Journal of Integrative Agriculture 2026, 25(8): 3368–3378

Available online at ScienceDirect

Journal of Integrative Agriculture



RESEARCH ARTICLE

Precise quantification of skeletal muscle fibers reveals the physiological basis for growth rate discrepancies in broilers

Shuang Gu[1, 2], Chaoyi Wang[1, 2], Qiang Huang[1, 2], Qiulian Wang[1, 2], Junying Li[1, 2, 3], Congjiao Sun[1, 2, 3], Chaoliang Wen[1, 2, 3], Ning Yang[1, 2, 3#]

[1] *State Key Laboratory of Animal Biotech Breeding/Frontier Science Center for Molecular Design Breeding, China Agricultural University, Beijing 100193, China*

[2] *National Engineering Laboratory for Animal Breeding/Key Laboratory of Animal Genetics, Breeding and Reproduction, Ministry of Agriculture and Rural Affairs/Department of Animal Genetics and Breeding, College of Animal Science and Technology, China Agricultural University, Beijing 100193, China*

[3] *Sanya Institute of China Agricultural University, Hainan 572025, China*

Highlights

- First precise quantification of total muscle fiber number in poultry skeletal muscle.
- Higher muscle fiber count drives rapid growth and high meat yield in fast-growing broilers.
- Total muscle fiber number serves as a key breeding target for improved broiler meat quality.

Abstract

Skeletal muscle is composed of multinucleated muscle fibers, which play a crucial role in determining the quality of meat products in livestock. Quantifying the total number of muscle fibers (TNM) is essential for understanding muscle composition, but this remains challenging in poultry, particularly since the considerable number of livestock complicates the preparation of tissue sections for analysis and makes the counting process laborious. Our previous study developed an automatic muscle fiber quantification tool powered by deep learning, named MyoV, which has addressed this bottleneck. This study employed the MyoV tool for accurately quantifying TNM in the pectoral muscles of slow-growing (SL), medium-growing (ML), and fast-growing (FL) broilers. The results showed that FL group exhibited higher growth performance compared to ML and SL groups from the embryonic to rearing stages. Processing of whole slide images of pectoral muscle revealed significantly higher TNM in FL and ML groups than in SL group (P<0.01). The TNM values of FL, ML and SL groups were 693,568.00±54,169.80, 652,122.00±65,822.60, and 539,778.57±40,722.94 at 7 days of age (D7), respectively; and 663,014.93±58,801.11, 645,784.76±80,204.34 and 507,280.29±98,092.16 at D35 for FL, ML and SL groups, respectively. Differences in the cross-sectional area (CSA) of muscle fibers among the three groups were consistent with the TNM results. A correlation analysis showed correlation coefficients of 0.73–0.89 between body weight (BW) and TNM and 0.78–0.87 between BW and CSA. These findings directly indicate that the number of muscle fibers in broilers is an important foundation for their rapid growth and development. This study precisely quantified the muscle fiber number of an important skeletal muscle in poultry for the first time, which provides direct evidence for the physiological basis of rapid development in broilers and offers important data support for further in-depth studies on muscle fiber development.

Keywords: broiler, growth performance, precision quantification, total number of muscle fibers

1. Introduction

The modern poultry industry is one of the most important industries within agriculture (Raheem *et al.* 2021) by providing high-quality animal protein to the human diet, including abundant and high-quality meat and eggs (Hegazy *et al.* 2021; Tavaniello *et al.* 2022). Decades of intensive artificial genetic selection has led to remarkable advancements in the growth performance and feed efficiency of modern broilers (Ahmadipour and Khajali 2019; Zuidhof 2020). Modern commercial broilers can usually reach a market weight of

Received 13 April 2024; Received in revised form 27 June 2024; Accepted 16 July 2024; Available online 20 August 2024
Shuang Gu, E-mail: 13592566171@163.com; #Correspondence Ning Yang, E-mail: nyang@cau.edu.cn

© 2026 CAAS. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/). Peer review under responsibility of Editorial Board of *Journal of Integrative Agriculture*.
doi: 10.1016/j.jia.2024.08.008

around 3 kg within 42 days (de Lima *et al.* 2019; Fan *et al.* 2020). Studies have shown that skeletal muscle is the largest organ in the animal body, making up over 40% of total body mass (Kritikaki *et al.* 2021). The fundamental building blocks of skeletal muscle are multinucleated muscle fibers, which are formed through the fusion of mononuclear myoblasts during embryonic development (Roman *et al.* 2017; Gu *et al.* 2022). The total number of muscle fibers (TNM) remains unchanged after birth (Ju *et al.* 2021). The histological characteristics of muscle fibers play a crucial role in determining the meat quality and palatability of livestock (Yu *et al.* 2018; Cheng H *et al.* 2021). Notably, TNM serves as the developmental basis for high muscle mass and quality (Shen *et al.* 2015). Studies have reported some correlation between muscle fiber density and muscle protein content (Zhang *et al.* 2020). The rapid growth rate of broiler breeds is closely associated with the rapid hypertrophy of muscle fibers after hatching, which results in a larger cross-sectional area (CSA) (Weng *et al.* 2022; Sultana *et al.* 2023) and lower density (Zhao *et al.* 2011) of muscle fibers compared to layers and native breeds. Our previous study demonstrated that myoblasts are in the process of fusion during the embryonic period until embryonic day 17 (E17) (Gu *et al.* 2022). Since the determination of TNM and CSA requires clear myofiber boundaries, quantifying these traits after hatching is reliable.

Studying TNM in livestock holds significant importance for exploring muscle development and promoting genetic selection. The TNM values of the extensor digitorum longus, tibialis anterior muscle or gastrocnemius in mice are usually used for quantification, as these muscle parts are relatively small in mice, with a lower TNM, which makes the quantification practically feasible (Porporato *et al.* 2013; Picchiarelli *et al.* 2019). Nevertheless, no studies to date have precisely quantified the TNM in poultry skeletal muscle. This is primarily because the substantial size of livestock makes it difficult to create tissue sections that encompass the entire array of muscle fibers and employ appropriate counting methods. Thus, the histological characteristics of livestock skeletal muscle are traditionally assessed by measuring the CSA and density of muscle fibers, in which the individual is represented by a few localized samples. However, relying on the measurements of a limited number of muscle fibers to represent the histological composition of thousands of skeletal muscle fibers is unreliable. Scheuermann *et al.* (2004) proposed a method for estimating the TNM by multiplying the estimated myofiber density with the CSA of a tissue block from the pectoral muscle. We performed a series of experiments to validate this estimation method and found noticeable errors between the estimated values and the actual muscle fiber counts of pectoral muscle in chickens, with the estimates being about 25% higher than the manually generated TNM (Gu *et al.* 2024).

Given these issues, accurately determining the TNM in poultry skeletal muscle has been a challenge. The recent advances in deep learning algorithms have achieved significant milestones in image recognition (Kermany *et al.* 2018; Hollandi *et al.* 2020; Cheng S *et al.* 2021; Yang *et al.* 2023), and provide a new opportunity to address the longstanding challenge of TNM quantification in livestock. Thus, we have developed the MyoV tool, which is based on deep learning, to automatically and accurately quantify muscle fibers (Gu *et al.* 2024). While many studies have indicated variations in the CSA of muscle fibers among chicken breeds with different growth rates, the differences in TNM have not been reported. Therefore, this study quantified the muscle fibers in the pectoral muscles of three different broiler breeds with varying growth rates for the first time using the big data analytic capabilities of MyoV, with the aim of filling the gap in the precise quantification of TNM. This study lays the groundwork for future studies aimed at selecting broilers with improved meat quality based on muscle fiber quantity.

2. Materials and methods

2.1. Experimental birds and sample collection

The fertilized eggs of slow-growing (SL), medium-growing (ML), and fast-growing (FL) broilers were obtained from the WOD168, WOD178, and WOD188 of Beijing Huadu Yukou Poultry Breeding Co., Ltd., China. All the experimental broiler chickens had Cornish ancestry, with lineage proportions of 50% for WOD168, 75% for WOD178 and 100% for WOD188. All fertilized eggs were sprayed and disinfected with 0.1% benzalkonium bromide solution before being placed into an incubator with a temperature of 37.8°C and relative humidity of 60%. Myoblast fusion is active from E14. A certain number of embryos from each experimental group were collected daily from E14 to E19 to measure their body weight (BW), pectoral muscle with sternum weight (PMW), and leg (drumstick with thigh) weight (LW). In addition, 20 males and 20 females from each experimental group were selected to measure BW, PMW and LW on the day of hatching (DOH). The remaining birds were separated by sex and raised in the same chamber together under the same conditions with *ad libitum* access to feed and water until 7 days of age (D7) and D35. At D7, 10 males and 10 females from each experimental group were selected for measurements of BW, PMW and LW. In addition, the pectoral major muscle samples were collected for H&E staining to assess muscle fiber histology. Finally, 20 males and 20 females from the three groups were weighed for the same indices at D35 and the pectoral major muscles were used for histological assessment.

2.2. Paraffin section preparation and H&E staining

The entire pectoral major muscles of the three groups at D7 were immersed in 4% paraformaldehyde for more than 48 h. The pectoral major muscles of the experimental groups at D35 were outlined along the line from the clavicular to the lateral midpoint of the muscle using the green Davidson® Tissue Dye (Bradley, USA). Subsequently, muscle segments of approximately 2 cm in length, encompassing the marked line, were excised parallel to the line at 1 cm intervals above and below it. These muscle strips were then fixed in 4% paraformaldehyde for a period exceeding 48 h. Then, the pectoral muscles were removed from the fixing solution and trimmed along the clavicle to the outer half, perpendicular to the direction of myofibers (Gu *et al.* 2024). Note that the specified cross-sections of the pectoral major muscle from

each individual at D7 were put onto a single glass slide, whereas the designated sections at D35 needed to be divided into 2–4 portions which were placed on the final glass slides. The trimmed tissues were placed into dehydration boxes in a dehydrator (DIAPATH, Italy) to dehydrate them with an ethanol gradient as follows: 75% alcohol for 4 h, 85% and 90% alcohol for 2 h and 95% alcohol for 1 h. They were then immersed in anhydrous ethanol I and anhydrous ethanol II for 30 min, alcoholic benzene and xylene II for 10 min, and 65°C melting paraffin I, II and III for 1 h each.

The paraffin-soaked muscle samples were placed into the embedding machine (Wuhan Junjie Electronics Co., Ltd., China). The melted wax blocks were placed into the embedding frame and the corresponding labels were affixed before the wax solidified. The trimmed wax blocks were placed on a –20°C freezing table and then sectioned at a thickness of 4 μm with a paraffin slicer (Shanghai Leica Instrument Co., Ltd., China). The slices were floated on a spreader with warm water (40°C) for expansion and were then picked up and fixed on the clean glass slides and baked in an oven (Tianjin Laibo Rui Instrument Equipment Co., Ltd., China) at 60°C. The slices were stained with hematoxylin solution for 5 min and then treated using hematoxylin differentiation solution and hematoxylin Scott tap bluing. The samples were washed with running water after each treatment. Slice samples were soaked and dehydrated in 85% and 95% alcohol for 5 min each and then stained with eosin dye for 5 min. The sections were then immersed in the 100% ethanol I, II and III for 5 min each and the xylene I and II for 5 min each to make them transparent, and then finally sealed with neutral gum. The stained sections were scanned using a scanner (3DHISTECH, Hungary) to capture the high-resolution whole slide images (WSIs).

2.3. Histological determination

The muscle fiber histological analysis was performed using our previously developed MyoV software (Gu *et al.* 2024). The basis for the precise quantification of TNM is that every muscle fiber within the WSIs is completely transversely sectioned. The pectoral major muscle tissue is relatively small, so slight deviations during the trimming process can lead to oblique sections of some muscle fibers within the WSIs at D7. This resulted in several WSIs that were not suitable for the statistical analysis of TNM. Thus, the number of available WSIs at D7 was less than at D35. First, the WSIs of the pectoral muscles obtained at D7 and D35 were segmented into smaller, high-resolution image tiles of 2,048×2,048 pixels. This approach facilitated a more detailed and precise analysis of the muscle fiber numbers within the muscle samples. Following segmentation, the juvenile stage model, which is designed for early developmental muscle fibers, and the mature stage model, which is tailored for the later stages of muscle growth, were employed to quantify muscle fibers within the pectoral muscles of each individual at D7 and D35. Note that the muscle fibers truncated at the upper and left edges of the image tiles were predicted, while those at the right and lower edges were not, which prevented individual muscle fibers from being detected multiple times. The overall aggregate count of muscle fibers across all image tiles within a WSI at D7 for each sample of the three groups was the TNM. Similarly, the sum of muscle fiber counts from all image tiles within several WSIs for each individual at D35 was also treated as the TNM.

Previous studies typically selected 6–10 local visual fields of myofiber sections from individual slides to determine the CSA, which may introduce errors due to the limited sampling size. However, the number of image tiles of WSIs ranges from several hundred to several thousand per individual sample. To enhance the reliability of our results, a subset of 100 image tiles for each individual was randomly chosen for the assessment of CSA based on their relatively uniform distribution of muscle fibers. Specifically, each image tile at D7 contained an average of approximately 700 muscle fibers, while each image tile at D35 featured around 155 muscle fibers on average. Using the MyoV models as described above, CSA assessment was performed on these selected image tiles. The mean CSA of the 100 image tiles was calculated as that individual's average CSA.

2.4. Statistical analysis

The statistical analysis was conducted using R software (v.4.2.3), with the results presented as mean±SD. One-way analysis of variance was employed for statistical comparisons among multiple experimental groups and intergroup differences were tested with LSD multiple comparisons. Statistical significance was defined at a *P*-value of <0.05.

3. Results

3.1. Broilers with varying growth rates exhibited differential development during the embryonic period

To evaluate the development of chick embryos, the BW, PMW, and LW growth of the three experimental groups was monitored from E14 to E19. The BW of the chick embryos in all groups showed a linear growth trend with an average daily gain of around 4 g from E14 to E16 (Fig. 1-A). In addition, the BW values of both FL and ML groups were higher than in the SL group, with a significant difference observed at E14 (P<0.05) (Fig. 1-A). The FL and ML groups had BW of (24.81±2.06) g and (24.83±1.14) g at E17, significantly higher than (23.21±2.19) g of the SL group (P<0.01). The BW of ML groups (33.79±1.52) g was higher than that of FL group (33.19±2.62) g at E19, but the differences among the three groups were not significant (Fig. 1-A).

The growth trends of PMW and LW during the embryonic period were consistent with BW. The results showed that FL had a higher PMW than ML and SL groups at E16 (P<0.05) (Fig. 1-B). At E17, the PMW of the FL group was (1.44±0.17) g and significantly higher than the SL group (P<0.01) (Fig. 1-B). The LW values of the three groups showed significant differences at E14 and E15, with (1.65±0.09) g for FL group and (1.61±0.15) g for ML group at E14, which were significantly higher than the (1.41±0.03) g of SL group (P<0.05) (Fig. 1-C). At E15, LW of the FL group was significantly higher than the ML group (P<0.01), while the difference between SL and ML groups was not significant. Furthermore, the FL and ML groups exhibited significantly

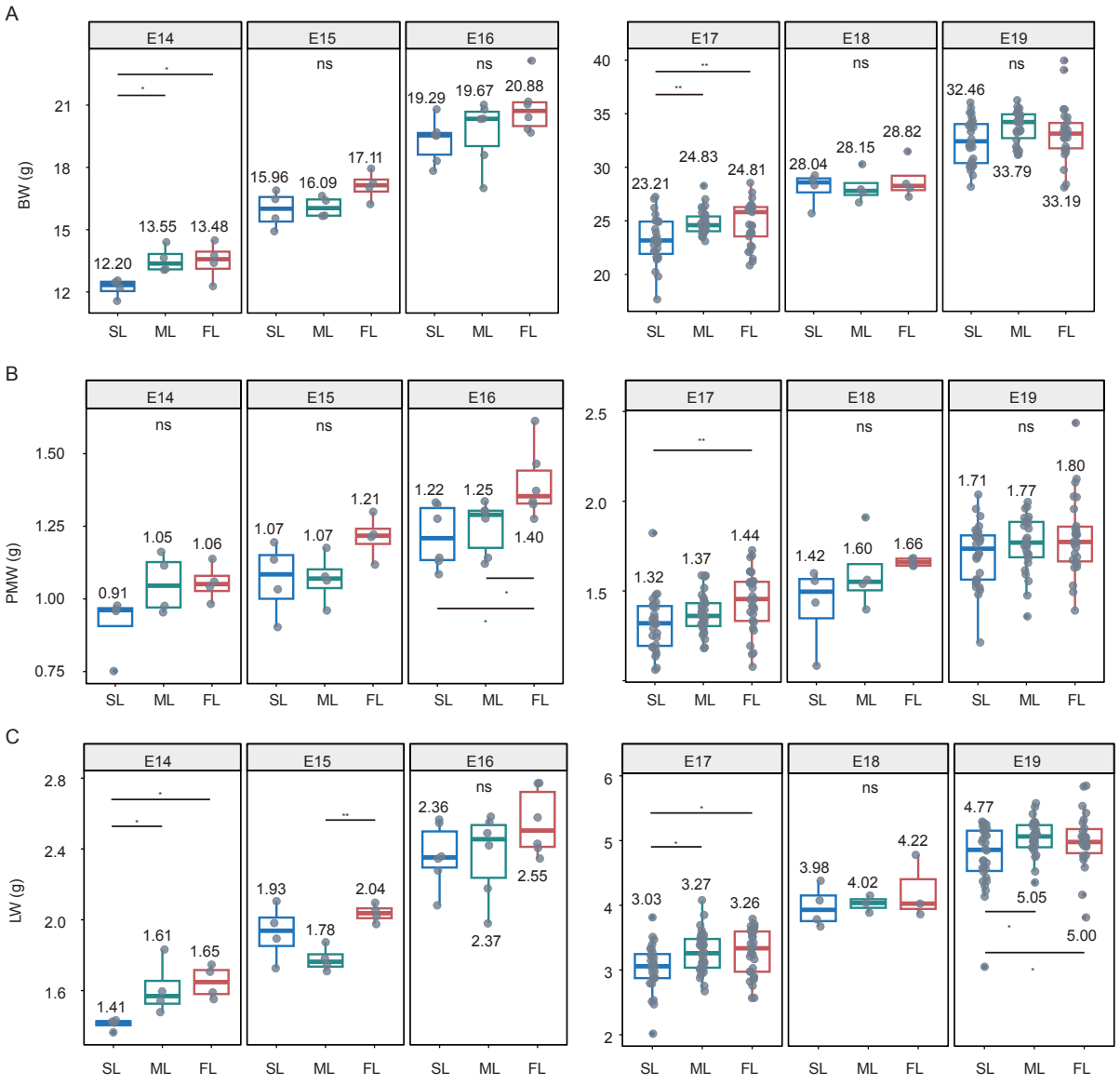


Fig. 1 Embryonic growth performance of broiler chickens with various growth rates. A, difference analysis of body weight (BW) among the slow-growing (SL), medium-growing (ML) and fast-growing broilers (FL) from embryonic day 14 (E14) to E19. B, difference analysis of pectoral muscle with sternum weight (PMW) among the three groups from E14 to E19. C, difference analysis of leg (drumstick with thigh) weight (LW) among the three groups from E14 to E19. Each experimental group had sample sizes of 4, 4, 6 and 4 at E14, E15, E16, and E18, respectively. At E17, the sample sizes for the SL, ML, and FL groups were 27, 31, and 29, respectively, while at E19, those were 30, 26, and 26, respectively. All values are expressed as mean±SEM. One-way analysis of variance was employed for statistical comparisons among multiple experimental groups, and intergroup differences were tested with LSD multiple comparisons. The underlined text indicates the results of the statistical tests for differences between the two experimental groups, and the same applies below. **, extremely significant differences between groups (P<0.01); *, significant differences between groups (P<0.05); ns, a non-significant difference between groups (P>0.05).

higher LW than the SL group at both E17 and E19 (P<0.05) (Fig. 1-C).

3.2. Developmental disparities among broilers increased during the rearing period

The performance results showed a faster developmental rate of broiler breeds after hatching, and the BW of the three experimental groups showed considerable differences (Fig. 2-A and B). The BW of the SL group at DOH was higher than those of the ML and FL groups, with the BW of (45.26±3.39) g for males of the SL group being significantly higher than the (41.28±3.36) g of the ML group and (41.87±4.21) g of the FL group (P<0.01) (Table 1). However, the PMW and LW at this time point showed no significant differences among the three experimental groups. The developmental speeds of the FL and ML groups were faster than that of the SL group within a week after hatching. The BW, PMW, and LW in both males and females of FL and ML groups were significantly higher compared to the SL group (P<0.01) (Table 1).

There were no differences in growth performance between males and females until D35. The differences

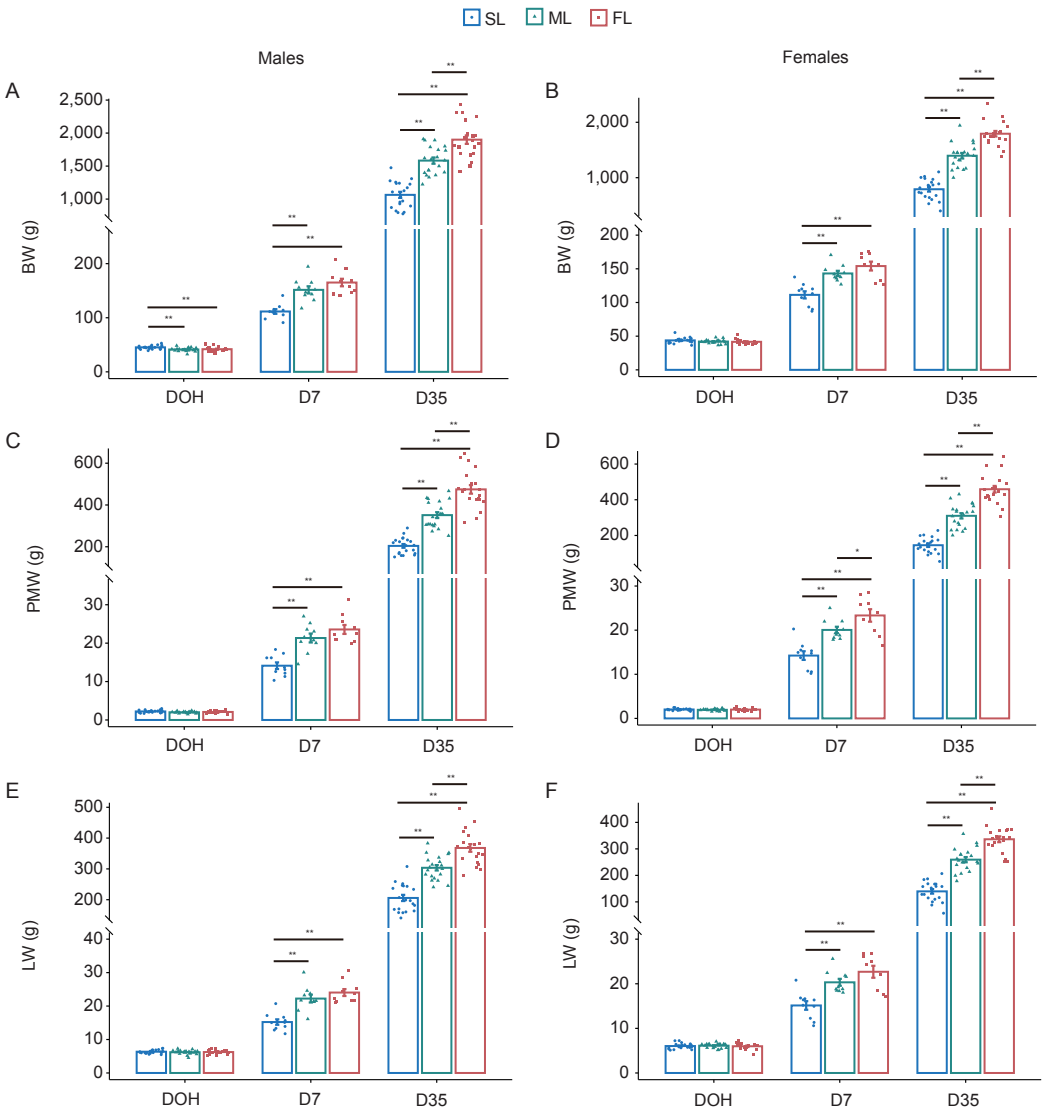


Fig. 2 Growth performance after hatching among broiler chickens with various growth rates. A, changes in body weight (BW) among the males of the slow-growing (SL), medium-growing (ML) and fast-growing (FL) broilers from the day of hatching (DOH) to 35 days of age (D35). B, changes in BW among the females of SL, ML and FL groups from DOH to D35. C, changes in pectoral muscle with sternum weight (PMW) among males of the three groups from DOH to D35. D, changes in PMW among females of the three groups from DOH to D35. E, changes in leg (drumstick with thigh) weight (LW) among males of the three broiler groups from DOH to D35. F, changes in LW among females of the three broiler groups from DOH to D35. The sample sizes of males and females for each group at DOH, D7 and D35 were 20, 10, and 20, respectively. One-way analysis of variance was employed for statistical comparisons among multiple experimental groups, and intergroup differences were tested with LSD multiple comparisons. **, extremely significant differences between groups (P<0.01).

in weight results at D35 among the three experimental groups became more pronounced, and the BW of the FL groups was significantly higher than that of the ML group (P<0.01), and the ML group was significantly higher than the SL group (P<0.01) (Table 1). Furthermore, the BW of males and females of the FL group showed no significant difference at (1,900.51±286.10) g and (1,793.54±221.96) g, respectively. The BW of males in the ML group was significantly higher than that of females (P<0.05). However, the BW of the SL group was (1,064.27±205.82) g, which was significantly higher than the BW of females at (791.48±188.92) g (P<0.01). The differences in PMW and LW among the three groups were consistent with BW, showing that FL group developed faster than the ML and SL groups (P<0.01) (Fig. 2-C–F). In addition, the PMW of the SL group showed a significant difference between males and females (P<0.01), while the differences between males and females in the other two groups were not significant (Table 1). For LW, the males of the SL and ML groups were significantly higher than the females (P<0.01), but the difference in the FL group was not significant (Table 1).

3.3. Rapid growth of broilers based on the total number of muscle fibers

Quantification of TNM and CSA for the three experimental groups was performed using the MyoV image processing

Table 1 Growth performance of broilers after hatching

Sex	Day[1)]	Trait (g)[2)]	Breed[3)]	N	Mean±SD	CV (%)	Minimum	Maximum
Male	DOH	BW	SL	20	45.26±3.39 A	7.49	39.21	52.90
			ML	20	41.28±3.36 B	8.15	32.57	48.39
			FL	20	41.87±4.21 B	10.06	34.20	50.90
		PMW	SL	20	2.22±0.34	15.35	1.70	2.98
			ML	20	2.00±0.23	11.27	1.50	2.45
			FL	21	2.09±0.31	14.89	1.52	2.84
		LW	SL	20	6.36±0.54	8.52	5.48	7.39
			ML	20	6.20±0.63	10.13	4.53	7.29
			FL	21	6.26±0.62	9.93	5.14	7.40
	D7	BW	SL	10	111.56±13.50 B	12.10	91.03	141.15
			ML	10	151.53±20.94 A	13.82	117.47	194.57
			FL	10	165.07±21.87 A	13.25	140.50	208.28
		PMW	SL	10	14.13±2.72 B	19.24	10.31	18.36
			ML	10	21.34±3.66 A	17.17	14.54	26.99
			FL	10	23.57±3.68 A	15.62	19.99	31.48
		LW	SL	10	15.22±2.57 B	16.91	11.73	20.73
			ML	10	22.24±3.70 A	16.63	16.13	30.09
			FL	10	24.04±3.26 A	13.56	21.01	30.58
	D35	BW	SL	20	1,064.27±205.82 C	19.34	776.90	1,475.00
			ML	20	1,583.14±211.24 B	13.34	1,223.50	1,909.60
			FL	20	1,900.51±286.10 A	15.05	1,419.20	2,439.20
		PMW	SL	20	204.12±39.74 C	19.47	151.20	289.80
			ML	20	351.44±62.77 B	17.86	252.70	467.00
			FL	20	474.71±91.68 A	19.31	315.80	644.80
		LW	SL	20	205.74±44.75 C	21.75	140.80	307.80
			ML	20	303.59±39.14 B	12.89	240.80	383.20
			FL	20	367.98±55.56 A	15.10	277.20	497.40
Female	DOH	BW	SL	20	43.74±4.06	9.28	36.42	55.41
			ML	20	42.01±2.95	7.02	36.69	48.32
			FL	19	41.62±3.71	8.91	36.95	53.00
		PMW	SL	20	2.03±0.22	10.81	1.59	2.48
			ML	20	1.95±0.21	11.02	1.56	2.35
			FL	19	2.00±0.31	15.49	1.47	2.79
		LW	SL	20	6.04±0.62	10.29	5.12	7.25
			ML	20	6.12±0.51	8.33	5.15	7.06
			FL	19	5.99±0.72	12.00	4.11	7.22
	D7	BW	SL	10	111.32±16.78 B	15.07	87.01	137.84
			ML	10	143.10±12.44 A	8.69	126.90	170.58
			FL	9	154.09±19.69 A	12.78	127.06	175.15
		PMW	SL	10	14.24±3.11 B	21.80	10.19	20.26
			ML	10	20.05±2.30 Ab	11.45	17.84	25.03
			FL	9	23.33±4.25 Aa	18.23	16.63	28.61
		LW	SL	10	15.14±3.05 B	20.13	10.60	20.81
			ML	10	20.33±2.46 A	12.09	18.03	25.61
			FL	9	22.69±4.02 A	17.74	17.04	26.81
	D35	BW	SL	20	791.48±188.92 C	23.87	400.40	1,104.40
			ML	20	1,396.37±223.42 B	16.00	1,000.10	1,941.90
			FL	20	1,793.54±221.96 A	12.38	1,375.50	2,345.10
		PMW	SL	20	145.47±43.81 C	30.12	54.60	228.10
			ML	20	310.07±66.52 B	21.45	200.90	431.00
			FL	20	459.04±82.68 A	18.01	305.70	643.20
		LW	SL	20	139.66±38.05 C	27.25	57.20	206.80
			ML	20	259.32±43.70 B	16.85	178.60	356.80
			FL	20	336.31±49.11 A	14.60	252.80	454.00

[1)] DOH, day of hatching; D7 and D35, 7 days old and 35 days old, respectively.
[2)] BW, body weight; PMW, pectoral muscle with sternum weight; LW, leg (drumstick with thigh) weight.
[3)] SL, ML, and FL, the slow-growing, medium-growing and fast-growing, respectively.
All values are expressed as mean±SD of *n* observations. Different capital letters (A, B, C) denote extremely significant differences between groups (P<0.01), while different lower-case letters (a, b) denote significant differences between groups (P<0.05).

software. Visual examples of the segmentation results at D35 indicated the good performance in predicting the muscle fibers and the reliability of the TNM and CSA results (Fig. 3-A). To avoid redundancy and potential errors, muscle fibers located along the right and bottom edges of each image tile were excluded from detection, thereby ensuring that no muscle fibers situated at the peripheries of the image tiles of WSIs were counted more than once. At D7, the TNM values of pectoral muscle in the FL and ML groups were 693,568.00±54,169.80 and 652,122.00±65,822.60, both significantly more than the TNM of 539,778.57± 40,722.94 in the SL group (P<0.01) (Table 2). Like D7, the between-group differences at D35 showed that the TNM of the FL group was significantly higher than that of the SL group (P<0.01), and the TNM of the ML group was also significantly higher than that of the SL group (P<0.05) (Fig. 3-B).

There were certain differences in TNM between males and females in the SL and FL groups at D35. The TNM of males in the SL group was 569,788.27±77,254.13, which was significantly higher than the 457,931.89±84,687.28 of females (P<0.01) (Table 3). The TNM of males of the FL group (696,958.80±43,900.37) was significantly higher than that of females (629,071.07±52,591.79) (P<0.01). The speed of muscle fiber hypertrophy in broiler chickens after hatching also varied among the groups. The CSA values of the FL and ML groups at D7 were (125.17±19.76) μm^2 and (111.18±19.15) μm^2, which were significantly higher than that of the SL group at (84.54±21.00) μm^2. By D35, the differences among the three experimental groups were all highly significant (P<0.01) (Fig. 3-C). Significant differences in CSA between males and females were only observed in the SL group (P<0.05), while the differences were not significant in the other two groups (Table 3).

3.4. Histological characteristics of muscle fibers are closely related to growth traits

The BW of broilers across the three experimental groups at D7 showed strong positive correlations with PMW, TNM and CSA (Fig. 4-A–C). The strengths of these relationships were quantified by the Pearson correlation coefficient, which revealed a notably high correlation between BW and TNM (R=0.89) and a slightly lower but still significant correlation between BW and CSA (R=0.78).

Extending the observations to D35, which had a greater sample size, the

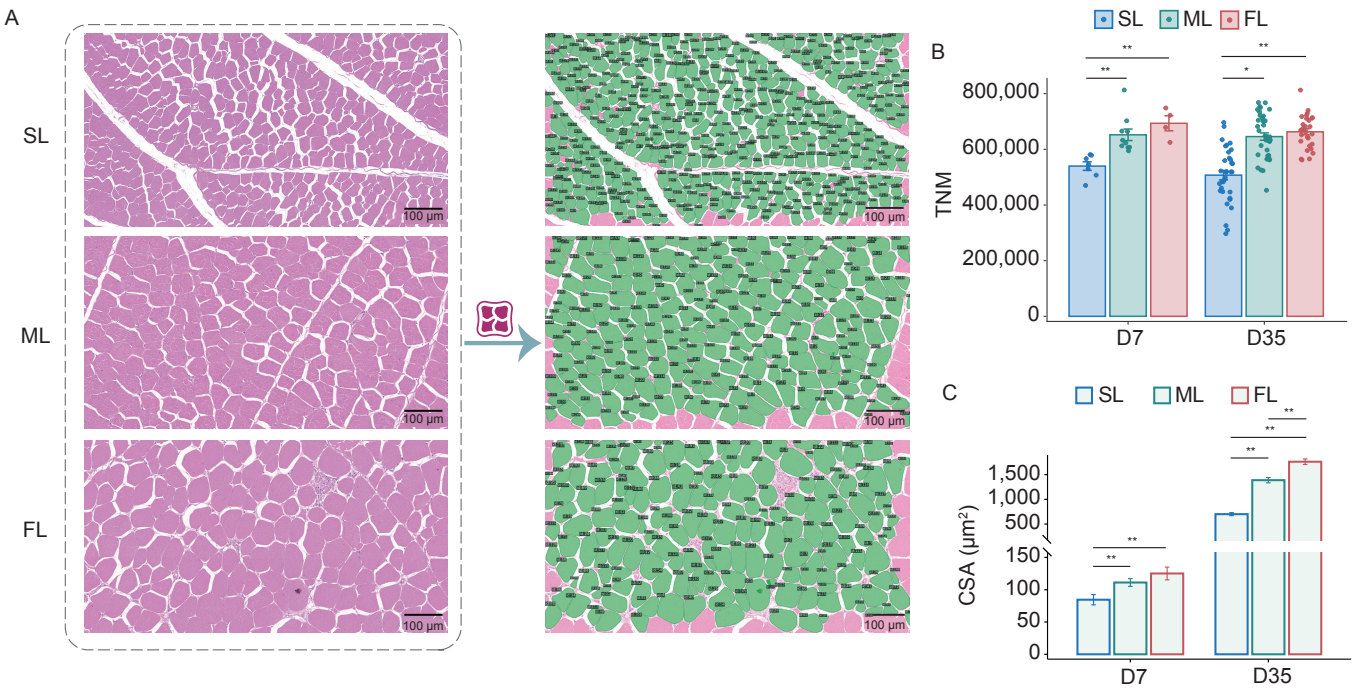


Fig. 3 Histological analysis of different broiler chickens. A, visualized results of the slow-growing (SL), medium-growing (ML) and fast-growing (FL) broilers at 35 days of age (D35). The images on the left are the original images and the images on the right are the pictures processed with MyoV software. The green boxes represent the predicted muscle fibers. Scale bar=100 μm. B, total number of muscle fibers (TNM) in pectoral muscles among different broiler breeds at D7 and D35. C, cross-sectional area (CSA) of muscle fibers among the three experimental groups at D7 and D35. The sample sizes of SL, ML, and FL groups at D7 were 7, 10, and 4, respectively, while those at D35 were 34, 37, and 30, respectively. One-way analysis of variance was employed for statistical comparisons among multiple experimental groups, and intergroup differences were tested with LSD multiple comparisons. **, extremely significant differences between groups (P<0.01); *, significant differences between groups (P<0.05).

Table 2 Characteristics of muscle fibers at 7 days of age (D7)

Trait[1)]	Breed[2)]	N[3)]	Mean±SD	CV (%)	Minimum	Maximum
TNM	SL	7	539,778.57±40,722.94 B	7.54	470,300	580,874
	ML	10	652,122.00±65,822.60 A	10.09	594,560	813,086
	FL	4	693,568.00±54,169.80 A	7.81	624,720	748,486
CSA (μm²)	SL	7	84.54±21.00 Bb	24.84	54.25	114.17
	ML	10	111.18±19.15 ABa	17.23	73.80	146.37
	FL	4	125.17±19.76 A	15.79	101.72	148.80

[1)] TNM, total number of muscle fibers; CSA, cross-sectional area.
[2)] SL, ML, and FL, slow-growing, medium-growing and fast-growing, respectively.
[3)] The 7 individuals of SL group included 4 males and 3 females; the 10 individuals of ML group were 4 males and 6 females; the 4 individuals of FL group were all males.
Different capital letters (A, B) denote extremely significant differences between groups (P<0.01), while different lower-case letters (a, b) denote significant differences between groups (P<0.05).

correlations were further analyzed. A strikingly strong correlation was identified between BW and PMW, with a correlation coefficient of 0.98 (Fig. 4-D). Furthermore, the correlations between BW and both TNM and CSA exhibited some differences from those observed at D7. The correlation coefficient between BW and TNM was moderately reduced to 0.73, while the correlation between BW and CSA was strengthened to 0.87 at D35 (Fig. 4-E and F). These findings underscore the dynamic nature of the relationships between broiler body weight and muscle characteristics at different developmental stages.

4. Discussion

Muscle fiber number and size are the main determinants of skeletal muscle mass, and the histological characteristics of muscle fibers are closely related to the meat shear force and tenderness in livestock and poultry (Xia *et al.* 2017; Qi *et al.* 2019). Muscle fiber characteristics such as muscle fiber diameter and density are often used to assess morphology and development in studies on muscle development and meat quality (Yao *et al.* 2016). However, the local statistical histological characteristics of muscle fibers cannot adequately represent individual animals due to the limited number of

Table 3 Characteristics of muscle fibers at 35 days of age (D35)

Breed[1)]	Trait[2)]	Sex	N	Mean±SD	CV (%)	Minimum	Maximum
SL	TNM	M	15	569,788.27±77,254.13 A	13.56	446,588	696,336
		F	19	457,931.89±84,687.28 B	18.49	296,896	589,794
	CSA (μm²)	M	15	780.27±189.28 a	24.26	415.61	1,069.66
		F	19	637.60±147.61 b	23.15	419.70	917.46
ML	TNM	M	19	670,675.05±73,103.65	10.90	533,146	768,066
		F	18	619,511.67±80,873.16	13.05	452,888	753,428
	CSA (μm²)	M	19	1,406.53±288.16	20.49	852.78	1,932.54
		F	18	1,367.8±363.71	26.59	785.44	1,881.35
FL	TNM	M	15	696,958.80±43,900.37 A	6.30	618,726	812,830
		F	15	629,071.07±52,591.79 B	8.36	561,940	739,984
	CSA (μm²)	M	15	1,748.22±279.46	15.99	1,420.98	2,438.02
		F	15	1,773.03±343.86	19.39	1,275.15	2,745.43

[1)] SL, ML, and FL, the slow-growing, medium-growing and fast-growing, respectively.
[2)] TNM, total number of muscle fibers; CSA, cross-sectional area.
Different capital letters (A, B) denote extremely significant differences between groups (P<0.01), while different lower-case letters (a, b) denote significant differences between groups (P<0.05).

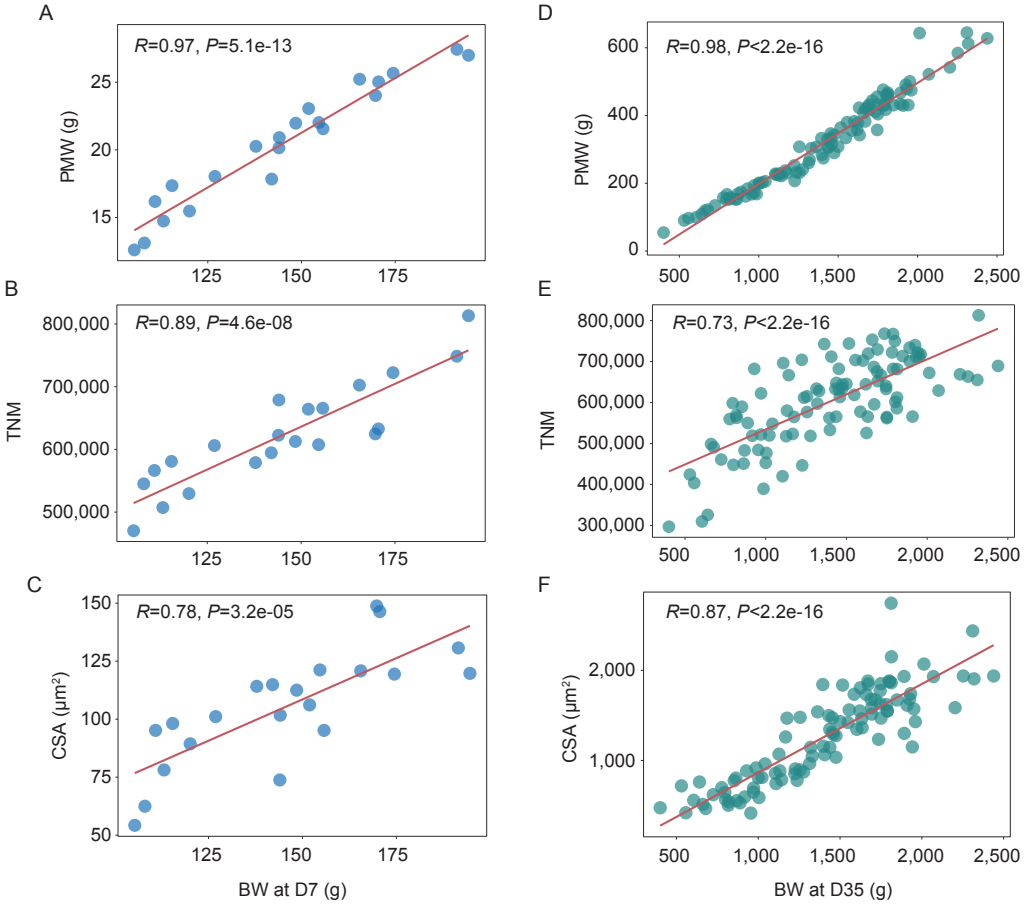


Fig. 4 Correlations between growth performance and the histological properties of muscle fibers. A, Pearson correlation coefficients between body weight (BW) and pectoral muscle with sternum weight (PMW) at 7 days of age (D7) (R=0.97, P=5.1e-13, n=21). B, Pearson correlation coefficients between BW and total number of muscle fibers (TNM) at D7 (R=0.89, P=4.6e-08, n=21). C, Pearson correlation coefficients between BW and cross-sectional area (CSA) at D7 (R =0.78, P=3.2e-05, n=21). D, Pearson correlation coefficients between BW and PMW at D35 (R=0.98, P<2.2e-16, n=101). E, Pearson correlation coefficients between BW and TNM at D35 (R=0.73, P<2.2e-16, n=101). F, Pearson correlation coefficients between BW and CSA at D35 (R=0.87, P<2.2e-16, n=101). Each point represents a sample from one individual.

local visual fields for the statistics. The TNM provides the most effective phenotypic data for muscle development. Nevertheless, a previous study estimated the total number of pectoral muscle fibers by multiplying the average density of muscle fibers obtained from five local views by the CSA of the pectoral muscle in chickens, resulting in an approximately 25% difference from the true value (Gu *et al.* 2024). The pectoral muscle of FL broiler chickens accounts

for 25% of the body weight, with the pectoral major muscle being its primary muscular component (Wen *et al.* 2017; Leskovec *et al.* 2019). The leg muscle comprises various smaller skeletal muscles with distinct functions, such as the gastrocnemius, tibialis anterior, and soleus muscles. Therefore, this study used the panoramic image processing method and tool we previously developed to accurately quantify the growth performance and total number of pectoral major muscle fibers in chickens with various growth rates. In addition, to ensure accuracy and representativeness, the CSA of muscle fibers was determined by selecting 100 image tiles, each containing more than 100 muscle fibers, thereby providing a comprehensive assessment of muscle fiber size and distribution.

Muscle fibers are formed through the fusion of mononuclear myoblasts during embryonic development (Dormoy-Raclet *et al.* 2013), with E12-E16 being a critical period when myoblasts actively fuse with both each other and myotubes to form muscle fibers (Kimura and Yoshioka 2014; Singh *et al.* 2018). Consequently, this study started with the determination of chick embryo weights among different chicken breeds from E14. The results revealed that the three chicken breeds exhibited different growth potentials during the embryonic period. This might be explained by the fact that the genetic selection of chickens with divergent growth rates resulted in considerable differences in the mechanisms of development and metabolism during embryogenesis (Buzała *et al.* 2015). This suggests varying rates and quantities of myoblast fusion and muscle fiber establishment in different broiler embryos. Interestingly, the growth rates of all three experimental groups declined in the late embryonic stage, possibly due to the energy provided by pectoral muscles for the body. There are two main types of myofibers: type I myofibers and type II myofibers, of which type II myofibers are type IIa, IIb and IIx (Wang *et al.* 2018). The mitochondrial contents and oxidative capabilities of type I and IIa myofibers are relatively high, while glycolytic metabolism is predominant in type IIb and IIx myofibers (Wang *et al.* 2022). Among the muscle fibers found in chicken pectoral muscle, only the type IIb myofibers that rely on glycolysis for energy provision are present (Xiong *et al.* 2020; Chodová *et al.* 2021). The greater metabolic rates of modern embryos may deplete embryonic nutrient reserves in the prenatal period because of insufficient nutrient reserves (Yair *et al.* 2013). Such embryos need the mobilization of protein from the pectoral muscle to provide energy for the organism through glycolysis/gluconeogenesis and protein processing, resulting in slower development and growth rates of the pectoral muscle (Peng *et al.* 2018). These findings indicate that the quantity of chicken muscle fibers may depend on the strong fusion of myoblasts before E16. Although the muscle fiber size may decrease during the late embryonic stage, the number of muscle fibers remains constant.

The growth performance of the three chicken breeds exhibited significant differences with the extension of the feeding period (P<0.01), and differences between males and females were observed at D35. Generally, male broilers show faster growth and achieve a higher final body weight compared to female broilers (Duangnumsawang *et al.* 2022). This could be related to the production of testosterone in the testes. Testosterone is one of the drivers of sex differences, and it has the effects of protein anabolism and promotion of muscle mass development (Zhao *et al.* 2008; Ohtsuki *et al.* 2019). The number of muscle fibers is determined primarily before birth, while the diameter of myofibers increases after birth (Li *et al.* 2016). The number and size of skeletal muscle fibers are principally reflected in muscle growth and development, thereby directly affecting meat quality (Li *et al.* 2021). However, as far as we know, no existing studies have quantified the skeletal muscle fiber number precisely in poultry. Thus, we provide a precise quantitative assessment of the TNM and CSA of pectoral muscles among broiler breeds with various growth rates for the first time.

This study confirmed findings on the consistent differences of TNM among the three broiler breeds at two time points after hatching. Specifically, the TNM in the pectoral muscle of the FL group was the highest, reaching approximately 700,000, and exceeding the TNM in the ML group of around 650,000. In contrast, the TNM in the SL group was the lowest, at only around 500,000. In addition, the differences in CSA among the experimental groups were in accordance with TNM, with the CSA of FL group being the largest and that of SL group being the smallest. This was consistent with previous reports that broilers have a larger CSA than local chickens and layers (Huo *et al.* 2022; Kim *et al.* 2022). These results directly demonstrate that the characteristics of rapid muscle mass and growth rates in broiler chickens are associated with greater numbers and faster hypertrophy of skeletal muscle fibers. Furthermore, the BW of broiler chickens has a stronger relationship with TNM at D7 and with CSA at D35. The number of muscle fibers is thought to remain constant after birth (Gu *et al.* 2016), unless there is muscle regeneration after injury (Kim *et al.* 2021). Broiler chickens exhibit rapid development after D21, accompanied by hypertrophy of the muscle fibers (Wu *et al.* 2020). Combining the growth performance of broiler chickens with the histological characteristics of muscle fibers, our results suggest that the quantity of muscle fibers forms the foundation for the growth and development of broiler chickens. To a certain extent, the increase in muscle fiber number plays a more significant role in enhancing muscle mass compared to the effect of CSA.

5. Conclusion

This study has pioneered the precise quantification of growth performance in broiler chickens with different growth rates, including the TNM, thereby filling a gap in the accurate determination of total muscle fiber count. The results reveal that FL broiler chickens have a greater number of muscle fibers in the skeletal muscles with a faster rate of hypertrophy. The findings directly demonstrate the significance of muscle fiber quantity in relation to muscle mass and muscle development, thereby providing crucial data support for future studies on muscle fiber development.

Acknowledgements

We thank Beijing Huadu Yukou Poultry Industry Co., Ltd.,

China for providing the experimental fertile eggs of WOD168, WOD178 and WOD188. This work was supported by the Key Research and Development Program of Hainan Province, China (ZDYF2023XDNY036) and the National Key Research and Development Program of China (2022YFF1000204).

Declaration of competing interest

The authors declare that they have no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that they did not use AI in the preparation and writing of this manuscript.

Ethical approval

All experimental procedures involving the broilers used in this study were conducted under the guidelines established by the Ministry of Science and Technology of China. Ethical approval was granted by the Animal Welfare Committee of China Agricultural University (AW71802202-1-2) and the experiments were conducted following the protocols outlined in the "Guide for Care and Use of Laboratory Animals" of China Agricultural University.

References

Ahmadipour B, Khajali F. 2019. Expression of antioxidant genes in broiler chickens fed nettle (*Urtica dioica*) and its link with pulmonary hypertension. *Animal Nutrition*, **5**, 264–269.

Buzała M, Janicki B, Czarnecki R. 2015. Consequences of different growth rates in broiler breeder and layer hens on embryogenesis, metabolism and metabolic rate: A review. *Poultry Science*, **94**, 728–733.

Cheng H, Song S, Kim G D. 2021. Frozen/thawed meat quality associated with muscle fiber characteristics of porcine longissimus thoracis et lumborum, psoas major, semimembranosus, and semitendinosus muscles. *Scientific Reports*, **11**, 13354.

Cheng S, Liu S, Yu J, Rao G, Xiao Y, Han W, Zhu W, Lv X, Li N, Cai J, Wang Z, Feng X, Yang F, Geng X, Ma J, Li X, Wei Z, Zhang X, Quan T, Zeng S, *et al.* 2021. Robust whole slide image analysis for cervical cancer screening using deep learning. *Nature Communications*, **12**, 5639.

Chodová D, Tůmová E, Ketta M, Skřivanová V. 2021. Breast meat quality in males and females of fast-, medium- and slow-growing chickens fed diets of 2 protein levels. *Poultry Science*, **100**, 100997.

Dormoy-Raclet V, Cammas A, Celona B, Lian X J, van der Giessen K, Zivojnovic M, Brunelli S, Riuzzi F, Sorci G, Wilhelm B T, Di Marco S, Donato R, Bianchi M E, Gallouzi I E. 2013. HuR and miR-1192 regulate myogenesis by modulating the translation of HMGB1 mRNA. *Nature Communications*, **4**, 2388.

Duangnumsawang Y, Zentek J, Vahjen W, Tarradas J, Goodarzi B F. 2022. Alterations in bacterial metabolites, cytokines, and mucosal integrity in the caecum of broilers caused by feed additives and host-related factors. *Frontiers in Physiology*, **13**, 935870.

Fan L, Wang Y, Jiang N, Chen M, Gao L, Li K, Gao Y, Cui H, Pan Q, Liu C, Zhang Y, Wang X, Qi X. 2020. Novel variant infectious bursal disease virus suppresses Newcastle disease vaccination in broiler and layer chickens. *Poultry Science*, **99**, 6542–6548.

Gu J M, Wang D J, Peterson J M, Shintaku J, Liyanarachchi S, Coppola V, Frakes A E, Kaspar B K, Cornelison D D, Guttridge D C. 2016. An NF-κB--EphrinA5-dependent communication between NG2(+) interstitial cells and myoblasts promotes muscle growth in neonates. *Developmental Cell*, **36**, 215–224.

Gu S, Wen C, Li J, Liu H, Huang Q, Zheng J, Sun C, Yang N. 2022. Temporal expression of myogenic regulatory genes in different chicken breeds during embryonic development. *International Journal of Molecular Sciences*, **23**, 10115.

Gu S, Wen C, Xiao Z, Huang Q, Jiang Z, Liu H, Gao J, Li J, Sun C, Yang N. 2024. MyoV: A deep learning-based tool for the automated quantification of muscle fibers. *Briefings in Bioinformatics*, **25**, bbad528.

Hegazy A, Yehia N, Hassan A, El-Saadony M T, Aboelenin S M, Soliman M M, Tolba H. 2021. The potency of newly development H5N8 and H9N2 avian influenza vaccines against the isolated strains in laying hens from Egypt during 2019. *Saudi Journal of Biological Sciences*, **28**, 5310–5316.

Hollandi R, Szkalisity A, Toth T, Tasnadi E, Molnar C, Mathe B, Grexa I, Molnar J, Balind A, Gorbe M, Kovacs M, Migh E, Goodman A, Balassa T, Koos K, Wang W, Caicedo J C, Bara N, Kovacs F, Paavolainen L, *et al.* 2020. NucleAIzer: A parameter-free deep learning framework for nucleus segmentation using image style transfer. *Cell Systems*, **10**, 453–458.

Huo W, Weng K, Li Y, Zhang Y, Zhang Y, Xu Q, Chen G. 2022. Comparison of muscle fiber characteristics and glycolytic potential between slow- and fast-growing broilers. *Poultry Science*, **101**, 101649.

Ju X, Liu Y, Shan Y, Ji G, Zhang M, Tu Y, Zou J, Chen X, Geng Z, Shu J. 2021. Analysis of potential regulatory LncRNAs and CircRNAs in the oxidative myofiber and glycolytic myofiber of chickens. *Scientific Reports*, **11**, 20861.

Kermany D S, Goldbaum M, Cai W, Valentim C, Liang H, Baxter S L, Mckeown A, Yang G, Wu X, Yan F, Dong J, Prasadha M K, Pei J, Ting M, Zhu J, Li C, Hewett S, Dong J, Ziyar I, Shi A, *et al.* 2018. Identifying medical diagnoses and treatable diseases by image-based deep learning. *Cell*, **172**, 1122–1131.

Kim D H, Choi Y M, Lee J, Shin S, Kim S, Suh Y, Lee K. 2022. Differential expression of MSTN isoforms in muscle between broiler and layer chickens. *Animals*, **12**, 539.

Kim H, Kwon Y T, Zhu C, Wu F, Kwon S, Yeo W H, Choo H J. 2021. Real-time functional assay of volumetric muscle loss injured mouse masseter muscles *via* nanomembrane electronics. *Advanced Science*, **8**, e2101037.

Kimura S, Yoshioka K. 2014. Parathyroid hormone and parathyroid hormone type-1 receptor accelerate myocyte differentiation. *Scientific Reports*, **4**, 5066.

Kritikaki E, Asterling R, Ward L, Padget K, Barreiro E, Simoes D. 2021. Exercise training-induced extracellular matrix protein adaptation in locomotor muscles: A systematic review. *Cells*, **10**, 1022.

Leskovec J, Levart A, Perić L, Đukić Stojčić M, Tomović V, Pirman T, Salobir J, Rezar V. 2019. Antioxidative effects of supplementing linseed oil-enriched diets with α-tocopherol, ascorbic acid, selenium, or their combination on carcass and meat quality in broilers. *Poultry Science*, **98**, 6733–6741.

Li H, Wang G, Hao Z, Zhang G, Qing Y, Liu S, Qing L, Pan W, Chen L, Liu G, Zhao R, Jia B, Zeng L, Guo J, Zhao L, Zhao H, Lv C, Xu K, Cheng W, Li H, *et al.* 2016. Generation of biallelic knock-out sheep *via* gene-editing and somatic cell nuclear transfer. *Scientific Reports*, **6**, 33675.

Li M, Zhang N, Zhang W, Hei W, Cai C, Yang Y, Lu C, Gao P, Guo X, Cao G, Li B. 2021. Comprehensive analysis of differentially expressed circRNAs and ceRNA regulatory network in porcine skeletal muscle. *BMC Genomics*, **22**, 320.

De Lima V A, Ceballos M C, Gregory N G, Da C M. 2019. Effect of different catching practices during manual upright handling on broiler welfare and behavior. *Poultry Science*, **98**, 4282–4289.

Ohtsuki M, Nishimura A, Kato T, Sokejima S, Shibata T, Okada H, Nagao-Nishiwaki R, Sudo A. 2019. Relationships between body mass index, lifestyle habits, and locomotive syndrome in young- and middle-aged adults: A cross-sectional survey of workers in Japan. *Journal of Occupational Health*, **61**, 311–319.

Peng M, Li S, He Q, Zhao J, Li L, Ma H. 2018. Proteomics reveals changes in hepatic proteins during chicken embryonic development: An alternative model to study human obesity. *BMC Genomics*, **19**, 29.

Picchiarelli G, Demestre M, Zuko A, Been M, Higelin J, Dieterlé S, Goy M A, Mallik M, Sellier C, Scekic-Zahirovic J, Zhang L, Rosenbohm A, Sijlmans C, Aly A, Mersmann S, Sanjuan-Ruiz I, Hübers A, Messaddeq N, Wagner M, van Bakel N, *et al.* 2019. FUS-mediated regulation of acetylcholine receptor transcription at neuromuscular junctions is compromised in amyotrophic lateral sclerosis. *Nature Neuroscience*, **22**, 1793–1805.

Porporato P E, Filigheddu N, Reano S, Ferrara M, Angelino E, Gnocchi

V F, Prodam F, Ronchi G, Fagoonee S, Fornaro M, Chianale F, Baldanzi G, Surico N, Sinigaglia F, Perroteau I, Smith R G, Sun Y, Geuna S, Graziani A. 2013. Acylated and unacylated ghrelin impair skeletal muscle atrophy in mice. *Journal of Clinical Investigation*, **123**, 611–622.

Qi K, Men X, Wu J, Xu Z. 2019. Rearing pattern alters porcine myofiber type, fat deposition, associated microbial communities and functional capacity. *BMC Microbiology*, **19**, 181.

Raheem M A, Jiangang H, Yin D, Xue M, Rehman K U, Rahim M A, Gu Y, Fu D, Song X, Tu J, Khan I M, Tipu M Y, Qi K. 2021. Response of lymphatic tissues to natural feed additives, curcumin (*Curcuma longa*) and black cumin seeds (*Nigella sativa*), in broilers against *Pasteurella multocida*. *Poultry Science*, **100**, 101005.

Roman W, Martins J P, Carvalho F A, Voituriez R, Abella J, Santos N C, Cadot B, Way M, Gomes E R. 2017. Myofibril contraction and crosslinking drive nuclear movement to the periphery of skeletal muscle. *Nature Cell Biology*, **19**, 1189–1201.

Scheuermann G N, Bilgili S F, Tuzun S, Mulvaney D R. 2004. Comparison of chicken genotypes: Myofiber number in pectoralis muscle and myostatin ontogeny. *Poultry Science*, **83**, 1404–1412.

Shen H, Grimston S, Civitelli R, Thomopoulos S. 2015. Deletion of connexin43 in osteoblasts/osteocytes leads to impaired muscle formation in mice. *Journal of Bone and Mineral Research*, **30**, 596–605.

Singh A J, Chang C N, Ma H Y, Ramsey S A, Filtz T M, Kioussi C. 2018. FACS-Seq analysis of Pax3-derived cells identifies non-myogenic lineages in the embryonic forelimb. *Scientific Reports*, **8**, 7670.

Sultana N, Islam R, Bhakta S, Saha J A, Islam S S, Hashem M A. 2023. Role of Clove and Tulsi on broiler health and meat production. *Saudi Journal of Biological Sciences*, **30**, 103654.

Tavaniello S, Slawinska A, Sirri F, Wu M, De Marzo D, Siwek M, Maiorano G. 2022. Performance and meat quality traits of slow-growing chickens stimulated *in ovo* with galactooligosaccharides and exposed to heat stress. *Poultry Science*, **101**, 101972.

Wang L, Chen M, Xu M, Li J, Feng P, He R, Zhu Y, Li H, Lin J, Zhang C. 2018. Ratio of creatine kinase to alanine aminotransferase as a biomarker of acute liver injury in dystrophinopathy. *Disease Markers*, **2018**, 6484610.

Wang Q, Qiu X, Liu T, Ahn C, Horowitz J F, Lin J D. 2022. The hepatokine TSK maintains myofiber integrity and exercise endurance and contributes to muscle regeneration. *JCI Insight*, **7**, e154746.

Wen C, Jiang X, Ding L, Wang T, Zhou Y. 2017. Effects of dietary methionine on breast muscle growth, myogenic gene expression and IGF-I signaling in fast- and slow-growing broilers. *Scientific Reports*, **7**, 1924.

Weng K, Huo W, Li Y, Zhang Y, Zhang Y, Chen G, Xu Q. 2022. Fiber characteristics and meat quality of different muscular tissues from slow- and fast-growing broilers. *Poultry Science*, **101**, 101537.

Wu S, Chen X, Li T, Ren H, Zheng L, Yang X. 2020. Changes in the gut microbiota mediate the differential regulatory effects of two glucose oxidases produced by *Aspergillus niger* and *Penicillium amagasakiense* on the meat quality and growth performance of broilers. *Journal of Animal Science and Biotechnology*, **11**, 73.

Xia J, Fan H, Chang T, Xu L, Zhang W, Song Y, Zhu B, Zhang L, Gao X, Chen Y, Li J, Gao H. 2017. Searching for new loci and candidate genes for economically important traits through gene-based association analysis of Simmental cattle. *Scientific Reports*, **7**, 42048.

Xiong Y, Fan L, Hao Y, Cheng Y, Chang Y, Wang J, Lin H, Song G, Qu Y, Lei F. 2020. Physiological and genetic convergence supports hypoxia resistance in high-altitude songbirds. *PLoS Genetics*, **16**, e1009270.

Yair R, Shahar R, Uni Z. 2013. Prenatal nutritional manipulation by *in ovo* enrichment influences bone structure, composition, and mechanical properties. *Journal of Animal Science*, **91**, 2784–2793.

Yang Y, He H, Wang J, Chen L, Xu Y, Ge C, Li S. 2023. Blood quality evaluation *via* on-chip classification of cell morphology using a deep learning algorithm. *Lab On a Chip*, **23**, 2113–2121.

Yao Y, Norris E H, Mason C E, Strickland S. 2016. Laminin regulates PDGFRβ(+) cell stemness and muscle development. *Nature Communications*, **7**, 11415.

Yu Q, Tian X, Shao L, Xu L, Dai R, Li X. 2018. Label-free proteomic strategy to compare the proteome differences between longissimus lumborum and psoas major muscles during early postmortem periods. *Food Chemistry*, **269**, 427–435.

Zhang G, Chen F, Wu P, Li T, He M, Yin X, Shi H, Duan Y, Zhang T, Wang J, Xie K, Dai G. 2020. MicroRNA-7 targets the KLF4 gene to regulate the proliferation and differentiation of chicken primary myoblasts. *Frontiers in Genetics*, **11**, 842.

Zhao G P, Cui H X, Liu R R, Zheng M Q, Chen J L, Wen J. 2011. Comparison of breast muscle meat quality in 2 broiler breeds. *Poultry Science*, **90**, 2355–2359.

Zhao W, Pan J, Wang X, Wu Y, Bauman W A, Cardozo C P. 2008. Expression of the muscle atrophy factor muscle atrophy F-box is suppressed by testosterone. *Endocrinology*, **149**, 5449–5460.

Zuidhof M J. 2020. Multiphasic poultry growth models: Method and application. *Poultry Science*, **99**, 5607–5614.

Executive Editor-in-Chief Kui Li
Managing Editor Yanyan Sun