb 500 broiler chickens

SNPs	AGE	ALLELE	BDW (g)	CG (cm)	TL (cm)
55A>G	Day-old	A	42.01±3.06	11.33±0.33	4.33±0.33
		G	42.00±2.00	11.00±0.01	4.50±0.50
	Week 6	A	1824.00±83.21^{b}	35.67±1.86^{b}	17.67±1.86^{a}
		G	2340.00±76.00^{a}	36.00±3.00^{a}	16.50±1.50^{b}
	Week 12	A	4827.33±307.82^{b}	53.33±1.86^{b}	23.67±0.33^{b}
		G	5837.00±663.00^{a}	56.00±1.00^{a}	28.50±3.50^{a}

^{ab} = Means occupying the same column with each age having different superscripts are significantly different (P< 0.05). **SNPs** = Single Nucleotide Polymorphisms, **BDW** = Body Weight, **CG** = Chest Girth, **TL** = Thigh Length, **A** = Adenine and **G** = Guanine.

Table 8: Effects of single nucleotide polymorphisms exon 1 of *MSTN* gene on growth parameters of Ross 308 broiler chickens

	AGE	ALLELE	BDW (g)	CG (cm)	TL (cm)
3A>G	Day-old	A	46.67±2.67^{a}	11.33±0.33	4.00±0.58^{a}
		G	42.00±0.01^{b}	11.00±0.01	3.50±0.50^{b}
	Week 6	A	1913.33±51.54^{a}	34.67±1.20^{a}	17.67±1.67^{b}
		G	1879.00±87.00^{b}	33.50±0.50^{b}	20.00±0.01^{a}
	Week 12	A	5292.00±463.53^{b}	53.67±1.67^{a}	24.33±1.45
		G	5516.00±516.00^{a}	46.50±0.50^{b}	24.00±0.01
49A>G	Day-old	A	47.00±5.00^{a}	11.00±0.01	4.33±0.01^{a}
		G	43.33±0.67^{b}	11.33±0.33	3.00±0.33^{b}
	Week 6	A	1950.00±36.00^{a}	35.50±1.50^{a}	20.50±0.50^{a}
		G	1832.67±38.68^{b}	33.33±0.33^{b}	17.33±1.33^{b}
	Week 12	A	5600.00±600.00^{a}	49.00±3.00^{b}	25.50±1.50^{a}
		G	5236.00±408.84^{b}	52.00±2.89^{a}	23.33±0.67^{b}
50A>C	Day-old	A	43.00±1.00^{b}	11.50±0.50	4.00±0.01^{a}
		C	46.00±3.06^{a}	11.00±0.01	3.67±0.67^{b}
	Week 6	A	1951.00±59.00	33.50±0.50^{b}	18.00±2.00^{b}
		C	1932.00±69.35	34.67±1.20^{a}	19.00±1.53^{a}
	Week 12	A	5354.00±678.00^{b}	52.00±5.00^{a}	23.00±1.00^{b}
		C	5400.00±400.00^{a}	50.00±2.00^{b}	25.00±1.00^{a}
66A>G	Day-old	A	46.00±3.06^{a}	11.33±0.33	3.33±0.33^{b}
		G	43.00±1.00^{b}	11.00±0.01	4.50±0.50^{a}
	Week 6	A	1970.00±69.90^{a}	35.00±1.00^{a}	19.00±1.53^{a}
		G	1894.00±2.00^{b}	33.00±0.00^{b}	18.00±2.00^{b}
	Week 12	A	5292.00±463.53^{b}	51.67±3.18^{a}	25.33±1.45^{a}
		G	5516.00±516.00^{a}	49.50±2.50^{b}	24.00±0.01^{b}

^{ab} = Means occupying the same column with each age having different superscripts are significantly different (P< 0.05). **SNPs** = Single Nucleotide Polymorphisms, **BDW** = Body Weight, **CG** = Chest Girth, **TL** = Thigh Length, **A** = Adenine, **G** = Guanine and **C** = Cytosine.

Discussion

The *Myostatin* (*MSTN*) gene, which regulates skeletal muscle growth, is of interest in poultry genetics for its impact on growth traits and productivity. The lower frequency of transversion mutations in exon 1 of the *Myostatin* gene in this study aligns with the work of Lesk (2002) and Mulekanle (2022), who reported that transition mutations were more common than transversions in the animal genome. The higher-than-expected substitution rates of transitions might be due to chance relative to those of transversions. These authors opined that selection disfavoured transversions, as non-synonymous or transversion mutations are less likely to conserve biochemical properties of the original amino acid.

A large number of single-nucleotide polymorphisms that were identified in exon 1 of the *MSTN* gene across Arbor Acres, Arbor Acres Plus, Cobb 500 and Ross 308 while the higher number of SNPs in *MSTN* exon 1 in all four chicken genotypes compared favourably with the polymorphism in the findings of Zhang *et al.* (2019). The presence of SNPs in the gene indicated that this region is highly polymorphic. The nucleotide sequence analysis of the gene revealed that SNPs showed variations among the chickens used. The SNPs substitutions in the study include C>T, A>C and C>G in which C>T and A>C had significant association with growth parameters in the chicken types. Different SNPs have been associated with various traits in poultry, as evidence that SNPs play a vital role in gene expression and consequently the manifestation of either the protein or growth trait, according

to Zhao *et al.* (2021).

In Arbor Acres chickens, six SNPs were identified and significant effects were observed at loci 65A>G and 329C>T. It was observed that the adenine at 65A>G associated with higher BDW, CG and TL at week 12, indicating that the adenine may confer superior muscle development which agreed with the findings of Ling *et al*. (2015), who reported that specific *MSTN* mutations, particularly in exon 1, positively influenced muscle hypertrophy and growth traits in broiler strains. Similarly, Zhang *et al*. (2019) identified mutations in *MSTN* that led to improved carcass yield and skeletal muscle mass, supporting the observations in Arbor Acres broilers.

The five SNPs that were identified in Arbor Acres Plus chickens, with significant had effects observed, particularly at position 64A>G, which supports Wang *et al.* (2022). Broilers carrying the guanine exhibited higher body weight, better chest girth and thigh length at week 6, affirming the role of *MSTN* polymorphisms in enhancing growth performance. This result corroborates the findings of Luo *et al*. (2017), who associated *MSTN* polymorphisms in Arbor Acres Plus chickens with superior breast muscle growth and better skeletal development. This consistency strengthens the relevance of *MSTN* genetic markers in selective breeding for meat production improvement. Five SNPs as observed in Cobb broilers with notable effects at the 55A>G locus agreed with the report of He *et al.* (2022) and broilers with the guanine allele exhibited significantly higher body weight and chest girth at 6^{th} and 12^{th} weeks. These results agreed with the findings of Wang *et al.* (2022), that *MSTN* polymorphisms in Cobb broilers were linked to enhanced body weight gain and better overall carcass traits. These current findings further corroborate with the general concept presented by Obiora *et al.* (2022), that *MSTN* gene inhibition promotes muscle fibre proliferation and mass deposition in broilers.

The highest number of SNPs were observed in Ross strain, identifying 11 polymorphisms which aligned with the report of Zhang *et al.* (2019). Significant effects on growth parameters were noted at loci 3A>G, 49A>G, 50C>A, and 66A>G. Guanine at 3A>G was associated with superior thigh length and body weight at 6^{th} and 12^{th} weeks respectively. Adenine at 49A>G was linked to higher body weight and thigh length across different ages. These observations are in agreement with the report of Ayuti *et al.* (2024) who observed that Ross strain with specific *MSTN* mutations had increased growth rates and higher breast muscle yield compared to other strains. Furthermore, *MSTN* gene mutations significantly enhanced thigh muscle development in fast-growing broilers like Ross.

In addition to identifying SNP effects on growth parameters, broiler performances in 6^{th} and 12^{th} weeks across the studied strains were compared. Generally, it was observed that BDW, CG and TL continued to increase across ages; however, the rate of growth differed among strains. The Ross and Cobb broilers showed substantial increases in body weight and chest girth between 6^{th} and 12^{th} weeks, supporting earlier findings by Luo *et al*. (2017), who reported that these strains possess superior late-growth potential linked to favourable *MSTN* gene variations. In contrast, Arbor Acres and Arbor Acres Plus strains demonstrated moderate gains, suggesting that their optimal economic weight might be achieved earlier than 12 weeks.

Conclusion and Recommendation

The identified SNPs demonstrated variable but significant effects on BDW, CG and TL. Generally, it was found that beneficial alleles (mainly Adenine or Guanine depending on the locus and strain) promoted higher BDW, larger CG and longer TL. Ross 308, having the highest number of SNPs, suggested that genetic variation in the strain contributed to differences in growth performance. This result validates the use of *MSTN* gene polymorphisms as potential genetic markers for broiler selection aimed at improving growth performance. Thus, Ross is considered

the most suitable strain due to its greater frequency of beneficial *Myostatin* alleles, which are linked to improved growth characteristics and a higher growth rate.

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