Egypt. J. Vet. Sci. **Vol. 55,** No. 5, pp. 1397-1407 (2024)

Egyptian Journal of Veterinary Sciences

https://ejvs.journals.ekb.eg/

Evaluating Candidate Gene Expressions, pH Levels, and Growth Hormone Gene Polymorphisms in Egyptian Chickens : Investigations for Meat Color Regulation and Abdominal Fat Deposition

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THE present study investigated genetic factors influencing meat quality in Fayoumi, Matrouh, Mamourah, and domesticated Leghorn chickens focusing on evaluated gene expression of thromboxane A synthase 1 (TBXAS1), matrix metallopeptidase 27 (MMP27), solute carrier family 2 member 6 (SLC2A6), and glycerophosphodiester phosphodiesterase domain containing 5 (GDPD5) genes that associated with meat color, and explored the growth hormone (GH) gene polymorphisms and their association with abdominal fat deposition (AbFW). Our results demonstrated distinct meat color characteristics among the breeds. Lightness of meat color was evident in the breast of Mamourah and the thigh of Fayoumi that associated with lower expression levels of SLC2A6, MMP27, and TBXAS1. Matrouh exhibited higher levels of redness in both breast and thigh that correlated with a slight elevation in TBXAS1 in breast meat but a down-regulation in thigh meat. The highest levels of yellowness were observed in the breast of Matrouh and Mamourah and the thigh of Fayoumi and Leghorn that linked to up-regulation of GDPD5, followed by TBXAS1. GH gene identified three different alleles (A, B, and C) with five possible genotypes (AA, AB, BB, AC, and CC). The AB genotype was predominant in Matrouh (0.6) with a higher AbFW (0.64) and the AC genotype in Fayoumi (0.8) with a higher AbFW (0.55), whereas Mamourah and Leghorn chickens predominantly had the AA genotype (0.9 and 0.6, respectively), which was associated with lower AbFW (0.2 and 0.18, respectively). The pH level was acidic in all breeds. This research offers valuable insights into bird selection strategies aimed at enhancing meat quality.

Keywords: Body fat, Chicken meat quality, Gene expression, Growth hormone gene, Meat color

Introduction

Chicken meat quality holds significant economic importance in the food industry and is a critical factor influencing consumer preferences. Meat quality is influenced by various factors, including meat color, pH levels, and fat content [1]. Meat color in chicken is a vital visual indicator that consumers used for selecting, evaluating, and accepting meat products for consumption, storage, and retail display. Furthermore, meat color provides valuable insights into the biochemical, physiological, and microbiological conditions within chicken muscle tissues. The regulation of meat color involves complex interactions between biochemical and biological processes during the transition from muscle to meat [2]. Meat color encompasses various shades, including lightness, darkness, paleness, redness, yellowness, and brownness [3]. Dark meat in chickens has been associated with higher levels of total pigment, iron, myoglobin, redness and lower values of lightness and yellowness, occasionally dark

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(Received 18/12/2023, accepted 31/01/2024)
DOI: 10.21608/EJVS.2024.256508.1733

meat is condemned due to cyanosis, which may be linked to conditions such as ascites or emaciation [3, 4]. In contrast, pale meat is characterized by lower levels of total pigments, iron, myoglobin, and redness, along with higher values of lightness, yellowness, and lipid content. Pale broiler meat often displays poor water-holding capacity, and the incidence of pale color may result from the leakage of heme pigments during chilling and storage with ice [3, 4]. Yellowness and lightness of chicken meat are closely associated with the lipid content in muscle tissues, particularly yellowness is linked to lipid oxidation pathways and carotenoid metabolism, with significant differences observed between chickens with white and yellow feathers [1]. Notably, yellowness meat color is also associated with intramuscular fat (IFM) content, with higher IFM levels corresponding to yellowness in capon breast meat [1, 5]. The lightness of chicken meat color is influenced by factors such as protein composition in the surrounding medium (including sarcoplasm and extracellular space), cytoskeleton lysis, and muscle structure. Changes in muscle structure can lead to reduced oxygen content, impacting the lightness color [2, 6]. Matrix metallopeptidase 27 (MMP27) is a protein that degrades the extracellular matrix, a network of substances that support the tissues and regulates fatty acid metabolism. This degradation changes the extracellular space, subsequently, affecting the lightness of meat color by altering the chemical state of myoglobin, the pigment that gives meat its color [1]. Additionally, the solute carrier family 2 member 6 (SLC2A6) proteins, associated with glycolysis and glucose transport, has been reported to influence the lightness of meat color by regulating the glucose metabolism in the muscle tissue [1, 7]. The redness of the meat color is primarily attributed to the presence of myoglobin pigment in chicken muscle tissue. Heme iron, mainly found in myoglobin, contributes to both desirable bright redness and undesirable brown meat color [8, 9]. Redness can also occur due to oxygen diffusion into muscle tissues, with myoglobin concentration, oxygen consumption, and myoglobin oxygenation rates being associated with the development of redness meat color [1, 2].

The relationship between meat color and pH levels, especially concer ning darkness, paleness, and lightness color traits, has been established. Dark and pale meat is characterized by higher pH values, while light meat exhibits lower pH levels [3, 4]. Additionally, the pH levels also influence the redness and yellowness of broiler breast meat, as reported by Fletcher [3].

The trait of chicken meat color was revealed to be determined, controlled and regulated by genetic factors. Several candidate genes were identified to be associated with incidence the various meat colors, especially including TBXAS1 (Thromboxane A synthase1), GDPD5 (Glycerophosphodiester phosph-ordiestrase domain containing 5), MMP27 (Matrix metallopeptidase 27) and SLC2A6 (Solute carrier family 2 member 6) [1]. The meat color trait is governed by genetic factors, and several candidate genes. Notably, TBXAS1, GDPD5, MMP27, and SLC2A6 have been associated with different aspects of meat color regulation [1]. TBXAS1 is linked to redness meat color. This gene encodes thromboxane A2 that plays a role in platelet aggregation, with its expression influenced by iron levels [10]. GDPD5 is associated with yellowness meat color. This gene encodes a glycerophosphodiester phosphodiesterase and is involved in glycerol metabolism and phosphoric diester hydrolase activity [11]. MMP27 and SLC2A6 are connected to lightness meat color, with MMP27 contributing to extracellular matrix breakdown and SLC2A6 being involved in glycolysis and glucose transport [1, 7]. While body fat is essential for imparting taste and flavor to meat, excessive adipose tissue poses challenges in the poultry industry. Elevated fat levels can lead to inefficient feed utilization, impacting acceptability, carcass yield, productivity, and consumer health [12, 13]. Studies have consistently shown that fat deposition increases in tandem with the growth rate of birds, with fast-growing chickens exhibiting faster and earlier fat accumulation compared to their slow-growing counterparts [12-14]. This growth trait is closely associated with genes related to the growth hormone axis, such as the growth hormone receptor and insulin-like growth factors [15]. These genes play a pivotal role in various physiological processes involving growth, body composition and fat deposition is a significant component of body composition [13, 15]. Genetic variants in these growth-related genes have been observed to influence various economically important traits in chickens [15-17]. Genetic variants within the GH gene have been associated with various economically important traits in chickens. Polymorphisms in intron 1 of the GH gene have been linked to egg production, resistance to Marek's disease and avian leukosis in White Leghorn chickens [18], laying performance in Chinese native chickens [19]. GH/*MSP*I sites observed in Bantam, Bantamized White Leghorn, and White Leghorn chickens were found to be related to body weight and egg production [15]. Therefore, our study was designed to assess mRNA expression levels of candidate genes associated with meat color (TBXAS1, MMP27, SLC2A6, and GDPD5) and to investigate polymorphisms within the GH gene and their potential correlations with abdominal fat deposition in four different chicken breeds: Fayoumi, Matrouh, Mamourah, and Leghorn.

Materials and Methods

Chickens

This study followed institutional, national, and international guidelines for the ethical treatment and use of animals. The ethical protocols and animal care procedures complied with the Medical Research Ethical Committee (MREC) of National Research Centre number 094110623. Four distinct chicken populations were used for this study: Fayoumi (an Egyptian native strain), Matrouh (a hybrid strain derived from Dokki-4 and Leghorn strains), Mamourah (a hybrid strain resulting from Alexandria and Dokki-4 strains), and Leghorn (an imported breed domesticated in Egypt). All chickens were weighed on the first day of age and raised under uniform environmental and management conditions. The experimental diets were formulated in accordance with the NRC requirements. The diet consisted of yellow maize, soybean cake, full-fat extruded soybean, guar korma cake, dicalcium phosphate, mixed vitamins and minerals, hydroxymethionine, analog calcium, sodium bicarbonate, calcium carbonate, and table salt. Nutritional analysis of these ingredients revealed a crude protein content of not less than 23%, crude fat content of not less than 5.76%, crude fiber content of not exceeding 4.2%, and an energy content of not less than 3000 Kilocalories/kg diet. Live body weights were measured at the end of the experiment that lasted for 60 days to determine the growth rate (final weight - initial weight / initial weight). Also, blood samples were collected from 80 broilers via standard venipuncture of the brachial veins and transferred to blood collection tubes containing heparin anticoagulant factor. These tubes were stored at -20°C prior to DNA isolation for evaluating the GH gene polymorphisms, then the chickens were humanely slaughtered. Their carcasses were carefully eviscerated and dissected in accordance with ethical practices, and a total of 20 breast samples and 10 thigh samples were collected from each of the chicken breeds to evaluate the gene expression of aforementioned genes. Tissue samples from these organs were promptly excised, snap-frozen in liquid nitrogen, and subsequently stored at -80ºC until use.

Meat color and pH measurements

The meat quality indices for meat color and pH were assessed 30 minutes post-slaughter. Meat color in both breast and thigh muscles was determined using Hunter Instruments with a D65 illuminant and a 10º standard observer for the color space system. The color measurements were expressed using three scales (L*, a*, b*). L* scale indicates lightness vs. darkness, with lower values (0-50) representing dark and higher values (51-100) representing light. a* scale represents red vs. green, where positive values indicate red and negative values indicate green. b* scale reflects yellow vs. blue, with positive values indicating yellow and negative values indicating blue. Additionally, the pH values of both breast and thigh muscles were determined using Hanna Instruments™ Basic Benchtop pH meters (pH ^{211}, Microprocessor pH Meter) [1].

Abdominal fat weight (AbFW)

Abdominal fats were collected from all birds and weighed as part of the meat quality characterization process, following the approach described in previous studies [1, 14].

Gene expression analysis

RNA extraction

Total RNA was extracted from the tissues using TRIzol® reagent kit (Invitrogen, Germany), following the manufacturer's protocol. Briefly, 50 mg of tissue samples were individually homogenized in 750 µL of TRIzol® reagent in an autoclaved mortar and incubated for 5 min. at room temperature to facilitate the dissociation of nucleoprotein complexes. After incubation, 140 µL of chloroform was added to the homogenized samples, and the mixture was vigorously shaken before centrifugation at 12,000 xg for 15 min. at 4°C. Subsequently, RNA in the aqueous phase was carefully separated, precipitated by adding 600 µL of 100% isopropyl alcohol, and then centrifuged at 12,000 xg for 10 min. at 4°C. The RNA pellet was washed with 70% DEPC ethanol and dissolved in RNase-free water at 60°C. RNA concentration and quality were determined using a nanodrop-1000 spectrophotometer (Thermo Scientific, Rockford, IL, USA). The absorbance ratio at 260/280 nm was confirmed to be between 1.8 and 2.1 for all samples. All RNA samples were treated with DNaseI (Invitrogen) to eliminate any contaminating DNA and then stored at -80°C until further use.

Complementary DNA (cDNA) synthesis

To synthesize cDNA, the isolated RNA was reverse-transcribed using the oligo$(dT)_{15}$ primer and the maxime RT PreMix Kit (iNtRON Biotechnology, Korea, Cat. No. 25081/96 tubes). The reaction volume was 20 µL and prepared according to the kit instructions. The reverse transcription (RT) reaction was conducted for 60 minutes at 45°C, followed by a 5-minute RTase inactivation step at 95°C. The PCR products containing the cDNA were stored at -20°C until use for DNA amplification [14, 20, 21]. The primer sequences for SLC2A6, MMP27, TBXAS1, GDPD5, and RPL32 genes are provided in Table (1) and were sourced from Sun *et al*. [1].

TABLE 1. Primer sequences for gene expression analysis and PCR-RFLP genotyping

Gene Name	Primer Sequences
SLC2A6	F: TTCCTTGGGGTTGTGGAGTT
	R: AAGACATTCCCAGCGCAGAT
MMP27	F: CCAACCGTCCCTACATCACC
	R: ACTCGCCACAAGTGTCTTCC
TBXAS1	F: GCTGTGCTGGGAGAAGATGT
	R: CACTGTGCCAGCTTTCAGTG
GDPD5	F: TTTTTATATTCCAGAAGTGGCGCT
	R: TCTTGACATCCCGACTGGAC
RPL32	F: AGTTCATCCGCCACCAGTCTGAT
	R: GCTTCGTCTTCTTGTTGCTCCCATA
GH	F: CTAAAGGACCTGGAAGAAGGG
	R: AACTTGTCGTAGGTGGGTCTG

***F: forward primers R: Reverse primers**

Determination of mRNA expression using Real-Time PCR

Real-Time PCR was conducted employing the Fast SYBR Green Master Mix (Topreal TM PCR 2X pre Mix with low ROX from Enzynomics, Korea) to assess the relative mRNA expression of TBXAS1, MMP27, SLC2A6, and GDPD5 genes. The final primer concentration was set at 10 Pmol. The triplicate amplification reaction volume totaled 20 µL, comprising 1 µL of cDNA, 1 µL of each forward and reverse primer, 10 µL of SYBR green master mix, and 7 µL of water. A Step One Plus instrument (Agilent Stratagene MX3000p) was utilized. The PCR protocol involved an initial activation step at 95°C for 15 min, denaturation at 95°C for 10s, annealing/extension at 60°C for 15s, and elongation at 72°C for 30s, with a total of forty amplification cycles. Melting curve analysis was also performed for each target marker. To normalize the Ct values, the endogenous control RPL32 was employed. The relative quantification of the target to the reference was determined using the ΔCt method.

DNA extraction and PCR amplification

Genomic DNA was extracted from whole blood samples using the salting-out method with some modifications [22]. The purity and relative integrity of genomic DNA were assessed through 1.5% agarose gel electrophoresis. Additionally, the concentration of DNA was quantified using a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, Wal/ham, MA, USA), resulting in final DNA concentrations ranging from 2-10 ng/µL. The forward and reverse primer sequences used for GH gene amplification were designed by Kulibaba *et al*. [23] (Table 1). Amplification was carried out using DreamTaq PCR Master Mix (Thermo Scientific) with a programmable Tertsik thermal cycler (DNA Technology, Russia). The amplification protocol included one cycle of denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 56°C for 1 min, and elongation at 72°C for 1 min, and a final elongation cycle at 72°C for 10 min. The final reaction volume was 20 µL, with primer concentrations of 0.2 µM.

PCR-RFLP and genotypes of GH gene

The amplified fragments were subjected to restriction endonucleases *MSP*I following the manufacturer's standard procedures (Thermo Scientific). The restriction products were separated on a 1.5% agarose gel at 150 V for 40 min. The fragments were visualized using ethidium bromide under ultraviolet light. The size of restriction fragments was determined using molecular weight markers M100 and M200 (IsoGene, Russia) through the PCR-RFLP procedure. The polymorphism of the chicken GH gene (GH/*MSP*I) was genotyped.

Statistical analysis

All data, including the weights of birds at the first day of age and at 60 days of age, gene expression assay results, and genetic polymorphism variations, were analyzed using the General Linear Models (GLM) procedure of the Statistical Analysis System (SAS), as outlined by Saxe and Weitz [24]. Post hoc Scheffe tests were conducted to assess significant differences between groups. The values are presented as mean ± SEM, and statements of significance were based on a probability level of $P<0.05$.

Results

Differences in meat color between breast and thigh muscles of four chicken breeds (Fayoumi, Matrouh, Mamourah, and Leghorn) are summarized in Table 2. Lightness color was significantly higher in Mamourah chicken breast muscles and Fayoumi thigh muscles compared to other breeds. Redness color was notably higher in Matrouh chicken breast muscles, followed by Mamourah, compared to the other two breeds. Matrouh also showed the highest redness in thigh muscles. Yellowness color was significantly greater in Matrouh chicken breast muscles compared to other breeds, while Fayoumi and Leghorn had slightly increased yellowness in thigh muscles compared to the other two breeds. Fayoumi had the highest value. Additionally, pH values (Table 2) showed a slight increase in Leghorn and Fayoumi breast muscles compared to the other breeds, while Mamourah and Matrouh had slightly higher pH values in thigh muscles compared to the other two breeds.

TABLE 2. Color lightness (L*), redness (a*) and yellowness (b*), as well as pH values in chicken meat of different four breeds

Breed	Meat color						pH	
	L*		a*		b*			
	Breast	Thigh	Breast	Thigh	Breast	Thigh	Breast	Thigh
Fayoumi	48.1 ± 0.9^{b}	54.8 ± 0.8^{c}	4.5 ± 0.2^{a}	7.9 ± 0.4^{a}	14.3 ± 0.8^{ab}	15.9 ± 0.6^{a}	5.5 ± 0.2^{a}	5.1 ± 0.4^{a}
Matrouh	43.9 ± 0.5^{a}	46.9 ± 0.7^{a}	10.7 ± 0.4^{c}	15.8 ± 0.5^{c}	16.4 ± 0.8^{c}	14.4 ± 0.7^{a}	5.3 ± 0.4^{a}	5.4 ± 0.5^{a}
Mamourah	51.1 ± 0.8^{c}	49.5 ± 0.6^{b}	10.5 ± 0.6^{c}	8.9 ± 0.4^{a}	15.9 ± 0.4^{bc}	14.1 ± 0.4^{a}	4.9 ± 0.9^{a}	5.6 ± 0.4^{a}
Leghorn	46.3 ± 0.9^{ab}	45.6 ± 0.6^{a}	6.3 ± 0.3^{b}	11.6 ± 1.0^{b}	12.9 ± 0.5^{a}	15.5 ± 0.4^{a}	5.5 ± 0.4^{a}	5.0 ± 0.5^{a}

*Data are expressed as the mean ± SE. Means with different superscript letters indicate significant difference.

Growth rate and performance

The results in Table 3 show a significantly higher growth rate in Matrouh and Fayoumi breeds compared to Mamourah and Leghorn breeds. Matrouh breed exhibited the highest growth rate, while Mamourah breed had the lowest growth rate. Regarding the assessment of growth, particularly in terms of abdominal fat (AbFW), the results indicate that Matrouh and Fayoumi breeds had significantly higher AbFW compared to Mamourah and Leghorn breeds. Matrouh chickens had the highest AbFW, while Leghorn chickens had the lowest.

TABLE 3. Values of body weight, growth rate and abdominal fat weight in different four chicken breeds

Breed	BW at first day	BW after 2 months	Growth rate	AbFW
Fayoumi	31.2 ± 0.9^{a}	724.2 ± 22.9^{b}	0.22 ± 0.02^{b}	0.55 ± 0.50^{b}
Matrouh	29.7 ± 0.3^{a}	740.5 ± 16.7^{b}	0.24 ± 0.01^{b}	0.64 ± 0.64^{b}
Mamourah	38.3 ± 0.4^{a}	534.3 ± 18.5^{a}	0.13 ± 0.01^{a}	0.20 ± 0.21^{a}
Leghorn	32.0 ± 0.6^{a}	499.7 ± 30.3^{a}	0.15 ± 0.02^{a}	0.18 ± 0.18^{a}

*Data are expressed as the mean ± SE. Means with different superscript letters indicate significant difference. BW: Body weight, AbFW: Abdominal fat weight.

Gene expression in breast tissue

In the breast tissue, gene expression analysis revealed significant differences among the breeds. TBXAS1 mRNA expression was notably up-regulated in Fayoumi and Leghorn breeds compared to Matrouh and Mamourah, with the latter displaying the lowest expression levels. MMP27, on the other hand, was significantly elevated in Mamourah and reduced in the other three breeds, with Matrouh chickens exhibiting the lowest expression. SLC2A6 showed a significant increase ($P<0.05$ or $P<0.01$) inMamourah compared to the other three breeds, while Matrouh birds had the lowest SLC2A6 expression. Additionally, GDPD5 gene expression was significantly higher in Mamourah, followed by Fayoumi, in comparison to the other two breeds (Fig. 1).

Gene expression in thigh tissues

Gene expression in thigh tissues revealed distinct patterns among the chicken breeds. TBXAS1 showed significant up-regulation in Leghorn, followed by Fayoumi, while it was down-regulated in Matrouh and Mamourah, with Matrouh birds exhibiting the lowest expression levels.

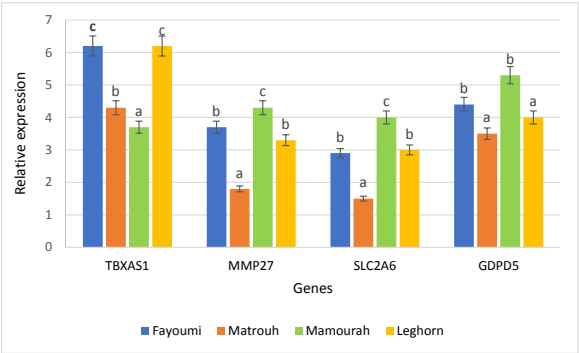

Fig. 1. Relative mRNA expression levels of four candidate genes (TBXAS1, MMP27, SLC2A6, and GDPD5) in breast fillet muscles of four chicken breeds (Fayoumi, Matrouh, Mamourah, and Leghorn) at 60 days old.

MMP27 and SLC2A6expressions displayed similar trends, increasing in Mamourah and Leghorn, and decreasing in Fayoumi and Matrouh, with the latter breeddemonstrating the lowest expression levels. Conversely, the relative levels of GDPD5 mRNA expression were significantly higher in Fayoumi, followed by Leghorn, when compared to Matrouh and Mamourah, with Mamourah chickens displaying the lowest expression levels. MMP27 gene expression was generally downregulated in most breeds, except for a slight increase in Mamourah, with the lowest levels observed in Matrouh (Fig. 2).

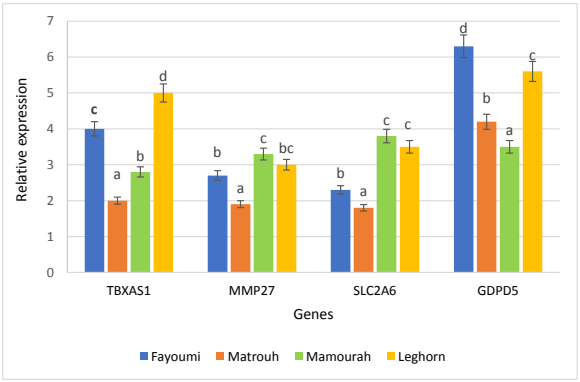

Fig. 2. Relative mRNA expression levels of four candidate genes (TBXAS1, MMP27, SLC2A6, and GDPD5) in thigh muscles of four chicken breeds (Fayoumi, Matrouh, Mamourah, and Leghorn) at 60 days old.

*MSP*I polymorphism of GH gene and nucleotide sequence analysis

The expected PCR product sizes were achieved at 1172 bp (Fig. 3). Polymorphism of the GH gene was assessed by digesting the PCR products with *MSP*I, which cuts at 5'-CCGG-3' sequence. This yielded five distinct fragment patterns (Fig. 4). Subsequent sequencing and alignment of the fragments with the GH gene sequence from GenBank (accession number: D10484.1) identified three alleles (A, B, and C) and five genotypes (AA, AB, BB, AC, and CC) (Fig. 5).

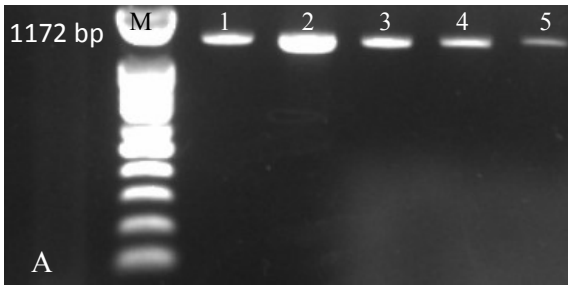

Fig. 3. Lanes: 1-9: PCR products of GH gene at size 1172 bp. Lane M: 100 bp ladder.

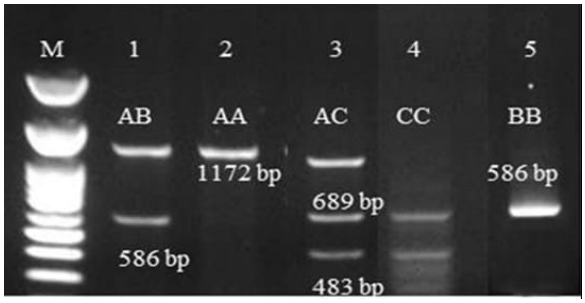

Fig. 4. PCR-RFLP patterns for GH gene with *Msp*Idigestion, where Lane 1 indicates the genotype AB, Lane 2 indicates genotype AA, Lane 3 indicates genotype AC, Lane 4 indicates genotype CC, and Lane 5 indicates genotype BB, Lane M: 100 bp ladder.

Genotype frequencies of GH gene and their association with AbFW

Genotype frequency analysis revealed distinct patterns among the breeds. Fayoumi chickens showed a high frequency of the AC genotype (0.8) and a lower frequency of the AA genotype (0.2). Genotypes AA, BB, and CC were absent in Fayoumi. Matrouh chickens had a prevalent AB genotype (0.6) and a less frequent AA genotype (0.4), with genotypes AA, BB, and CC not observed. Mamourah breed predominantly had the AA genotype (0.9) and a low frequency of the BB genotype (0.1). Genotypes AB, AC, and CC were not present in Mamourah. The Leghorn breed exhibited a high frequency of genotype AA (0.6) and a low frequency of genotype AB (0.3) and genotype CC (0.1), with genotypes AC and BB being absent (Table 4). The results showed that high levels of AbFW were significantly associated with the AB genotype in Matrouh chickens (0.64) and the AC genotype in Fayoumi chickens (0.55). Conversely, low levels of AbFW were linked to the presence of the AA genotype in Mamourah (0.2) and Leghorn (0.18) chickens.

Fig. 5. Nucleotide sequence analysis and alignment of five different genotypes of GH gene obtained from PCR-RFLP with *Msp*I enzyme, compared to GH gene sequences from GenBank (accession number: D10484.1). (*) indicates AC and CC genotypes, where allele A is not cut (1172 bp) and allele C is cut at position 483 bp, yielding two fragments of 483 and 689 bp. (**) indicates AA, AB, and BB genotypes, where allele A is 1172 bp and allele B is cut at position 586 bp, yielding two fragments with the same size.

Discussion

Differences in chicken meat color can result from various factors, including breed type, age, and environmental conditions. In our experiment, all chicken breeds were raised under identical environmental and management conditions from day one until they reached 60 days of age. Therefore, age and environmental factors, such as the nutritional system, were not considered significant contributors to meat color variations in this study. Instead, our focus was on genetic variations, particularly gene expression, as crucial determinants of meat color in chickens. Sun et al. [1] have reported that differential gene expression, especially for genes like TBXAS1,

MMP27, SLC2A6, and GDPD5, is closely linked to metabolic processes involving proteins, fatty acids, heme content, and glycolysis. These molecular markers likely play a pivotal role in regulating chicken meat color. In our study, we observed distinct meat color characteristics such as lightness, redness, and yellowness among different chicken breeds. Further investigation into the genetic factors underlying these color differences revealed interesting patterns. Lightness meat color was notably evident in the breast of Mamourah chickens and in the thigh of Fayoumi chickens. In the context of lightness breast meat color, the expression of GDPD5 was slightly elevated in Mamourah chickens. While, in lightness thigh color, GDPD5 followed by TBXAS1 were up-regulated in Fayoumi chickens. However, both breast and thigh meat showed lower expressions of SLC2A6 and MMP27.

TABLE 4. GH gene polymorphisms and their correlation with abdominal fat deposition in four distinct chicken breeds

	Genotype Frequency					Allele Frequency			
Breed	A/A	A/B	A/C	B/B	C/C	A	B	C	Fat deposition (AbFW)
Fayoumi	0.2	0	0.8	0	0	0.6	0	0.4	0.55 ± 0.50^{b}
Matrouh	0.4	0.6	0	0	0	0.7	0.3	0	0.64 ± 0.64^{b}
Mamourah	0.9	0	0	0.1	0	0.9	0.1	0	0.20 ± 0.21^{a}
Leghorn	0.6	0.3	0	0	0.1	0.75	0.15	0.1	0.18 ± 0.18^{a}

*AbFW: Abdominal fat weight

These findings shed light on the intricate genetic and metabolic processes contributing to meat color variation in different chicken breeds. The occurrence of lightness in meat color may be intricately related to the dissolution and turnover rate of proteins, alongside changes in amino acid metabolism processes, additionally, the gene MMP27 has been identified as encoding matrix metallopeptidase 27, a protein involved in the breakdown of the extracellular matrix (ECM). This breakdown leads to alterations in the extracellular space, ultimately influencing lightness in meat color in chickens [1]. Furthermore, TBXAS1 gene expression was significantly increased in chickens of the Arbor Acres breed with lighter meat color, so the authors suggested that TBXAS1, encoding for thromboxane A synthase 1, might play a role in regulating blood flow and oxygen delivery to the muscle tissue, affecting the meat quality [1].

Matrouh and Mamourah chickens exhibited higher levels of redness meat color in breast, whereas Matrouh followed by Leghorn possessed higher level of redness in thigh. Redness meat color in the breast was associated with a higher expression of TBXAS1 in Matrouh chickens, while Mamourah chickens exhibited a down-regulation in this gene's expression. Notably, Mamourah chickens had the highest expression of GDPD5 in this context. In the case of redness thigh meat color, Matrouh chickens displayed down-regulation of TBXAS1, MMP27, and SLC2A6, while Leghorn chickens exhibited up-regulation of GDPD5 and TBXAS1, with lower expressions of MMP27 and SLC2A6.Turning to the issue of redness in meat color in our study, it was more pronounced in both the breast and thigh of Matrouh chickens. This coloration was correlated with a slight elevation in TBXAS1 expression in the breast but down-regulation in the thigh. Interestingly, other studies have found that the down-regulation of TBXAS1 gene expression is associated with redness in meat color, both in rats [25] and chickens [1]. This reduction in mRNA expression is often linked to iron overload, a factor influencing redness color [2, 9]. Myoglobin, a pigment mainly composed of heme iron, has been identified as responsible for the appearance of desirable bright red meat color in chickens [1, 2]. Furthermore, TBXAS1 genes have been shown to encode thromboxane synthase A2, which plays a crucial role in platelet aggregation [10].

Regarding the yellowness meat color, this type of meat was detected in Matrouh and Mamourah breast and in Fayoumi and leghorn thigh. Yellowness breast and thigh meat color was discriminated with up-regulation of GDPD5 followed by TBXAS1, where yellowness breast meat color was linked to high expression of GDPD5 in Mamourah chickens, along with a slight elevation of TBXAS1 mRNA in Matrouh chickens. Our findings are in agreement with that reported by Sun *et al.* [1], who identified high significant increases of GDPD5 and TBXASI gene in different chicken breeds with yellowness of breast muscle. Lang *et al.* [11] and Sun *et al.* [1] demonstrated that the expression of GDPD5 gene was found to be correlated with glycerol metabolism activity that is considered as a major factor in determining the yellowness meat color. In addition, yellowness thigh meat color was linked to high expression of GDPD5 in Fayoumi and Leghorn chickens, along with a slight elevation of TBXAS1 mRNA. Nevertheless, both breast and thigh meat exhibited lower expressions of MMP27 and SLC2A6. Yellowness meat color in chickens

appeared to be influenced by glycolysis and cytoskeleton lysis, as well as pH changes in the protein composition within the surrounding medium, potentially the sarcoplasm extracellular space [2].

We examined the influence of pH on chicken meat color. The typical pH range for chicken meat falls between 5.6 to 6.0[1]. Previous studies in commercial processing plants linked lower pH values to lighter meat color [3, 4, 26] and higher pH values to darker meat color [3, 4, 27, 28]. Deviations in pH, whether too low or high, result in meat quality issues. Extremely low pH leads to a pale, soft, and exudative (PSE) condition, while high pH leads to a dark, firm, and dry (DFD) state, both impacting meat quality [29]. Lower pH reduces water-holding capacity and influences myoglobin, the primary meat pigment, contributing to light color [26], as reflected in our color measurements across all breeds.

Notably, Fayoumi chickens showed higher thigh meat lightness, correlating with lower pH values in thigh muscles. Mamourah chickens exhibited slightly higher breast meat lightness, along with lower pH values in breast muscles. Matrouh chickens demonstrated consistent pH values between breast and thigh meat, suggesting similar meat quality in both. However, Leghorn chickens showed similar lightness in both breast and thigh but higher thigh meat yellowness and higher pH values in breast muscles, suggesting a complex relationship between pH and meat color with likely involvement of other factors. Our pH results align with gene expression findings, reflecting underlying metabolic processes, including glycolysis, heme content, and lipid metabolism in muscle tissue [1, 30]. Overall, our findings underscore the pivotal role of pH in determining chicken meat color.

This study revealed a significant increase in growth rate and body weight, accompanied by a substantial elevation of AbFW in Matrouh chickens, followed by Fayoumi, compared to the other two breeds. These findings align with previous research conducted on various chicken breeds. Studies by Zhou *et al.* [17], Havenstein *et al.* [31], Cariborg *et al.* [32], and Li *et al.* [33] have consistently shown that fast-growing chickens tend to accumulate more fat compared to their slower-growing counterparts. Furthermore, D'Andre *et al.* [13] reported that an increase in growth rate in fast-growing chickens, such as White Rock Ross (WRR), was associated with a higher body fat percentage, including a concurrent increase in abdominal fat mass when compared to slow-growing Xinghua (XH) chickens. These findings collectively highlight the relationship between growth rate, body weight, and the accumulation of abdominal fat in chickens, emphasizing the impact of genetic factors and growth characteristics on fat deposition.

Regarding the polymorphisms in the GH gene, our study utilized PCR-RFLP to identify *MSP*I-polymorphisms in the fourth intron. This analysis revealed the presence of five different patterns, with subsequent sequence analysis uncovering three alleles labeled A, B, and C. These alleles results in five distinct genotypes: AA, BB, CC, AB, and AC. Our findings are consistent with prior research conducted by Shahnaz et al.[15] and Kulibaba *et al.* [23], which also identified *MSP*I polymorphisms in the fourth intron of the growth hormone gene across various chicken breeds. Their studies similarly observed five patterns and confirmed the existence of three alleles, A, B, and C, leading to five genotypes: AA, BB, CC, AB, and AC. In terms of the association between GH gene polymorphisms and AbFW, our results revealed a significant relationship. Specifically, we found that the presence of genotype AB and genotype AC was significantly associated with higher AbFW in Matrouh (0.64) and Fayoumi (0.55), respectively. Conversely, the low AbFW was correlated with the presence of genotype AA in Mamourah (0.2) and Leghorn (0.18). However, prior investigations into chickens haveidentified links between GH gene polymorphisms and increased body weight. Feng *et al.* [34] and Shahnaz *et al.* [15] reported significant associations between GH and GHR polymorphisms and increased body weight in certain strains of white Leghorn chickens. Additionally, Zhou *et al.* [17] used PCR-RFLP to identify polymorphisms in IGF-1 (IGFI-SNP1) in hybrid chicken breeds, demonstrating that broilers with homozygous genotypes exhibited higher growth rates, body weights, and AbFW compared to those with heterozygous genotypes. Conversely, Chen *et al.* [14] highlighted the significant expression of IGF2BP1 in XH chickens, which was accompanied by an increase in AbFW in birds fed a high-fat diet compared to those on a normal diet. These findings collectively underscore the complex relationship between genetic polymorphisms, growth traits, and abdominal fat deposition in chickens, shedding light on the potential role of the GH gene in regulating these physiological processes.

Conclusion

In conclusion, our study has firmly established a significant correlation between the expressions of candidate genes (TBXASI, MMP27, SLC2A6, and GDPD5) and the regulation of chicken meat color. Furthermore, we have identified GH gene polymorphisms and their associations with AbFW. pH level was found to be related to lightness meat color. This research offers promising prospects for elevating poultry meat quality in the industry and highlights a potential avenue for targeted breeding programs aimed at optimizing meat traits.

Competing Interests

The authors declare that they have no financial interests.

Funding statements

There's no financial/personal interest or belief that could affect the manuscript objectivity.

Acknowledgement

We acknowledge the National Research Centre-Giza, Egypt, for providing the equipment and laboratories that we used to perform this work.

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تقييم التعبيرات الجينية المرشحة، ومستويات الأس الهيدروجيني (pH)، وتعدد أشكال جين هرمون النمو في الدجاج المصري على تنظيم لون اللحوم وترسيب الدهون في البطن (AbFW)

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بحثت الدراسة الحالية في العوامل الوراثية التي تؤثر على جودة اللحوم في دجاج الفيومي ومطروح والمعمورة ودجاج الليجهورن المستأنس مع التركيز على درجة الحموضة المقاسة، وتقييم التعبير الجينى للجينات TBXAS، MMP27، SLC2A6، وGDPD5، وإستكشاف تعدد أشكال جين هرمون النمو (GH) وإرتباطه بترسب الدهون في البطن (AbFW). أظهرت النتائج خصائص لون اللحوم المميزة بين السلالات. وكانت درجة لون اللحم الفاتح واضحة في لحم الصدر فى سلالة المعمورة ولحم الورك فى سلالة الفيومي الذي إرتبط بإنخفاض مستويات التعبير الجينى في جيناتSLC2A6 وMMP27 وTBXAS1. أظهرت سلالة مطروح مستويات أعلى من درجة إحمرار اللحم في كل من الصدر والورك والتي إرتبطت بإرتفاع طفيف في التعبير الجينى لجينTBXAS1 في لحم الصدر وإنخفاضه في لحم الورك. ولوحظت أعلى مستويات من درجة لون اللحم الأصفر في لحم الصدر فى سلالة مطروح والمعمورة ولحم الورك فى سلالة الفيومي وليجهورن والتي إرتبطت بزيادة التعبير الجينى لجينGPDD5، يليه جينTBXAS1. حدد جين GH ثلاثة أليلات مختلفة (A، B، وC) مع خمسة أنماط وراثية محتملة (AA، AB، BB، AC، وCC). كان النمط الوراثي AB هو السائد في سلالة مطروح مع إرتفاع AbFW والنمط الجيني AC في سلالة الفيومي مع إرتفاع AbFW، في حين كان النمط الجيني AA هو الغالب لسلالات المعمورة والليجهورن. والذي كان مرتبطًا بإنخفاض AbFW. وكان مستوى الرقم الهيدروجيني حمضيًا في جميع السلالات. يقدم هذا البحث رؤى قيمة حول إستراتيجيات إختيار الطيور التي تهدف إلى تحسين جودة اللحوم.

الكلمات الدالة: دهون الجسم، جودة لحم الدجاج، التعبير الجيني، جين هرمون النمو، لون اللحم.