

Iraqi Journal of Veterinary Sciences



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Age effects on muscle-related gene expression and glycogen levels in broiler chickens (Gallus gallus domesticus)

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Article information

Article history:

Received 06 March 2025 Accepted 26 May 2025 Published 20 June 2025

Keywords:

Histology Factors Development Growth Fibers

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Abstract

Muscle regulatory factors MYF5, MYH7, and MYOG are essential for muscle growth and development; thus, they are considered applicant genes for meat production traits in chickens. This study investigated the levels of glycogen and the expression of MYF5, MYH7, and MYOG genes in the pectoralis major muscle in broiler chickens. Fresh specimens were collected from the pectoralis major muscle for this study. Special stains were employed for identifying the histological structure and glycogen content in the pectoralis major muscle. The highest mean of the muscle fiber area, thickness of the connective tissue layers, and muscle bundle area was recorded at 38 days. The muscle fibers of the pectoralis major showed a progressive increase in PAS staining intensity from 7 to 21 days old, followed by a decline at 38 days old. qPCR was employed to estimate the mRNA concentrations of MYF5, MYH7, and MYOG in the muscle fibers in the pectoralis major muscle. In fact, the expression at 7 days exhibited that the highest average expression was detected in MYF5, whereas at 21 days, it revealed that there were no significant changes in gene expression. On the other hand, the expression at 38 days exposed that the highest average expression was detected in MYOG. In conclusion, this study demonstrates the essential function of muscle regulatory factors in the growth and development of the pectoralis major muscle in broiler chickens. The analysis showed a strong correlation between gene expression at various growth stages and the development of muscle fibers.

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Introduction

Broiler chickens are an essential part of the global diet, providing a rich source of protein for many people worldwide, with high nutritional value and at acceptable prices when compared to the prices of meat and other animal derivatives (1-3). The broiler chickens are distinguished by their rapid growth and efficient feed-to-meat conversion, contributing significantly to the evolution of the poultry industry as a reliable source of affordable animal protein globally (4,5). Consumer preference for broiler meat stems from its lower fat content, higher protein, and digestibility (3). Skeletal muscles in broilers play a vital role in meat production, such as the pectoral muscles and thigh muscles, as these muscles represent the largest portion of meat mass

in poultry raised for consumption purposes (5). Growth is one of the important traits that researchers consider when improving poultry production, as the growth rate and size of poultry greatly affect productivity in the poultry industry. Therefore, studying the genetic factors that influence these traits is essential in poultry farming (6). Additionally, gene expression helps in understanding how different genes influence the process of muscle fiber formation, thus affecting meat quality and the nature of muscle growth in chickens (7). Muscle regulatory factors (MRFs), which include *MYF5*, *Myf6* (*MRF4*), *MyoD*, and *MYOG* (MYOGenin), are essential for muscle growth and development; thus, they are considered candidate genes for meat production traits in chickens (8,9).

This study focused on exploring age-dependent changes in skeletal muscle growth of broiler chickens using a combined histochemical and expression approach of the MYH7, MYF5, and MYOG genes. Investigating these genes yields valuable insights into the molecular mechanisms underlying muscle fiber formation and growth.

Materials and methods

Ethical approval

The investigation was ethically certified by the College of Veterinary Medicine ethics committee at the University of Al-Qadisiya, Iraq, resulting in the issuance of an ethical approval number, #5051, 24/11/2024.

Collection of specimens

The specimens were collected from a broiler farm in Al-Diwaniyah Governorate from October 15 to November 21, 2024. A total of 15 broiler chickens (Gallus gallus domesticus) were divided into three age groups: the first group at 7 days old, the second group at 21 days old, and the third group at 38 days (marketing age). Broiler chickens were fed a gradual ration of 22% CP starter feed and 18% CP finisher feed. Temperature was maintained at 31°C and progressively lowered to 24°C by week five. Humidity was maintained at 50-60%, and vaccinations against ND, IB, and Gumboro with booster doses to ensure disease resistance. Specimens were taken from the center of the pectoralis major muscle to investigate the histological structure and evaluate the glycogen and level assessment of the gene expression of MYF5, MYOG, and MYH7, which are key genes involved in muscle development, aligning with the study objectives. Specimens are directly preserved in 10% neutral buffered formalin (NBF) for histological study and in TRIZOL (SRCr Green-Zol reagent) for real-time polymerase chain reactions (RT-PCR) (4).

Histochemical process of tissue

Fixed specimens in 10% NBF for 48 hours, then washed with tap water for 2 hours. The specimens were processed

through dehydration by an ascending series of ethyl alcohols that started with 60, 70, 80, 90, and 100% 2 hours each time, clearing by xylene one time for 5 min, infiltration by paraffin wax twice for 2 h, embedding with paraffin, sectioning to 5 µm, mounting on a glass slide, and finally staining with Mayer's hematoxylin and eosin and Masson's trichrome to demonstrate the general histological structure of muscle and Periodic Acid-Schiff (PAS) to demonstrate the glycogen in muscular tissue. The tissue sections were then mounted and left to adhere for 24 hours (10). Histometric measurements were taken of the muscle fiber area, muscle bundle area, and connective tissue thickness were obtained post-imaging and evaluated utilizing the ImageJ software.

Gene expression study

Fresh specimens for gene expression were collected from three age groups. Specimens were taken from the same areas from which samples were taken in the histochemical study, about 100 mg weight each. It is placed directly in an Eppendorf container containing an adequate amount of TRIzol® total RNA extraction solution. It keeps it in the freezer until all samples are collected and testing is performed. The quantification of *MYF5*, *MYOG*, and *MYH7* gene normalized by housekeeping gene (GAPDH) in muscle tissue broiler chicken specimens was done by using the RT-qPCR technique, and this technique was carried out using primers described in table 1 (4).

The total RNA was extracted from pectoralis muscle tissue specimens using the TRIzol® reagent kit (Bioneer, Korea) following the manufacturer's instructions. The extracted RNA was quantified using a Nanodrop spectrophotometer and stored at -20°C. To assess RNA quality and measure its concentration (ng/ μ L) and purity by determining the absorbance ratio at 260 nm to 280 nm (4). To remove trace amounts of genomic DNA contamination from the extracted RNA, the total RNA was treated with DNase I enzyme (Promega, USA), and the cDNA synthesis was done using the M-MLV Reverse Transcriptase kit according to the manufacturer's protocol (4).

Table 1: Explain primers used in the current study with their amplicon number

Primer		Sequence (5'-3')	Product size	Amplicon	
MVE5 gana	F	CAAAGCCTGCAAGAGGAAATCC	103bp	NIM 001020262 2	
MYF5 gene	R	CAAGGTCTCGAATGCTTGGTTC	1030p	NM_001030363.2	
MVOC gana	F	AAAACTGAGCTGGCGCAAAG	144bp	NIM 204194.2	
MYOG gene	R	GGAAAGGATTTGGGCGGTTTC	1440p	NM_204184.2	
MVII7 como	F	TGCTGCTCATCACCAACAAC	100hm	NIM 001001202.2	
MYH7 gene	R	AAGCACTATCGGTTGCCAAC	108bp	NM_001001302.2	
CADDII cono	F	TGGCATTGCACTGAATGACC	0.6hm	NIM 204205.2	
GAPDH gene	R	TCAAGTCCACAACACGGTTG	86bp	NM_204305.2	

The qPCR master mix was prepared using the GoTaq® qPCR Master Mix kit (Promega, USA) with SYBR Green dye for real-time PCR amplification of the target genes

MYF5, MYOG, and MYH7 and the housekeeping gene GAPDH (4,11). The relative gene expression levels (fold change) were calculated using the Δ CT method with

GAPDH as the reference gene. The equation used for this analysis was: Gene expression ratio = $2^{-(CT(Housekeeping gene) - CT(Target gene))}$.

Statistical analysis

All data were analyzed using IBM SPSS Statistics (v26.0). Normality was confirmed via Shapiro-Wilk tests (P>0.05) and homogeneity of variance via Levene's test. For normally distributed parameters: Muscle fiber area, muscle bundle area, connective tissue thickness, as well as MYF5, MYOG, and MYH7 expression levels, we performed oneway ANOVA with LSD post-hoc testing (α =0.05). Data are presented as mean \pm SEM (n=5/group). Effect sizes were calculated using partial eta-squared (η^2).

Result

This study focuses on histological structure, glycogen level, and mRNA expression of MYH7, MYOG, and MYF5 proteins in the pectoralis major muscle of broiler chickens across three age groups. Microscopically, the pectoralis major muscle consists of muscle fibers. The muscle fibers were elongated, cylindrical, and multinucleated, with oval nuclei positioned peripherally beneath the sarcolemma, with a light and dark cross striation (Figure 1). The area of muscle fiber in the pectoralis major muscle exhibited a progressive increase with age. The highest mean fiber area was recorded at 38-day-old, whereas the lowest was observed at 7-day-old. Statistical analysis revealed a highly significant difference in the muscle fiber area at 38-day-old compared to both the 7day-old (p = 0.0006) and 21-day-old (p = 0.0004). Additionally, a significant difference was detected between the 21-day-old and 7-day-old (p = 0.003), as presented in figures 2 and 3.

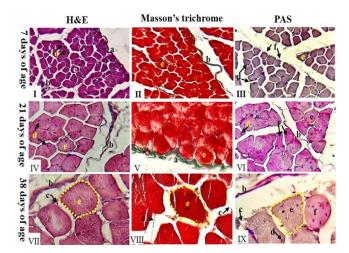


Figure 1: histological structure of the pectoralis major muscle of broiler chicken. (a) epimysium, (b) perimysium, (C) Endomysium, (d) nucleus, € muscle fibers, (f) glycogen. 400x.

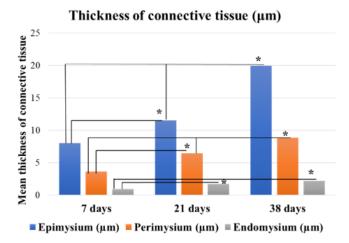


Figure 2: Represented the thickness of the connective tissue layers in the pectoralis major muscle at 7, 21, and 38 days of age of the broiler chickens. Histometric measurements were evaluated utilizing the ImageJ software. Data is expressed as mean \pm standard error (n = 5). * P<0.05.

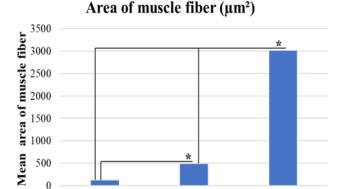


Figure 3: Represented the area of muscle fiber in the pectoralis major muscle at 7, 21, and 38 days of age of the broiler chickens. Histometric measurements were evaluated utilizing the ImageJ software. Data is expressed as mean \pm standard error (n = 5). * P<0.05.

21 days

38 days

7 days

The pectoralis major muscle was externally covered by a dense irregular connective tissue layer, the epimysium, which encased the entire muscle mass. From this layer, connective tissue septa extended inward, forming the perimysium, a dense, irregular connective tissue layer that contained small blood vessels surrounding the muscle fascicles to organize the muscle fibers within bundles. Each muscle fiber was surrounded by a thin layer of loose connective tissue accompanying the blood vessels and nerves that supplied the muscle tissue (Figure 1). The thickness of the connective tissue layers in the pectoralis major muscle increased with age. The epimysium exhibited

the highest mean thickness at 38-day-old, while the lowest was observed at 7-day-old. Statistical analysis demonstrated a highly significant difference between the 38-day-old compared to both the 7-day-old (p = 0.0004) and the 21-day-old (p = 0.0001).

Additionally, a significant difference was observed between the 21-day-old and 7-day-old (p = 0.0003). Similarly, the perimysium showed a notable thickening with age, with the highest mean thickness recorded in the 38-dayold and the lowest in the 7-day-old. Statistical analysis revealed a highly significant difference between the 38-dayold compared to both the 7-day-old (p = 0.0008) and the 21day-old (p = 0.0002). Furthermore, a significant difference was observed between the 21-day-old and 7-day-old (p = 0.0001). Regarding the endomysium, a gradual increase in thickness was detected with advancing age, with the highest mean thickness recorded in the 38-day-old and the lowest in the 7-day-old. Statistical analysis revealed a highly significant difference between the 38-day-old compared to both the 7-day-old (p = 0.0001) and between the 21-day-old and 7-day-old (p = 0.001). However, no significant difference was observed in the thickness of the endomysium between the 38-day-old and 21-day-old (p = 0.057), as presented in (Table 2 and Figure 2). The area of the muscle bundle of the pectoralis major muscle exhibited a significant increase with age. The highest mean muscle bundle area was recorded at 38 days old, whereas the lowest was observed at 7 days old. Statistical analysis revealed a highly significant difference in the muscle bundle area in the 38-day-old compared to both the 7-day-old (p = 0.0002) and 21-day-old (p = 0.0009). Additionally, a significant difference was observed between the 21-day-old and 7-day-old (p = 0.029), as presented in (Table 2 and Figure 4). PAS staining indicated variable expression of glycogen in the muscle fibers based on the age stage of broiler chickens. The glycogen content appeared as purple granules and was located between the myofibrils. Muscle fibers of the pectoralis major showed a progressive increase in PAS staining intensity from 7 to 21 days old (Figure 1), followed by a decline at 38 days old. By 21 days, the PAS staining reached its highest intensity, and glycogen granules showed a noticeable increase in size and distribution within the muscle fibers, indicating the highest level of glycogen accumulation at this stage (Figure 1).

Table 2: Parameters in the pectoralis major muscle at 7, 21, and 38 days of age of the broiler chickens

Parameters	7 days of age	21 days of age	38 days of age
Epimysium (µm)	7.990 ± 0.357 a	11.503 ± 0.349 b	19.935 ± 0.340 c
Perimysium (µm)	3.621 ± 0.149 a	6.450 ± 0.254 b	8.851 ± 0.446 c
Endomysium (µm)	0.956 ± 0.122 a	$1.737 \pm 0.144 \text{ b}$	$2.179 \pm 0.137 \text{ b}$
Area of muscle fiber (µm²)	119.02 ± 6.420 a	$486.903 \pm 22.334 \text{ b}$	3004.735 ± 186.999 c
Area of muscle bundle (µm²)	22747.126 ± 3352.132 a	$40702.178 \pm 3237.438 b$	$80021.122 \pm 8682.460 \text{ c}$

The values represent the mean \pm standard error for each group. (n = 5). P \le 0.05

Table 3: Gene expression of MYH7 and housekeeping gene analyzed using gene expression ratio method

Group	CT: MYH7	CT: GAPDH	Δ CT	Gene expression ratio (MYH7)	Mean± standard
	28.63	26.57	2.06	4.17	
	28.84	26.52	2.32	4.99	
7 days	29.67	26.22	3.45	10.93	6.554±1.44
	29.5	26.33	3.17	9.00	
	28.09	26.21	1.88	3.68	
	30.72	26.28	4.44	21.71	
	31.97	27.72	4.25	19.03	
21 days	31.64	27.97	3.67	12.73	21.124±2.99
·	31.37	26.99	4.38	20.82	
	31.6	26.63	4.97	31.34	
	32.52	26.63	5.89	59.30	
	32.22	26.4	5.82	56.49	
38 days	32.33	26.26	6.07	67.18	63.861±4.040
•	32.21	26.35	5.86	58.08	
	32.28	25.99	6.29	78.25	

The values represent the mean \pm standard error for 5 samples for each group. P \le 0.05.

Real-time PCR primers (RT-qPCR) were used to analyze their transcript expression in the pectoralis major muscle of broiler chickens. The RT-qPCR accuracy for experimental samples and housekeeping genes displayed identical curves

of amplification and melting peaks. The RT-qPCR amplification was extremely specific, with no nonspecific product amplification. Every experimental specimen displayed melting peaks between 79 and 80°C. MYH7, MYOG, and MYF5 protein expression at 7 days of age exhibited that the highest average expression was detected in MYF5, while the lowest was found in MYH7, as presented in (Tables 4 and 5). Therefore, statistical analysis revealed a highly significant difference in MYF5 compared to both MYH7 and MYOG (p-value = 0.0001 and 0.000228, respectively). Furthermore, there was no significant difference between MYH7 and MYOG in the pectoralis major muscle (p = 0.8486). (Figures 5-9). Moreover, the MYH7, MYOG, and MYF5 protein expression at 21 days of age revealed non-significant changes in gene expression. The

highest average expression was detected in MYF5, while the lowest was found in MYH7, as presented in (Table 3-5). So, statistical analysis revealed no significant difference in MYF5 compared to both MYH7 and MYOG (p-value = 0.4001 and 0.736, respectively). Additionally, there was no significant difference between MYH7 and MYOG in the pectoralis major muscle (p = 0.536). (Figures 5-9). Besides, the MYH7, MYOG, and MYF5 protein expression at 38 days of age exposed that the highest average expression was detected in MYOG, while the lowest was found in MYF5, as presented in (Tables 4 and 5). Therefore, statistical analysis revealed a highly significant difference in MYOG and MYH7 compared to MYF5 (p-value = 0.00041 and 0.0338, respectively) and no significant difference between the MYOG and the MYH7 (p-value = 0.1278) (Figures 5-9).

Table 4: Gene expression of MYOG and housekeeping gene analyzed using gene expression ratio method

Group	CT: MYOG	CT: GAPDH	ΔCΤ	Gene expression ratio (MYOG)	Mean± standard
	29.45	26.57	-2.88	7.36	
	30.4	26.52	-3.88	14.72	
7 days	29.79	26.22	-3.57	11.88	11.352±1.356
-	30.09	26.33	-3.76	13.55	
	29.42	26.21	-3.21	9.25	
	32.51	26.28	-6.23	75.06	
	32.18	27.72	-4.46	22.01	
21 days	32.07	27.97	-4.1	17.15	33.850±10.493
•	31.69	26.99	-4.7	25.99	
	31.49	26.63	-4.86	29.04	
	33.57	26.63	-6.94	122.79	
	33.29	26.4	-6.89	118.60	
38 days	33.14	26.26	-6.88	117.78	102.436±14.976
•	33.13	26.35	-6.78	109.90	
	31.42	25.99	-5.43	43.11	

The values represent the mean± standard error for 5 samples for each group. P≤0.05.

Table 5: Gene expression of MYF5 and housekeeping gene analyzed using gene expression ratio method

Group	CT: MYF5	CT: GAPDH	ΔCΤ	Gene expression ratio (MYF5)	Mean± standard
	33.33	26.57	-6.76	108.38	
	33.21	26.52	-6.69	103.25	
7 days	33.28	26.22	-7.06	133.44	222.812±67.996
	34.72	26.33	-8.39	335.46	
	34.97	26.21	-8.76	433.53	
	33.21	26.28	-6.93	121.94	
	31.99	27.72	-4.27	19.29	
21 days	31.51	27.97	-3.54	11.63	42.324±20.230
	31.63	26.99	-4.64	24.93	
	31.71	26.63	-5.08	33.82	
38 days	29.22	26.63	-2.59	6.02	
	30.26	26.4	-3.86	14.52	
	29.44	26.26	-3.18	9.06	9.676±1.496
	29.25	26.35	-2.9	7.46	
	29.49	25.99	-3.5	11.31	

The values represent the mean \pm standard error for 5 samples for each group. P \le 0.05.

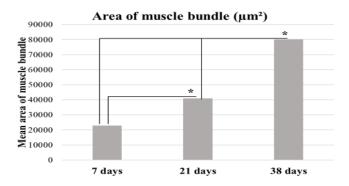


Figure 4: Represented the area of muscle fiber in the pectoralis major muscle at 7, 21, and 38 days of age of the broiler chickens. Histometric measurements were evaluated utilizing the ImageJ software. Data is expressed as mean \pm standard error (n = 5). * P<0.05.

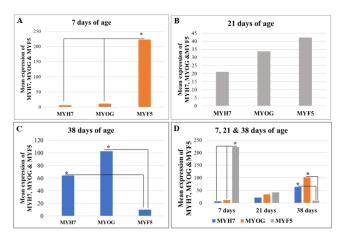


Figure 5: Represented the quantification of the MYH7, MYOG, and MYF5 transcripts in the pectoralis major muscle in broiler chicken using RT-qPCR. The expression of MYH7, MYOG, and MYF5 genes was normalized to the expression of GAPDH and is presented as gene expression ratios. Each column denotes the gene and age of the broiler chickens. Data is expressed as mean± standard error (n = 5). * P<0.05. (A) at 7 days of age, (B) at 21 days of age, (C) at 38 days of age, (D) at 7, 21 and 38 days of age.

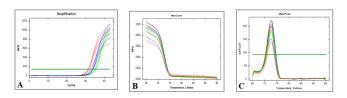


Figure 6: Represented the MYOG gene in the pectoralis major muscle. The green, blue, and red plots are for 7, 21, and 38 days of age, respectively. (A) qPCR plots of MYOG, (B) qPCR melt curve of MYOG, (C) qPCR melt peak of MYOG.

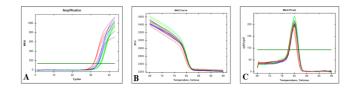


Figure 7: Represented the MYF5 gene in the pectoralis major muscle. The green, blue, and red plots are for 7, 21, and 38 days of age, respectively. (A) qPCR plots of MYF5, (B) qPCR melt curve of MYF5, (C) qPCR melt peak of MYF5.

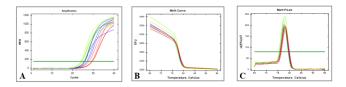


Figure 8: Represented the MYH7 gene in the pectoralis major muscle. The green, blue, and red plots are for 7, 21, and 38 days of age, respectively. (A) qPCR plots of MYH7, (B) qPCR melt curve of MYH7, (C) qPCR melt peak of MYH7.

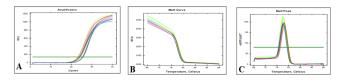


Figure 9: Represented the GAPDH gene in the pectoralis major muscle. The green, blue, and red plots are for 7, 21, and 38 days of age, respectively. (A) qPCR plots of GAPDH, (B) qPCR melt curve of GAPDH, (C) qPCR melt peak of GAPDH.

Discussion

The histological analysis of pectoralis major muscle fibers in broiler chickens across three age groups, 7, 21, and 38 days, revealed a consistent structural organization (12,13). However, muscle fiber area increased significantly among the different age groups (14-16). The significant increase in muscle fiber area with advancing age reflects the normal growth and development of the skeletal muscle and the dynamic process influenced by age. Additionally, the muscle bundle area increased with age. Although the overall histological organization of the pectoralis major muscle remained consistent, the thickness of the connective tissue layers increased with age (14-17). On the other hand, the thickness of connective tissue in broiler chickens decreased significantly with age (18). We believe the gradually increasing thickness of connective tissue layers in the pectoralis major muscle was explained by age-related mechanical and physiological demands.

As muscle mass and muscle fiber size increased with growth, the epimysium, perimysium, and endomysium obtained structural strengthening to preserve mechanical strength and support enlarged muscle fibers. Our results appear to show the muscle fibers stained with PAS stains, which demonstrated a difference in glycogen granule content among myofibrils in age groups (19). These variabilities in muscle glycogen are influenced by factors such as age and diet prior to slaughter. The muscle fibers of the pectoralis major exhibited a progressive increase in PAS staining intensity from day 7 to day 21, followed by a decline at day 38. These results are consistent with the findings reported that younger broilers primarily utilize glucose for glycogen synthesis and storage, whereas older broilers increasingly rely on glucose oxidation for energy production rather than storage (20). A few other studies have detected that feed withdrawal prior to slaughter or a low-protein diet significantly reduces postmortem glycogen content in the pectoralis major muscle of broilers (21,22). Additionally, glycogen levels were higher at hatching compared to day 5. Collectively, these findings suggest that changes in glycogen accumulation are part of the metabolic adaptations occurring in muscles at different developmental stages (23).

Our results for mRNA expression of proteins MYF5, MYOG, and MYH7 in the pectoralis major muscle across different developmental stages were analyzed. The expression results of MYF5 were at the highest level at 7 days of age, reaching its lowest level at 38 days. This pattern may indicate the crucial role of MYF5 in the early stages of muscle development. On the other hand, the MYOG showed a progressive increase with age. With its expression at the lowest level at 7 days of age and reaching the highest level at 38 days. This pattern suggests a potential role of MYOG in differentiation regulating muscle and maturation, particularly in the later stages of development. These results are consistent with the findings of (9,24,25).

In contrast, in chicken breast muscle, the *MYOG* was highly expressed during embryonic stages but was undetectable post-hatch, suggesting its role in early myogenic differentiation. In contrast, *MYF5* expression persisted until day 7 post-hatch but was absent in adult muscles, indicating its involvement in early postnatal muscle development (26). The observed differences may be a result of different conditions that either activate or suppress genes at specific times.

In this study, the gene expression analysis of MYH7 in the pectoralis major muscle showed a progressive increase with age. The expression was lowest at 7 days, followed by the highest expression level at 38 days, indicating a significant age-dependent increase in MYH7 expression. So that the current study has reported the muscle fibers of the pectoralis major contain slow-twitch fibers. Myosin Heavy Chain 7, which encodes the β -myosin heavy chain in slow-twitch muscle fibers (27,28). However, this result contradicts the finding in broiler chickens that MYH7 expression was not

detected in the pectoralis major muscle, indicating that this muscle consists entirely of fast-twitch type fibers with no slow-twitch fibers (29-40).

Additionally, in ducks, the pectoralis muscle is predominantly composed of fast-twitch fibers with a weak or absent expression of the *MYH7* gene, which is associated with slow-twitch fibers. It is possible that the variations in MYH7 gene expression between our findings in chickens and other birds were caused by the types of breeds of broiler chickens differing in how muscle-related genes were regulated. Additionally, dietary differences might have affected gene expression and muscle growth (41-50).

Conclusions

This study provides valuable insights into the role of muscle regulatory factors (MYF5, MYH7, and MYOG) in muscle growth and development in broiler chickens. The results demonstrate a progressive development in muscle structure, glycogen content, and gene expression across different ages. Notably, A strong correlation was established between muscle tissue development and gene expression, indicating the significant role of these regulatory factors in improving meat production and quality.

Acknowledgments

The authors express gratitude to the members of the Department of Anatomy & Histology, College of Veterinary Medicine, University of Al-Qadisiya, for their collaboration.

Conflict of interest

None

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تأثيرات العمر على التعبير الجيني المرتبط بالعضلات ومستويات الجليكوجين في دجاج التسمين

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الخلاصة

تعد عوامل التنظيم العضلي MYOG و MYH7 و MYF5 ضرورية لنمو العضلات وتطورها، ولذلك تعتبر جينات مرشحة لصفات إنتاج اللحوم في الدجاج. قامت هذه الدر اسة بالتحقيق في مستويات الجليكوجينّ وتعبير جينات MYOG و MYF5 و MYF5 في عضلة الصدر الكبرى في دجاج التسمين. تم جمع عينات طازجة من عضلة الصدر الكبرى لدَّجاج النَّسمين. استخدمت صبغات خاصة لتحديد البنية النسجية ومحتوى الجليكوجين في عضلة الصدر الكبرى. سجلت أعلى متوسط لمساحة الألياف العضائية وسمك طبقات النسيج الضام ومساحة الحزم العضلية عند عمر ٣٨ يوماً وأظهرت ألياف عضلة الصدر الكبرى زيادة تدريجية في شدة التلون بصبغة PAS من عمر ٧ إلى ٢١ يوماً، تلاها انخفاض عند عمر ٣٨ يوماً. تم استخدام تقنية تفاعل البوليمير از المتسلسل الكمي لتقدير تراكيز الرنا الرسولي لجينات MYOG و MYF5 في ألياف عضلة الصدر الكبرى. في الواقع، أظهر التعبير الجيني عند عمرً ٧ أيام أن أعلى متوسط للتعبير كان في MYF5 . أما عند عمر ٢١ يومأ، لوحظ عدم وجود تغيرات معنوية في التعبير الجيني. من ناحية أخرى، أظهر التعبير الجيني عند عمر ٣٨ يوماً أن أعلى متوسط للتعبير كان في MYOG، في حين كان الأدنى في MYF5. الاستنتاج، توضح هذه الدراسة الدور الأساسي لعوامل التنظيم العضلي، وتحديدا MYOG و MYH7 و MYF5، في نمو وتطور عضلة الصدر الكبرى في دجاج التسمين. كما أظهر التّحليل وجود ارتباط قوي بين التعبير الجيني في مختلف مراحل النمو وتطور الألياف العضلية