Research Article

# Screening for SNPs in *MSTN* and *IGF-2* genes and its Relationship with Body Weight and Carcass Traits in Black Bronze Turkey

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**Abstract** | This study was conducted to evaluate the relationship between variant body weight and carcass traits of Black Bronze turkey with the single nucleotide polymorphism in the coding region of the *MSTN* gene and *IGF-2* (3'-UTR), using DNA sequencing. Fifty turkeys were categorized into high and low body weight. Blood samples were collected from each bird for DNA extraction. Four birds were selected randomly/group to be slaughtered for carcass traits. In *MSTN,* only six non-synonymous SNPs were recorded leading to four amino acid substitution in exon 1 and 2 amino acids substitution in exon 2. In low body weight, only one non-synonymous mutation was recorded at exon 2. The 3'UTR-*IGF-2* sequence showed 14 SNPs in high body weight and three SNPs in low body weight. The carcass weight percentage didn't change significantly for high or low body weight. While carcass cuts percentage increased in high body weight birds compared to low weight. We conclude that no association was present between the SNPs recorded neither at *MSTN* coding region nor at *IGF-2*-3'UTR and the body weight or carcass traits. Further studies are needed to associate these SNPs with different growth stages of turkey.

**Keywords** | Black Bronze turkey; *MSTN*; *IGF-2*; SNPs; Body weight

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## INTRODUCTION

The turkey (*Meleagris gallopavo*) is an important meat producing poultry species. It comes in the second rank after chicken as meat producing bird. Demand of turkey meat increases very rapidly. In this context, more attention was concerned to increase commercial turkey farms and get genetic programs aimed mainly to improve bird's body weight at marketing age (20-24 weeks of age). Whereas body weight is directly linked to muscle development, the genetic mechanism controlling the growth of the muscle must be studied. Multiple genetic factors control and regulate muscle growth mechanism (Jawasreh et al., 2019). Myostatin (*MSTN*), a member of the transforming growth factor β (*TGF–β*) family, is an important regulator of muscle mass (as reviewed in Chen et al., 2021). It is a limiting element for muscular growth and helps muscle fibers maintain their protein balanced (Baldwin et al., 2013; Lee, 2004). *MSTN* sequence and function appear to be substantially conserved among vertebrate species, and

its role during chicken myogenesis like that seen in mammals (Grade et al., 2019).

Also, Insulin-like Growth Factor 2 (*IGF-2*) gene regulates body and muscle growth (Duclos et al., 1999; Kadakia and Josefson, 2016). It has a role in the proliferation, differentiation, and metabolism of a variety of myogenic cell lines from various species (Aboalola and Han, 2017; Florini and Ewton, 1996). The turkey *IGF-2* mRNA codes for 187-amino-acid precursor protein that share greater than 95% nucleotide and amino acid identity with chicken *IGF-2* and 60–70% homology with mammalian *IGF-2* sequences (Richards et al., 2005). Numerous studies have found links between *IGF-2* gene polymorphism and economic traits in various species (Abo-Al-Ela et al., 2014; Ali et al., 2021; Ramadan et al., 2020).

The use of genetic markers-based selection, especially for the productive trait, is an attempt to improve the economic traits. Single nucleotide polymorphism (SNPs) marker is an important marker for assisted selection (MAS), it implies variation in a single nucleotide of DNA. If it occurs in the coding region of a gene, it can change the phenotypic of an individual within the same species, and this can provide beneficial character. Also, SNPs were recorded at untranslated regions (UTR) of the gene and were associated with productive traits (as reviewed in Udoh et al., 2021). As the genetic variation is important for strategic breeding programs and genetic improvement, this study aims to screen the presence of a relationship between marketing weight as well as carcass traits and the single nucleotide polymorphisms at the coding region of the *MSTN* and 3`-UTR of *IGF-2* genes in Black Bronze turkey using DNA sequencing.

## MATERIALS AND METHODS

### Ethical Statement

The experiment and all procedures and management conditions used in this study were approved by the local ethics committee of animal use, Faculty of Veterinary Medicine, Alexandria University- Institutional Animal Care and Use Committee (AU-IACUC), Egypt.

### Experimental Samples And Carcass Characteristics

The Black Bronze turkey poults (*Meleagris gallopavo*) were reared at the Research Centre, Mahalet Mousa Station Kafr El Sheikh Province, Egypt. After hatching of eggs, fifty poults were selected randamly. The poults were brooded for eight weeks of age. The brooder house was cleaned, disinfected and a clean litter of sawdust was used. The heat during the brooding period was adjusted at 35 °C for first day and reduced gradually 3°C/week after one or two weeks until reach 21°C. After 8 weeks, poults were moved to wire-separated pens (one birds/$m^2$) individually. During the experimental period, food and water were introduced to bird's ad libtum with equal feeder and drinker spaces. All birds were fed the same food with the same quantity according to rearing stage, which was formulated to meet the National Research Council (NRC) recommended requirements Ingredients and Nutrient Composition of turkey rations at different ages were illustrated in Table 1 (NRC, 1994). The lighting program planned from the first day as 24 hrs of light until 7 days of age then decreased one hr/day until 15 days of age to reach 16 hrs light/day until 2 weeks of age. Body weights of fifty Black Bronze turkeys were estimated and categorized into two groups: high body weight (HBWT) ranged from 6700 to 5950 gm with average 6143.75 gm and low body weight (LBWT) between 4100 and 4500 gm with average 4263.636 gm. Whole blood (4 ml) were drawn from the wing vein of each bird. Blood samples were individually collected in tubes containing anti-coagulant then frozen immediately at -20°C until DNA extraction. Four birds were selected randomly from each group. The selected birds were prevented feed and water for 12 hrs prior to be slaughtered. The birds were left to complete bleeding then manually eviscerated to record the carcass weight. The weights of breast, thigh, drumstick, wings, intestine, giblet (liver, heart, and gizzard) were determined.

### DNA Extraction, PCR Amplification Of *MSTN* (Exon 1, 2 And 3) And *IGF-2* Genes.

DNA was extracted using G-spin™ Total DNA Extraction Kit (iNtRON Biotechnology, South Korea), according to manufacture instructions. The primers which were used for amplification of selected genes were shown in Table (2). Amplification of selected genes fragments were performed in 50 µl reaction volume containing 25 µl master mix, 5 µl genomic DNA, 1 µl of each primer (10 nm) and 18 µl $dH_2O$. The final reaction mixture was placed in a thermal cycler (Techne, TC-3000, USA). The PCR program was carried out by initial denaturation at 95°C for 5 min followed by 35 cycles of 95°C for 1 min for DNA denaturation, annealing at 55°C for 1 min) for 1 min, extension at 72°C for 1 min and final extension at 72°C for 10 min according to manufacture instructions of 2X TOPsimple™ DyeMIX (Enzynomics, South Korea) with few modifications. PCR products of each sample and 100 bp DNA ladder (GeneDirex Taiwan) were loaded in 2% agarose gel stained with ethidium bromide. The gel documentation system (Gel Doc. Alpha-chem. Imager, USA) was used to visualize and photographed electrophoresis gel fragments.

**Table 1:** Ingredients and Nutrient Composition of Rations used in this study

| Ingredient % | Starter | Grower | Finisher |
|---|---|---|---|
| Corn | 66.53 | 58.28 | 53.84 |
| Soybean meal 44% | 37.09 | 31.69 | 23.28 |
| Corn gluten 60 | 5 | 5 | 5 |
| Fat | 1.06 | 2.03 | 2.19 |
| Dicalciumphosphate[A] | 0.23 | 0.072 | 0.048 |
| Limestone | 1.8 | 1.5 | 1.308 |
| Premix | 0.25 | 0.2 | 0.15 |
| Lysin | 0.23 | 0.825 | 1 |
| Salt | 0.5 | 0.4 | 0.5 |
| Total | 100% | 100% | 100% |
| Nutrients | | | |
| ME (kcal/kg) | 2900 | 3000 | 3100 |
| Crude protein | 24 | 22 | 19 |
| Calcium | 1 | 0.85 | 0.75 |
| Available phosphorus | 0.5 | 0.42 | 0.38 |
| Methionine | 0.45 | 0.4 | 0.35 |
| Lysine | 1.5 | 1.3 | 1 |

* Provides per kg of diet: Mn, 80 mg; Zn, 60 mg; Fe, 60 mg; Cu, 5 mg; Co, 0.2 mg; I, 1 mg; Se, 0.15 mg; choline chloride, 200 mg, vitamin A, 12,000 IU; vitamin D3, 2,400 IU; vitamin E, 50 mg; vitamin K3, 4 mg; vitamin B1, 3 mg; vitamin B2, 6 mg; niacin, 25 mg; calcium-D-pantothenate, 10 mg; vitamin B6, 5 mg; vitamin B12, 0.03 mg; D-biotin, 0.05 mg; folic acid, 1 mg.

**Table 2:** List of primers used in this study.

| Primers | Sequence (5`→3`) | Amplicon size (bp) |
|---|---|---|
| *MSTN* (exon 1) | F: ATGCAAAAGCTAGCAGTCTATG<br>R: ACTCCGTAGGCATTGTGATAAT | 373 |
| *MSTN* (exon 2) | F: CTGATTTTCTTGTACAAATGGAG<br>R: CAATCCATCTTCACCCGGTC | 374 |
| *MSTN* (exon 3) | F: AACCCATTTTTAGAGGTCAGAG<br>R: TCATGAGCACCCGCAACGAT | 381 |
| *IGF-2* | F: AGGAATGAACTGTGACCGGC<br>R: AAGAAAGACACGGAATGGACTCT | 452 |

### PCR Product Purification, DNA Sequencing, And Statistical Data Analysis

PCR products were purified by using MEGAquick-spin™ total fragment DNA purification kit (Intron Biotechnology, South Korea) according to manufacture instruction. The DNA sequencing was performed for eight birds (four high and four low body weights) for each primer. The sequencing was done for purified PCR products in one direction (forward primer) by DNA sequencer (LGC Company, Germany). The sequence results were analyzed using Chromas 1.45 (http://www.technelysium.com.au). The sequences comparisons were done using the BLAST program from the national center of biotechnology information (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The alignment of obtained sequences was done by Clustalw program version 1.8, and the amino acid translation was done using the (ExPASy - Translate tool). The body and carcass weight percentages data were analyzed through one-way ANOVA, using the SAS (Statistical Analysis System, version 6, 4th Edition, SAS Institute, Cary, NC, USA.

## RESULTS

### *MSTN* (Exon 1, 2 And 3) Gene Polymorphism

The PCR products of *MSTN* exon 1 (373 bp), exon 2 (374 bp) and exon 3 (452 bp) from the studied birds were illustrated in Figure (1 a, b and c). Sequence blast of turkey *MSTN* exons 1, 2, and 3 with other poultry species revealed a similarity of 97, 98, 100 % with chicken, 94, 100, 100 % with duck, 93.9, 97.9, 100 with geese, and 97, 100, 100 % with a Japanese quail (data is not shown). The obtained sequences of *MSTN* e1, 2 and 3 of *Meleagris gallopavo* in large body weights and small body weights were submitted to GeneBank and received the accession No# MZ666302,

**Table 3:** The SNPs and amino acid substitutions recorded in exon 1, 2, and 3 of *MSTN* gene in high and low body weights turkey.

| *MSTN* exon 1 | | *MSTN* exon 2 | | *MSTN* exon 3 | |
|---|---|---|---|---|---|
| **HBWT** | **LBWT** | **HBWT** | **LBWT** | **HBWT** | **LB-WT** |
| 45 A>G (1H) Amino acid 15 I>F | 140 T>C (6L)- | 233 A>G (2H) | 339 A>C (7L) Amino acid 90 K>T | 830 G>A | - |
| 46 T >A (1H) | - | 396 C>A (2H) | - | 856 G>C | - |
| 50 A>T (1H) Amino acid 16 L>F | - | 431 G>C (2H) | - | 862 A>G | - |
| 51 G>A (1H) | - | 463 T>C (2H) Amino acid 109 K>T | - | 901 A>G | - |
| 52 T>G (1H) Amino acid 17 V>S | - | - | - | 919 C>A | - |
| 54 C>G (2H) | - | - | - | 940 C>T | - |
| 68 T>C (2H) | | | | 967 G>T | - |
| 96 G>A (2H) Amino acid change 32 A>T | - | - | - | 980 A>G | - |
| | - | - | - | 1003 C>T | - |
| 143 T>A(2H) | - | - | - | 1048 C>T | - |
| 240 T>C(2H) | - | - | - | 1099 C>T | - |
| 257G>A(2H) | - | - | - | 1105 T>C | - |
| 281 T>C (2H) | - | - | - | - | - |
| 296 C>T(2H) | - | - | - | - | - |
| 323 C>T (2H) | - | - | - | - | - |

HBWT: high body weight, LBWT: low body weight, A: adenine, G: genuine, C: cytosine, T: thymine, I: isoleucine, F: phenylalanine, L: leucine, V: valine, S: serine, k: lysine,and T: threonine.

**Table 4:** Body weight and carcass components of high and low body weight bronze turkey. The values were represented as Means ± standard error (SE).

| **Phenotype** | **HBWT** | **LBWT** |
|---|---|---|
| | Means ± SE | Means ± SE |
| Carcass | 68.813 ± 1.840 | 64.193 ± 2.19 |
| Edibles | | |
| Liver | 1.867 ± 0.251 | 1.851 ± 0.022 |
| Heart | 0.474 ± 0.038 | 0.436 ± 0.027 |
| Gizzard | 1.769 ± 0.202 | 1.332 ± 0.035 |
| Cuts | | |
| Breast | 28.680 ± 0.139 | 25.764 ± 1.114 |
| Drumstick | 4.798 ± 0.283 | 4.499 ± 0.173 |
| Thigh | 22.786 ± 1.362 | 20.395 ± 0.606 |
| Wing | 9.34 ± 0.439 | 8.25 ± 0.537 |

MZ666303, MZ666304 and MZ666305 for *MSTN e1* large body weights, MZ666306, MZ666307, MZ666308, and MZ666309 for *MSTN e1* small body weights. MZ666310, MZ666311, MZ666312, and MZ666313 for *MSTN e2* large body weights, and MZ666314, MZ666315, MZ666316, and MZ666317 for small body weights. MZ666318, MZ666319, MZ666320, and MZ666321 for *MSTN e3* large body

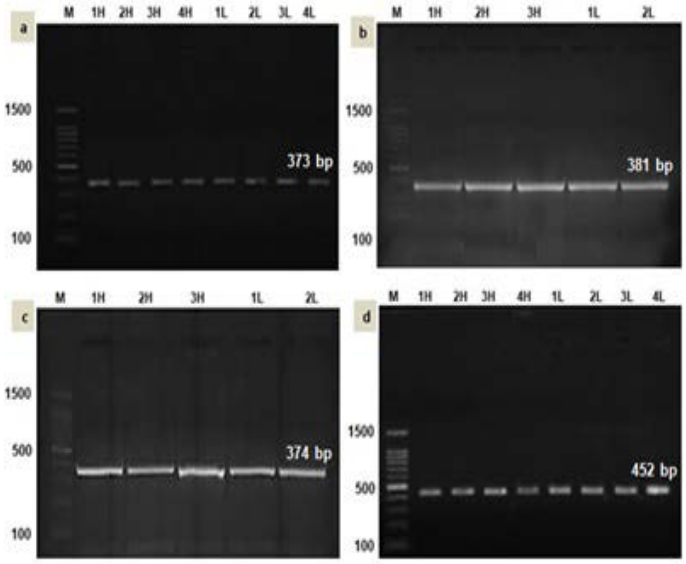


**Figure 1**: The PCR products of *MSTN* gene, (a) exon 1, 373 bp. (b), exon 2, 374 bp. (c) exon 3, 381bp. (d). *IGF-2* PCR products (452 bp).

**MSTN-exon.1**

DFSHPVALDGSSQPTENAEKDGLCNACTWRQNTKSSRIEAIKIQILSKL
RLEQAPNISRDVIKQLLPKAPPLQELIDQYDVQRDDSSDGSLEDDDYHA
TTETIITM

**MSTN-exon.2**

CCFFKFSSKIQYNKVVKAQLWIYLRQVQKPTTVFVQILRLIKPMKDGTR
YTGIRSLKLDMNPGTGIWQSIDVKTVLQNWLKQPESNLGIEIKAFDENG
RDLAVTFPGPGEDGL

**MSTN-exon.3**

KRSRRDFGLDCDEHSTESRCCRYPLTVDFEAFGWDWIIAPKRYKANYCS
GECEFVFLQKYPHTHLVHQANPRGSAGPCCTPTKMSPINMLYFNGKEQI
IYGKIPAMVVDRC

**Figure 2**: Amino acid sequence of *MSTN* gene of exon 1(106 a.a), exon 2 (113 a.a), and exon 3 (111 a.a) for one representative bird.


**Figure 3:** *Meleagris gallopavo, IGF-2* mRNA-3'UTR (452 bp). a) sequence alignment among the high and low body weight birds. b) phylogenetic tree constructed based on the sequence of high and low body weight bird.

weights, and MZ666322, MZ666323, MZ666324, and MZ666325 for small body weights. Sequences alignments of high and low body weight birds revealed the presence of 32 SNPs (15, 5 and 12 in exon 1, 2 and 3 respectively) as shown in Table (3). Whereas 30 SNPs were detected in high body weight birds, most of them are synonymous mutations. Only six non-synonymous SNPs were detected, and they led to four amino acid substitution in exon 1 and two amino acids substitution in exon 2 (Table 3). No amino acid changes were detected at exon 3. In low body weight, only one non-synonymous mutation was recorded (339 A>C: 90 K>T) in exon 2. Most mutations were recorded in bird No.2 and then No.1 which had the higher body weight (6700 and 6450 gm), and at birds No. 6 and 7 for low body weight (4250 and 4350 gm).

The translated amino acids of the studied coding region of turkey *MSTN* gene are consisted of 330 amino acids (106, 113, 111 from exon 1, 2, 3 respectively) as shown in Figure 2. The amino acid alignment of *Meleagris gallopavo MSTN* gene was compar-ed to GeneBank accession number NP_001290090.1. Four non-synonymous mutations were detected at *MSTN* exon 1 (45 A>G, 50 A>G, 52 T>G and 96 G>A), led to change in four amino acids as shown in Table (3). One non-synonymous mutation was detected low body weight (339 A>C: amino acid 90 K>T). While there is no change in amino acid sequence of *MSTN* exon 3 was observed (Table 3).

## *IGF-2* -3'UTR Gene Polymorphism

The PCR of *IGF-2* products (452 bp) from the studied Black Bronze turkey is shown in Figure (1d). Sequence blast of turkey *IGF2* -3'UTR revealed a similarity 99 % with chicken, 100% with duck and quail. Sequence blast with GenBank showing 97% similarity with *Meleagris gallopavo* insulin-like growth factor 2 (*IGF-2*) mRNA-3' UTR, accessions No. JN942585.1. The obtained sequenc-

es of *IGF-2* of *Meleagris gallopavo* in large and small body weights were submitted to GeneBank and received the accession No. MZ666326, MZ666327, MZ666328, and MZ666329 for high body weight. MZ666330, MZ666331, MZ666332 and MZ666333 for low body weight individuals.

The Sequence alignment of *IGF-2* regulatory region (3'UTR) in all studied birds indicates the existence of 17 SNPs in high and low body weight individuals (Figure 3a). Twelve SNPs were recorded in one of high body weight birds (2H) including (901 C>G, 925 A>G, 1009 G>A, 1055 C>T, 1056 T>G,1084 C>T, 1123 A>C, 1142 T>C, 1146 C>T, 1151 G>A, 1197 C>A and 1230 T>A). Additional two SNPs were recorded in another high body weight bird (4H) (908 T>C and 916 C>T). Three SNPs were recorded in one bird with low body weight (5L) (919 G>C, 920 T>G and 921C>T). No SNP was recorded in other high and low body weight birds. The body weight of the high body weight birds with mutation is 6450, 6400gm, and 4250 for the low body weight bird.

Phylogenetic tree based on the sequence data of 3'UTR of *IGF-2* indicates clustering of the large body weight birds No. 1, 2, and 3, also clustering of the small body weight birds No. 6, 7, and 8 (Figure 3b).

### Carcass Traits

The results of statistical analysis of different carcass traits were shown in Table (4). The carcass weight percentage didn't change significantly for high or low body weight. The means of relative weight percentages of edible carcass components (liver, gizzard and heart) had non-significant difference between high and low body weight birds. The carcass cuts (breast, drumstick, thigh, and wing) percentage increased in high body weight in comparison to low weight bird.

## DISCUSSION

The turkey *(Meleagris gallopavo)* is an important farming species and the world's second-largest producer of poultry meat. Turkey genomic resources supply turkey breeders with the tools they need to develop commercial turkey breeds for economically significant features. The molecular characterization of the turkey *MSTN* and *IGF-2* genes have provided valuable hints for understanding how they are expressed and regulated. Myostatin gene has an important role in muscle development regulation (Matsakas and Diel, 2005). Sequence blast of turkey *MSTN* gene revealed high similarity with other poultry species, especially exon 3 which revealed 100 % similarity. Mcpherron and Lee, (1997) demonstrated high sequences similarity in *MSTN* gene and 100% identical in the C-terminal region across species, they also suggested a conserved function of myostatin among species.

We identified 32 SNPs in *MSTN* gene, but only six non-synonymous mutations were recorded in Black Bronze turkey (*Meleagris gallopavo*). A recent study showed that in Nigerian indigenous turkey, *MSTN* gene exon 1 had a moderate to a high degree of polymorphism (Fijabi et al., 2020). Additionally, Tanjung et al. (2019) detected 7 SNPs in myostatin gene exons in GAMA chicken which yield nine haplotypes. Some of them had different Myostatin protein sequences; others have normal protein. They sometimes demonstrated positive correlation, and other times a negative correlation with 49-days-old chicken body weight. Other studies demonstrated that *MSTN* polymorphisms have positive effect on the growth trait in broiler, geese and fish (Aboukila et al., 2021; Smołucha et al., 2019; Zhang et al., 2019). Although the results did not show a significant difference between the high and low markting weights of turkey. This study could suggeste that the role of myostatin in muscle growth and proliferation appeared during early growth stages of turkey and decreased with the marketing age. Ye et al. (2006) recorded that the increase in weight associated with *MSTN* gene mutations is associated with MSTN protein degradation, so that myoblast proliferation occurs continuously. Moreover, Lu et al. (2011) reported that *MSTN* gene thought to be one of the candidate genes, which influences duck growth and carcass quality. Khaerunnisa et al. (2016) demonstrated an association of myostatin gene polymorphism in Indonesian chickens with chicken carcass characteristics. For that the results of this study could use these nonsynonymous SNPs as a candidate marker for marker-assisted selection in turkey breeding either for high or low body weight (non-synonymous mutations in high and low body weights) and carcass traits, however, they need further investigation, especially their correlation with body weight at different growth stages of turkey. Sequence blast of turkey *IGF-2*-3'UTR revealed high similarity (99-100%) with other poultry species. McMurtry et al. (1997) demonstrated strong similarity in turkey *IGF-2* and chicken *IGF-2*, it showed >95% in nucleotides, and 100 % amino acid identity. Sequence alignment of turkey *IGF-2* in high and low body weight birds revealed the presence of 14 SNPs, which categorized the higher body weight birds in one cluster of the dendrogram, also the low body weight birds in another cluster, so we suggested that these SNPs may have importance and effect of turkey body weight. Moreover, Arnold et al. (2012) reported that SNPs discovered in the 3' UTR of genes can interfere with mRNA stability and translation by affecting polyadenylation and microRNA-mRNA interaction, and consequently, change the expression of *IGF-2* in turkey. Other studies reported that the C>G mutation in exon 2 of the *IGF-2* gene is common in chickens and

it was associated with some production performance, they proposed *IGF-2* gene as a marker gene for marker-assisted selection in chicken (Tang et al., 2010; Wang et al., 2005). In addition, Molee et al. (2018) demonstrated a significant association between *IGF-2* gene, body weight, and meat quality in chickens. On the other side, Nurcahya et al. (2020) showed that the different genotypes "CC, TC and TT" in the *IGF-2* gene has no relation to growth traits in SKKedu and KeduSK chickens. In context of present study, we concluded that there was no association between the SNPs found in the coding region of the *MSTN* gene or the 3'UTR of *IGF-2* and the body weight or carcass traits of Black Bronze turkeys. Further research is needed to link these SNPs to different stages of turkey growth.

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## CONFLICT OF INTEREST

There was no conflict of interest in regards to authors reporting their findings.

## FUNDING

This research did not receive any specific fund.

## NOVELTY STATEMENT

Nowadays production sector concerning with improving birds' body marketing weight. As turkey is consider meat producing bird. In this study we are screening two important muscle regulator genes (MSTN and IGF-2) in turkey and investigate thier relations with body weight and carcass traits.

## AUTHORS CONTRIBUTION

Experimental Design was conceived by WSH, HB and SIE. Samples were collected by HB and SIE. Practical work and data analysis were conducted by HB, WSH and AFE. All authors contributed to writing, revision and final proof-reading of the manuscript.

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