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Gaping conditions of the *Pectoralis minor* (tenders) in commercial broilers: Prevalence, histology, and gene expression

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ABSTRACT

Gaping is a recently described condition that affects the *Pectoralis minor* (tender) muscle of broiler chickens, characterized by post-mortem separation of myofiber that leads to meat depreciation and economic losses. In this study, we aimed at understanding prevalence, morphological features, and transcriptomics signatures of this poorly understood myopathy. Between July 2022 and January 2023, a total of 5,180 chicken tenders were collected from 32 flocks across two plants in the USA, handling light (2.7 kg) and heavy (4.1 kg) birds. The prevalence of moderate and severe gaping was 24.8 % and 53.7 %, respectively. The light bird plant had a lower prevalence of moderate gaping ($P < 0.001$), while the heavy bird plant had a lower prevalence of severe gaping ($P < 0.001$). Spaghetti meat prevalence from 8,000 fillets was 46.9 % for moderate and 8.3 % for severe cases, with no significant inter-plant differences. Use of peracetic acid treatment at the poultry plants significantly increased the prevalence of severe gaping. Physical and histological features, along with gene expression, were evaluated in 120 samples representative of three gaping severity tiers. Severely gaped tenders showed greater width compared to normal and moderately gaped tenders in both light and heavy birds ($P < 0.05$). An increase of 1 cm in tender width was associated with a 1.99-fold increase in the odds of classification into a more severe gaping category (95 % CI: 1.15 – 3.46). Affected muscles revealed histological evidence of myodegeneration, inflammation, and lipidosis with fibrosis. For one-unit increase in the myodegeneration score, samples had a 1.75-fold increase in the odds of being classified into a more severe gaping category (95 % CI: 1.37 – 2.23). Gene expression analysis using droplet digital PCR showed differential expression of 19 genes involved in oxidative stress response, cellular signaling, muscle development, and collagen formation between weight groups and myopathy categories. Notably, 21 out of 22 differentially expressed genes showed higher expression in light birds. This study provides the comprehensive description of gaping in broiler chickens and lays a crucial benchmark for assessment of future mitigating strategies.

Introduction

Broiler production has seen remarkable advancements in growth rate and muscle yield of the birds over the past few decades (Zuidhof et al., 2014; Siegel, 2023). However, this rapid progress has been accompanied by an increase in muscle abnormalities, collectively known as growth-related myopathies, which pose significant challenges to the poultry industry (Kuttappan et al., 2012; Barbut et al., 2024). Among these, woody breast (WB) and spaghetti meat (SM) in the *Pectoralis major* muscle have been well-documented and extensively studied (Mutryn et al., 2015; Abasht et al., 2016; Sihvo et al., 2017; Kuttappan et al., 2017; Papah et al., 2017; Baldi et al., 2018; Velleman et al., 2018; Chen et al., 2019; Wang et al., 2023, 2024). Studies show that the incidence of these myopathies increases with higher slaughter weights (Alnahhas et al., 2016; Santos et al., 2021), and morphological parameters such as breast weight and width tend to increase in breast fillets affected with WB (Dalgaard et al., 2018; Che et al., 2022a). These myopathies are characterized by distinct histological changes in muscle structure and composition, leading to altered meat quality and significant economic losses for producers (Sihvo et al., 2017; Baldi et al., 2019; Barbut, 2019;

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Barbut et al., 2024).

Gaping, also known as feathering, is a quality defect in broiler chicken *P. minor* tender muscles characterized by separations between muscle fiber bundles and tears in the tender. This newly described condition exhibits fiber separation patterns similar to SM and potentially impacts the quality and marketability of chicken tenders (Soglia et al., 2019; Mueller et al., 2023).

The etiology of these myopathies is multifactorial, involving genetic, nutritional, and environmental variables (Petracci et al., 2019). Research suggests that the rapid growth rate and increased muscle mass of modern broiler lines are primary contributing factors (Barbut et al., 2024), and high temperature during grow-out can be a risk factor for increased odds of developing SM and WB (Che et al., 2022b). At the molecular level, these myopathies are associated with alterations in gene expression patterns related to oxidative stress response, energy metabolism, muscle growth regulation, and extracellular matrix composition (Zambonelli et al., 2016; Malila et al., 2019; Lake and Abasht, 2020; Che et al., 2024). Understanding these molecular mechanisms is crucial for developing effective strategies to mitigate the impact of myopathies on poultry production and meat quality.

Despite the growing body of research on WB and SM, there is a lack of information regarding the gaping condition in the tender muscle. This knowledge gap requires a comprehensive investigation of its prevalence, physical characteristics, histological features, and molecular mechanisms. Moreover, comparing the gaping condition with SM in the *P. major,* which exhibits a similar macroscopic appearance, can provide valuable insights into potential shared pathological mechanisms and distinctive features. Thus, the aims of this study are to investigate the gaping condition in US commercial broiler chicken tenders, to examine its prevalence, physical and histological characteristics, and associated morphological features. It also analyzes the expression of 19 key genes related to breast myopathies, compares gaping prevalence in tenders to SM in breast fillets, and evaluates the influence of body weight and seasonal variations on these conditions.

Materials and methods

Sample size calculation and sample collection

No live animals were directly employed for this study and no IACUC was required. To estimate the prevalence of gaping in tenders and SM in breast muscles, every fifth tender and fillet, respectively, were collected (random selection) and scored from the conveyor belt approximately 3 h after slaughter, in the deboning area.

For gaping, the required sample size was calculated as follows:

$$\boldsymbol{n} = \frac{\boldsymbol{Z}^2\boldsymbol{P}(1-\boldsymbol{P})}{\mathrm{d}^2}$$

where n is the sample size, Z is the Z-score corresponding to the desired confidence level (1.96 for 95 % CI), P is the expected prevalence (10 %), and d is the desired precision (5 %). The calculation resulted in a sample size of 138 tenders per flock, which was increased to 140 to account for any potential missing data. The sample size for *P. major* was determined based on our previous study (Che et al., 2022b), which established a required sample size of 250 fillets per flock.

Between July 2022 and January 2023, tenders were randomly sampled (every fifth tender) from two USDA-inspected poultry processing plants on the East Coast, USA. Birds were of the same commercial genetic strain but differed in rearing duration to achieve market weights and in antimicrobial use practices. Specifically, the study evaluated 2,800 tenders from 20 flocks at a heavy bird plant (4.1 kg average live weight; 58 d age; no antibiotics birds) and 1,680 tenders from 12 flocks at a light bird plant (2.7 kg average live weight; 50 d age; organic birds). The two plants used similar transportation duration (approximately one h), lairage time (approximately two h), and line speed (140 birds per min), following USDA guidelines (USDA FSIS, 2022). The no antibiotics protocol was verified through USDA Process Verified Program audits (USDA AMS, 2025), and organic certification adhered to USDA organic standards (USDA AMS, 2023). Most tenders (n = 4,480) were sampled following peroxyacetic acid (PAA) treatment for microbial decontamination, which was carried out according to USDA FSIS regulations (USDA FSIS, 2024), with similar application parameters (i.e., concentration and duration) between the two plants. Additionally, 700 tenders from five flocks at the light bird plant were sampled before PAA treatment. In total, 5,180 tenders were examined.

During the same period, an additional 8,000 fillets were assessed for the presence of SM, consisting of 5,000 fillets from 20 flocks at the heavy bird plant and 3,000 fillets from 12 flocks at the light bird plant after PAA treatment. Following scoring, samples were returned to the production line, except for 120 tenders and 60 fillets, which were used for physical measurements and histology (tenders), and gene expression assessment (tenders and fillets), as described below.

Macroscopic scoring

During each visit, at least two members of the investigative team visited each of the two slaughterhouses and collaboratively scored each sample to minimize scoring variations. In instances where differing scores were recorded, the two scorers re-evaluated the fillets and tenders to reach a consensus. Gaping of tenders was scored according to previously established criteria (Soglia et al., 2019): Score 0 (normal): absence of gaping; Score 1 (moderate): one to two separation points, each less than 1.5 cm in length, or a single separation point between > 1.5 cm in length; Score 2 (severe): three or more separation points of any length, or two or more separation points, each greater than 1.5 cm in length (Fig. 1). The scoring criteria for SM were as follows: score 0 (normal), absence of SM; score 1 (moderate), presence of separation of muscle fibers on the surface of the fillet; score 2 (severe), deeper separation of muscle fibers on the fillets (Che et al., 2022a).

Physical measurements

For obtaining macroscopic measures, a subset of 120 tender samples were collected to represent normal (n = 40), moderate (n = 40), and severe (n = 40) gaping conditions, with half samples from each group derived from the heavy bird plant and half from the light bird plant during the warm season. Tenders were transported to a local laboratory in insulated containers filled with ice within one h of collection from both plants. Upon arrival at the laboratory, tenders were photographed

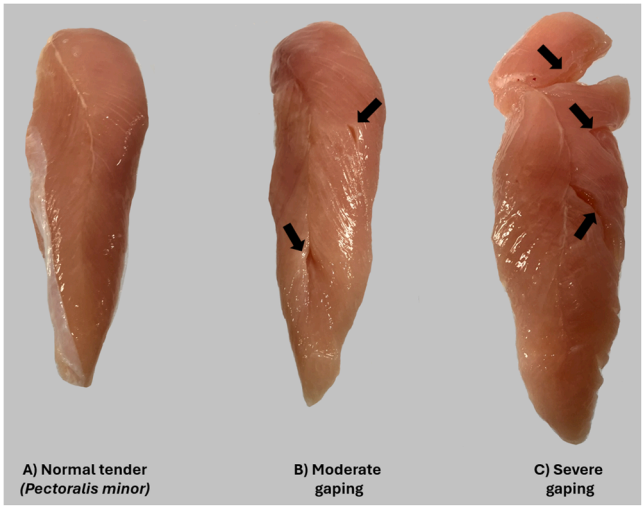

Fig. 1. Representative figure of normal tender (*Pectoralis minor)*, moderate gaping, and severe gaping.

using an iPhone camera and weighed using a digital scale (Model PM600, Mettler Toledo, Columbus, OH, USA). Digital images were analyzed using an image processing software (ImageJ, the National Institutes of Health, Bethesda, MD, USA) to measure surface area, length, and width of each sample, taken along the longest and widest axes of each tender's surface.

Histology

After photographs were obtained, the same tenders were utilized for histological assessment of gaping ($n = 120$). From each sample, a 1-cm^3 core was extracted from the cranial third of the tenders, fixed in 10 % formalin for 24 - 48 h, embedded in paraffin, and processed for routine hematoxylin and eosin (H&E) staining. Histological scoring was performed using a BX45 light microscope (BX45, Olympus Canada, Richmond Hill, ON, CA) with disposable slide-grids (Z688533, Sigma-Aldrich, St. Louis, MO, USA), as previously described (Che et al., 2022a). Briefly, six 4 mm^2 windows (each encompassing four 1 mm^2 squares) were identified in the upper left, upper right, middle right, middle left, lower left, and lower right areas of the tissue. For each of the 24 squares, the microscopic scores assessed myodegeneration, perivascular inflammation (PVI), and endomysial accumulation of fat and / or fibrous tissue (lipidosis/fibrosis, LF) on a scale of 0-3. A final histological score was calculated by adding the individual scores for each of the 24 squares, with a possible range of 0-216. All histological assessments were performed by a veterinarian (SC) blinded to experimental groups.

Droplet digital polymerase chain reaction

Gene expression analysis was conducted for 19 selected genes (Table 1) using droplet digital polymerase chain reaction (ddPCR), following a previously described method (Malila et al., 2019). These genes, associated with the onset of chicken breast myopathies, broadly represent four diverse biological processes: five genes involved in oxidative stress response, nine in cellular signaling pathways, four in muscle development, and one in collagen production.

A total of 48 samples were analyzed, representing four distinct myopathy groups (normal breast, severe SM, normal tender, and severe gaping) across two body weight categories (2.7 kg and 4.1 kg). Six specimens were selected for each myopathy group within each weight category. Fillets and tenders were obtained from the same cohort previously utilized for physical and histological assessments. Subsequent to scoring at processing plants, tissue samples (approximately 2 mg) were immediately collected and preserved in RNAlater for gene expression analysis. The muscle samples were shipped to the Food Biotechnology

Table 1
Primers designed for EVAGREEN-based droplet digital polymerase chain reaction.

Gene ID	Gene Annotation	NCBI Accession number	Sequence (5′ → 3′) (F: forward, R: reverse)	Amplicon length (bp)	Template quantity (ng/20 uL reaction)
Oxidative stress response					
HIF1A	Hypoxia inducible factor 1 subunit alpha	XR_001466725.2	F: ATCAGAGTGGTTGTCCAGCAG R: CAGTCCAAGCCCACCTTACT	111	25
SOD1	Superoxide dismutase 1	NM_205064.1	F: AAGGGAGGAGTGGCAGAAGTA R: CGAGGTCCAGCATTTCCAGTT	158	10
SOD2	Superoxide dismutase 2, mitochondrial	NM_204211.1	F: TGTATCAGTTGGTGTTCAAGGAT R: AGCAATGGAATGAGACCTGTT	129	10
SOD3	Superoxide dismutase 3, extracellular	XM_015285700.1	F: TACAAACCCAACCTCTTCGC R: GTTATTGCCCTTGCCCATGT	102	10
GSTM2	Glutathione S-transferase mu 2	NM_205090.1	F: GTGGACTTCCTGGCTTACGA R: GCCGTGTACCAGAAAATGG	173	10
Cellular signalling					
mTOR	Mechanistic target of rapamycin	XM_417614.6	F: GTTGGTTTGGGTTTCCAGGAC R: GATCAGGGCAATAATCCATCTGC	156	10
TGFB1	Transforming growth factor beta 1	NM_001318456.1	F: GACGATGAGTGGCTCTCCTTC R: GTGCTTCTTGGCAATGCTCT	195	10
PRKAA1	5′-AMP-activated protein kinase alpha-1 catalytic subunit	DQ302133.1	F: GAATGGATGGGACTTCTTGCC R: GGAATTTCTGGACTGAAGCCAA	116	25
PRKAA2	5′-AMP-activated protein kinase alpha-2 catalytic subunit	DQ340396.1	F: CAACCCTGAACCCATTCTTTTG R: TGTCTCATTCTCCTCCTTGCT	139	10
PRKAB2	5′-AMP-activated protein kinase beta-2 non-catalytic subunit	NM_001044662.1	F: CCATCCTGCTGTCCCATTATAC R: GAGCAAGGAAAGGCTGTTCTG	129	10
PRKAG3	5′-AMP-activated protein kinase gamma-3 non-catalytic subunit	NM_001031258.2	F: GCCAACTCCTGTCAACCTCG R: GGTGCCTACTGAGCATCCCT	97	10
LKB1	Liver kinase B 1 (Serine/threonine kinase 11)	NM_001045833.1	F: GGTCCTCCACTCTCAGACCTA R: GAGGTATGGGCACCAGAGTC	136	10
CAMKK2	Calcium/calmodulin dependent protein kinase kinase 2	XM_025155529.1	F: AAATCTCTTCGGTTCCCTGTC R: TTAACTCCACGGTCAGCACT	158	25
LITAF	Lipopolysaccharide-induced tumor necrosis factor-alpha factor	NM_204267.1	F: ACTATCCTCACCCCTACCCTGTC R: TGTTGGCATAGGCTGTCCTG	156	25
Muscle development and repair					
IGF1	Insulin like growth factor 1	NM_001004384	F: TCTCAACATCTCACATCTCT R: AAGCAGCACTTAACTAATTGT	135	10
MYOD1	Myogenic differentiation 1	NM_204214.2	F: AGGAAACCTGAGTGACAGTGG R: GACCTGCCTTTATAGCACTTGG	121	1
MYF5	Myogenic factor 5	NM_001030363.1	F: TGAACCAAGCATTCGAGACC R: AGTAGTTCTCCACCTGTTCCCT	141	10
MSTN	Myogenic differentiation 1 (myostatin)	NM_001001461.1	F: GAAACTTGACATGAACCCAGGC R: TGACAGCAAGATCTCGTCCAG	143	10
Collagen and connective tissue					
COL3A1	Collagen type 3, alpha 1	NM_205380.3	F: GCCAATTTCCAAAGCACTGGT R: TGACTTAGCCCTGTTTCTAGCC	128	1

Research Team (BIOTEC, Pathum Thani, Thailand) with a storage temperature kept below −20°C. Upon arrival, total RNA was isolated using TriReagent (Molecular Research Center, Inc., Cincinnati, OH, USA), followed by a DNase treatment (Promega Corporation, Madison, WI, USA) at 37 °C for 30 min, and purification using a column-based GeneJET RNA Cleanup and Concentration Micro Kit (Thermo Scientific, Inc., Rockford, IL, USA). Concentration and integrity of total RNA samples were determined using a Nanodrop spectrophotometer (Thermo Scientific, Inc.), and a 5200 Fragment Analyzer system (Agilent, Santa Clara, CA, USA), respectively. Only the samples with RNA quality number greater than 7.5 (RQN > 7.5) were proceeded to cDNA synthesis. Total RNA (1.5 μg) was converted into cDNA using The ImPromII Reverse Transcription System kit (Promega, Madison, WI, USA) with an oligo(dT) as a primer. The synthesized cDNA was then quantified using the Nanodrop spectrophotometer. The absolute abundance of transcripts was determined using specific primer sets for each gene, which spanned amplicons between 97 and 195 bp (Table 1). These primers were designed using GenBank (The National Center for Biotechnology Information, NCBI) sequences and the Primer-BLAST software. The potential for primer-dimer formation was assessed using OligoAnalyzer, and primers were selected based on a GC content of 40 – 60 %, a melting temperature of 50 – 65°C, and a ΔG (free energy) > –5 for the most stable estimated dimer.

To perform ddPCR, 20 μL-reaction contained 1X EVAGREEN supermix (Bio-Rad, Hercules, CA, USA), 0.25 μM of each forward and reverse primer, and 1 to 25 ng of cDNA template, depending on the target genes (Table 1). An equal volume of nuclease-free water was added instead of the cDNA for the no template control. A droplet emulsion of 20,000 nL-sized droplets was created using a QX100 droplet generator (Bio-Rad). Forty microliters of the droplets were then transferred to a 96-well plate and amplified using a standard thermocycler (T100 thermal cycler, Bio-Rad). The cycling conditions were as follows: initial enzyme activation at 95°C for 5 min, 40 cycles of denaturation at 95°C for 30 s and annealing/extension at 58°C for 1 min, followed by signal stabilization at 4°C for 5 min and 90°C for 5 min. After amplification, the intensity of the fluorescent signal from the droplets was measured using a QX200 droplet reader (Bio-Rad) with the assistance of the QuantaSoft droplet reader software (Bio-Rad). This allowed for the calculation of the initial concentration of targets, the expression of copies per 20 μL of reaction volume, and the calculation of copies per ng of template.

Statistical analysis

Descriptive statistics

Statistical analyses were conducted using R software (version 4.3.2, R Core Team, 2023) for computation and GraphPad Prism (version 10.3.1 for Windows) for visualization, respectively. Chi-square tests were employed to evaluate statistical differences in the overall prevalence of moderate and severe gaping, as well as moderate and severe SM, across plants from all 32 flocks. Spearman's rank correlation coefficient was employed to assess the relationship between the prevalence of severe gaping and SM across 32 flocks. The Mann-Whitney rank sum test was applied to test seasonal differences in severe SM and gaping prevalence between cold and warm season from 32 flocks. To compare the prevalence of gaping before and after PAA treatment, the Chi-square test with Bonferroni adjustment was used to analyze the prevalence of moderate and severe gaping in a subset of 5 flocks from the light bird plant.

Physical characteristics were compared using two-way analysis of variance (ANOVA) with gaping severity and body weight as factors. Data normality was confirmed using the Shapiro-Wilk test. When ANOVA indicated significant differences ($p < 0.05$), Tukey's post-hoc test with Holm's correction was performed for multiple comparisons. Differences in histological scores among gaping severity groups were analyzed using the Scheirer-Ray-Hare test followed by Dunn's post-hoc test with Holm's correction, due to non-normal distribution of the data (as determined by Shapiro-Wilk test). Gene abundance differences between light (2.7 kg) and heavy birds (4.1 kg) across the four myopathy categories were evaluated using Scheirer-Ray-Hare test followed by Dunn's post-hoc test with Holm's correction, due to non-normal distribution of the data. For all analyses, statistical significance was established at $p < 0.05$.

Exploratory statistics

A multivariable ordered logistic regression analysis was conducted to assess the association between the severity of gaping on tenders and potential risk factors. The severity of gaping, categorized as normal, moderate, or severe, served as the ordinal outcome variable. Potential risk factors included physical parameters (area, length, weight, and width of tenders) and histological scores of myodegeneration, perivascular infiltration, lipidosis/fibrosis, and sum of the histological score of tenders, taken separately.

Univariable screening was performed to identify variables demonstrating unconditional significance with a relaxed p-value of 0.2 for inclusion in the multivariable model. To mitigate multicollinearity in the multivariable models, pairwise correlation coefficients among all significant independent variables were examined using Spearman's rank test. In cases where two variables exhibited high correlation (rho > 0.70; $P < 0.05$), the more statistically significant P-value (i.e., smaller P-value) was selected for inclusion in the multivariable model. Subsequently, multivariable analysis was conducted using a manual backward elimination process. Variables with $P > 0.05$, as determined by the likelihood ratio test, were then removed to enhance model fitness.

Results

Prevalence

The prevalence of moderate and severe gaping in chicken tenders across both plants was 24.8 % (95 % CI: 23.6 – 26.0 %) and 53.7 % (95 % CI: 52.4 – 55.1 %), respectively (n = 5,180). At the heavy bird plant, which processed birds averaging 4.1 kg, moderate gaping was observed in 29.3 % (95 % CI: 27.7 – 31.0 %) and severe gaping in 38.4 % (95 % CI: 36.6 – 40.2 %) of samples. At the light bird plant, which processed birds averaging 2.7 kg, showed 19.4 % (95 % CI: 17.9 – 21.1 %) moderate and 71.8 % (95 % CI: 70.0 – 73.6 %) severe gaping. Chi-square tests revealed significant differences between the plants: the light bird plant had a lower prevalence of moderate gaping ($P < 0.001$), while the heavy bird plant had a lower prevalence of severe gaping ($P < 0.001$).

The prevalence of moderate and severe SM across both plants was 46.9 % (95 % CI: 44.2 – 49.6 %) and 8.3 % (95 % CI: 7.5 – 9.2 %), respectively. In the heavy bird plant, moderate SM was observed in 22.8 % (95 % CI: 21.6 – 24.0), while severe SM affected 8.3 % (95 % CI: 7.6 – 9.1). The light bird plant showed similar results, with 24.1 % (95 % CI: 22.6 – 25.6) moderate SM and 8.2 % (95 % CI: 7.3 – 9.3) severe SM. Statistical analysis indicated no significant difference in the prevalence of either moderate or severe SM (Chi-square test; $P = 0.139$ and 0.867, respectively) between the plants.

The prevalence of severe SM (n = 8,000) and severe gaping (n = 5,180) was further stratified by seasons and processing weights, with distribution presented in Fig. 2 and Supplementary Table 1. Across all seasons (Fig. 2A), severe SM ranged from 1.6 % to 27.6 %, whereas the prevalence of severe gaping varied more widely, spanning from 11.4 % to up to 80.0 % in certain flocks (Supplementary Table 1). The plot revealed a very weak inverse correlation (Spearman's rho = –0.052) between severe SM and severe gaping (Fig. 2A), which however was not significant ($P = 0.775$). During the cold season, severe SM prevalence was generally lower than in the warm season; however, their difference was not significant (Mann-Whitney rank sum test; $P = 0.318$). Similarly, seasonality did not significantly affect severe gaping prevalence (Mann-Whitney rank sum test: $P = 0.108$).

During the cold season (Fig. 2B), light birds (2.7 kg, green circles)

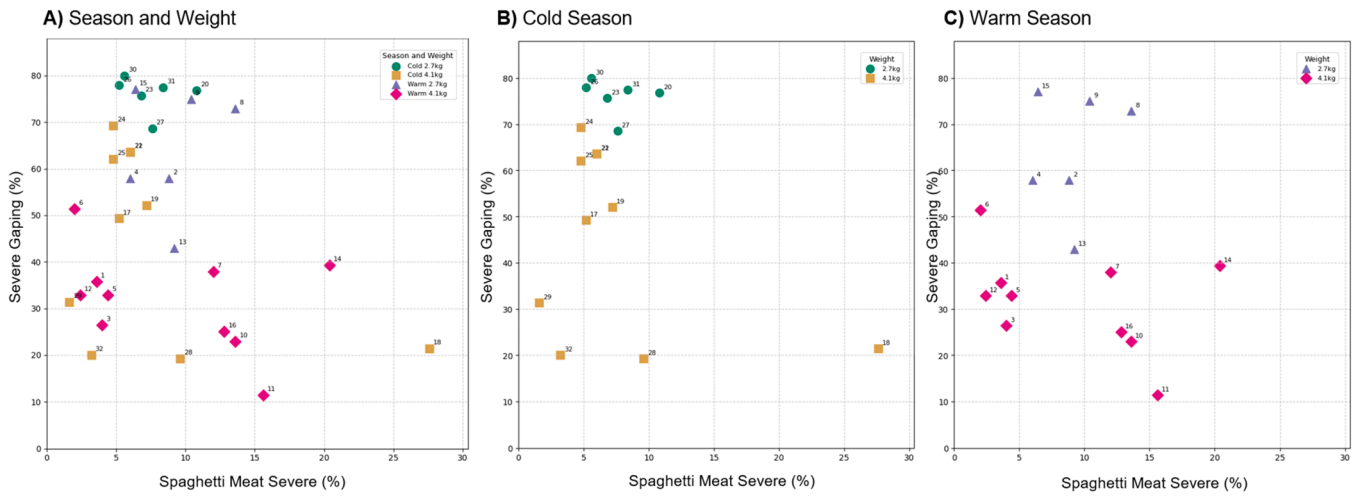

Fig. 2. Scatterplot of flock-level prevalence of severe spaghetti meat (SM, x-axis) versus severe gaping (y-axis), in flocks evaluated across two seasons (July 2022 to January 2023) and stratified by processing weights. (**A**) All seasons combined; (**B**) Cold season (October 2022 - January 2023); (**C**) Warm season (July - September 2022). Markers represent: green circles - cold season, 2.7 kg chickens; gold squares - cold season, 4.1 kg chickens; purple triangles - warm season, 2.7 kg chickens; pink diamonds - warm season, 4.1 kg chickens. Note: Flocks #21 and #22 overlap in Panels A and B.

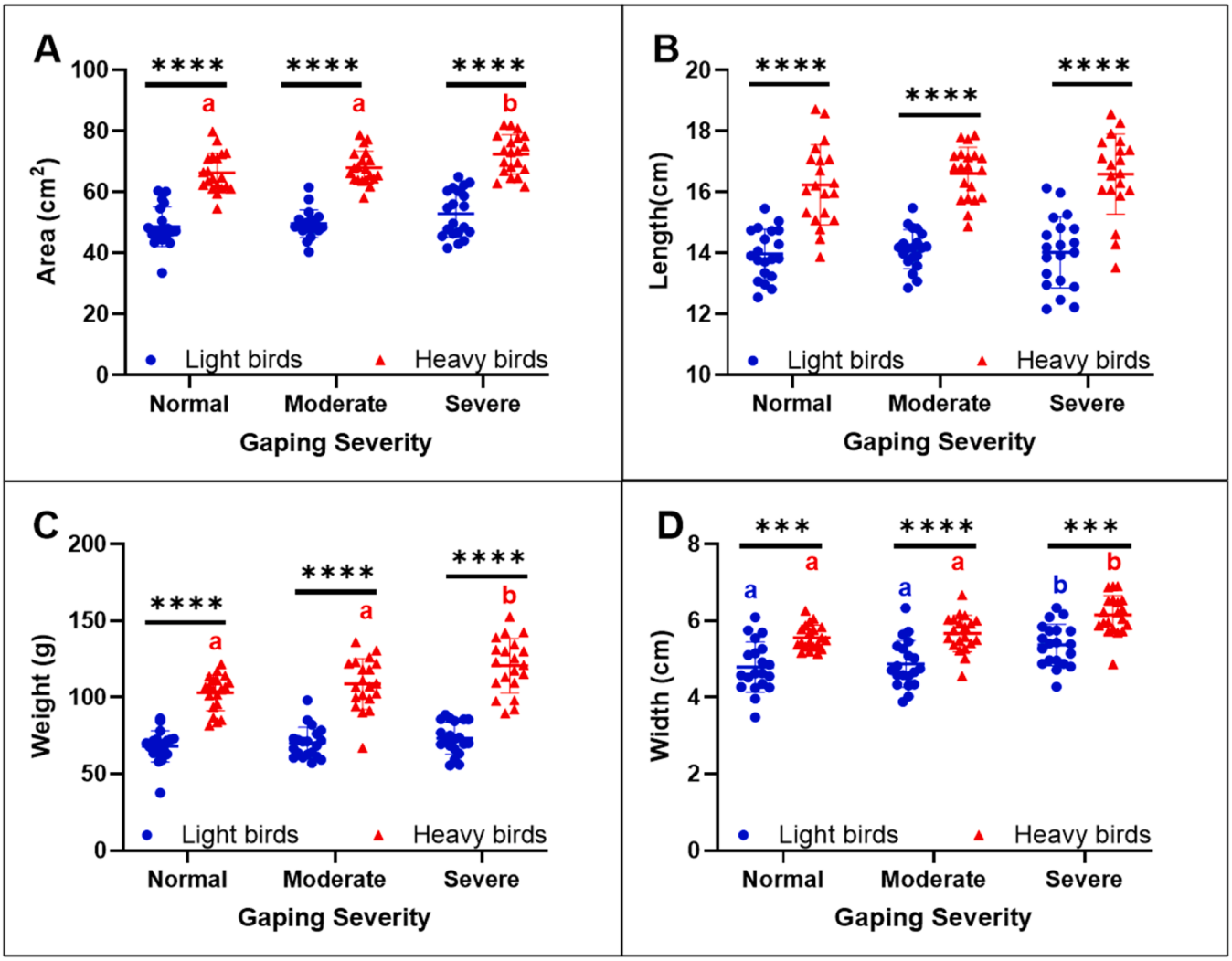

Fig. 3. Physical characteristics of *Pectoralis minor* tender muscles in relation to gaping severity in broiler chickens. Measurements of (A) area (cm^2), (B) length (cm), (C) weight (g), and (D) width (cm) were compared between light (2.7 kg) and heavy (4.1 kg) birds across three gaping severity categories ($n = 20$ per group, total N = 120). Asterisks indicate significant differences between weight groups (***$p < 0.001$, ****$p < 0.0001$) and different lowercase letters denote significant differences among gaping severity groups within each weight category (Two-way ANOVA followed by Tukey's post-hoc test with Holm's correction, $p < 0.05$).

showed a distinct pattern characterized by high percentages of severe gaping (68.6 – 80 %) combined with low percentages of severe SM (5.2 – 10.8 %). Notably, heavy birds tended to exhibit lower percentage of severe gaping compared to light birds in this season (Mann-Whitney rank sum test: $P = 0.001$). During the warm season (Fig. 2C), a similar pattern was observed, with light birds showing high percentages of severe gaping (Mann-Whitney rank sum test: $P = 0.001$). However, no statistical differences were observed in the prevalence of severe SM between light and heavy birds (Mann-Whitney rank sum test: $P = 0.815$).

In a subset of 5 flocks ($n = 1,400$) sampled from the light bird plant, the prevalence of moderate gaping decreased from 17.3 % (95 % CI: 14.7 – 20.3 %) to 13.0 % (95 % CI: 10.7 – 15.7 %) as assessed in samples before and after PAA treatment, although this difference was not significant (Chi-square test; $P = 0.025$, Bonferroni adjusted significance level: 0.025). Conversely, the prevalence of severe gaping significantly increased from 71.0 % (95 % CI: 67.5 – 74.2 %) to 81.3 % (95 % CI: 78.2 – 84.0 %) after PAA treatment (Chi-square test; $P < 0.001$).

Physical measurements and histological findings

The physical parameters of tender muscles, including area, length, weight, and width, were significantly greater in heavy birds (4.1 kg) compared to light birds (2.7 kg) across all gaping severity categories ($P < 0.001$, Fig. 3). Severely gaped tenders showed a greater area (Fig. 3A) compared to normal tenders in both light birds ($P = 0.08$) and heavy birds ($P = 0.007$). Similarly, severely gaped tenders had significantly higher weights (Fig. 3C) compared to both normal and moderately gaped tenders in heavy birds ($P < 0.001$ and $P = 0.013$, respectively). Additionally, severely gaped tenders showed greater width (Fig. 3D) compared to normal and moderately gaped tenders in both light birds ($P = 0.002$, $P = 0.01$, respectively) and heavy birds ($P = 0.002$ and $P = 0.013$, respectively). No interaction was observed between the weight and gaping severities.

Histological analysis revealed that tender tissues displayed changes of varying severity, which affected the myofibers, such as myodegeneration and necrosis, or the interstitium, such as inflammation, lipid infiltration, and fibrosis, or both. The degeneration and necrosis of myofibers were characterized by the loss of structural integrity, centralization of nuclei, and small round fibers (Fig. 4A). Inflammation was characterized by endomysial accumulation of lymphocytes, macrophages, and scattered heterophils (Fig. 4B). The interstitial lesions were characterized by the accumulation of loose connective tissue, which in some areas was associated with adipocytes, expanding the endomysium and occasionally replacing myofibers (Fig. 4C).

Histological evaluation of tender tissues revealed differences between light and heavy broiler chickens. The median myodegeneration score was significantly higher in heavy birds compared to light birds ($P = 0.003$, Table 2). Myodegeneration showed means scores of 2.2 ± 4.0 in light birds and 2.7 ± 3.6 in heavy bird, with maximum observed scores of 20 and 24, respectively. Perivascular infiltration showed means scores of 0.4 ± 0.8 in light birds and 0.7 ± 2.4 in heavy bird, with maximum observed scores of 5 and 18, respectively. Lipidosis & fibrosis showed means scores of 1.4 ± 1.8 in light birds and 1.6 ± 1.9 in heavy

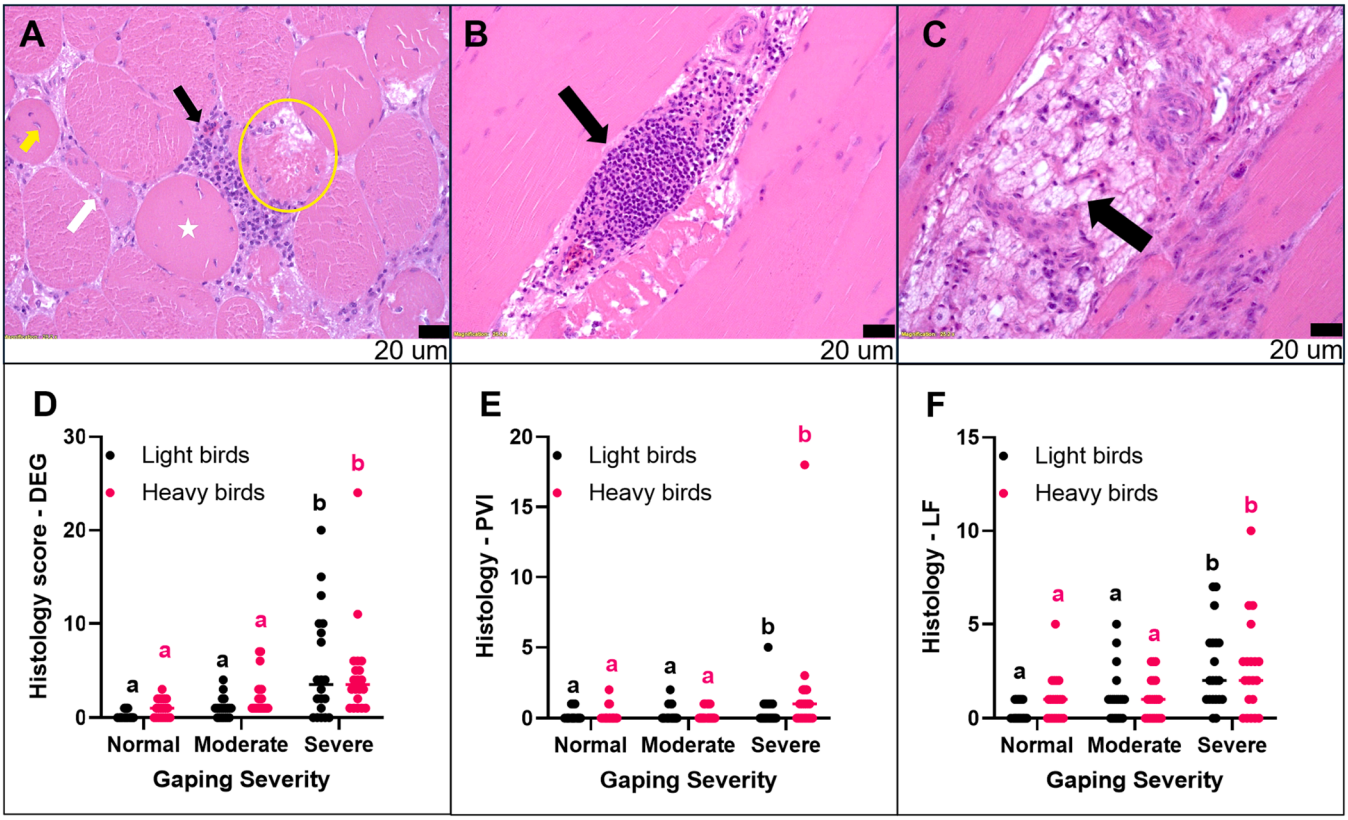

Fig. 4. Histopathological features of *Pectoralis minor* (tender) muscle associated with macroscopic gaping severity in (2.1 kg) and heavy (4.1 kg). broiler chickens. (A-C) Representative hematoxylin and eosin-stained sections (scale bars: 20 μm). (A) Myodegeneration characterized by cytoplasmic vacuoles and floccular necrosis (yellow circle), inflammatory cell infiltration in the interstitium (black arrow), rounded fiber morphology (white star), fiber size variation (white arrow), and centralized nuclei (yellow arrow) (B) Perivascular inflammatory cell infiltration (black arrow). (C) Fibrofatty tissue accumulation (black arrow). (D-F) Plots with median values comparing histological scores between light (2.7 kg) and heavy (4.1 kg) broiler chickens across gaping conditions ($n = 20$ per group, total N = 120): (D) myodegeneration (DEG), (E) perivascular infiltration (PVI), and (F) lipidosis and fibrosis (LF). Different lowercase letters above data points indicate statistically significant differences between gaping severity groups within each body weight category (Scheirer-Ray-Hare test followed by Dunn's post-hoc test with Holm's correction; $p < 0.05$). No statistical differences were observed between body weight groups in any histological category.

Table 2
Histopathological evaluation of *Pectoralis minor* muscles from broiler chickens (n = 120) of two weight classes: light (2.7 kg, n = 60) and heavy (4.1 kg, n = 60). Each sample was scored for myodegeneration, perivascular infiltration, and lipidosis & fibrosis.

Histology	Plant	Min	Mean	Median	Max	SD
Myodegeneration	Light bird	0	2.2	1.0[a]	20	4.0
	Heavy bird	0	2.7	1.5[b]	24	3.6
Perivascular infiltration	Light bird	0	0.4	0.0	5	0.8
	Heavy bird	0	0.7	0.0	18	2.4
Lipidosis & fibrosis	Light bird	0	1.4	1.0	7	1.8
	Heavy bird	0	1.6	1.0	10	1.9
Sum	Light bird	0	4.0	2.0	22	5.5
	Heavy bird	0	4.9	3.0	52	7.1

[a,b] Median values followed by different superscript letters indicate significant differences (P = 0.003, Mann-Whitney rank-sum test).

bird, with maximum observed scores of 7 and 10, respectively. The sum of all histological parameters showed higher median values in heavy birds (3.0) compared to light birds (2.0), with considerable variation in both groups (SD = 7.1 and 5.5, respectively).

When stratified by macroscopic gaping severity, myodegeneration scores were significantly higher in severely gaped tenders compared to normal ($P < 0.001$) and moderate ($P = 0.017$) tenders in light birds. In heavy birds, severely gaped tenders showed significantly higher myodegeneration scores compared to normal tenders ($P < 0.001$; Fig. 4D). For perivascular infiltration, severely gaped tenders showed significantly higher histology scores compared to normal tenders in heavy birds ($P = 0.025$; Fig. 4E). For lipids & fibrosis accumulation, both light and heavy bird groups showed significantly higher scores in severely gaped tenders compared to normal ones with $P < 0.001$ and $P = 0.007$, respectively (Fig. 4F). However, no significant differences in histological scores were observed when comparing between weight categories within each gaping severity group. No interaction effect was detected between weight and gaping severities.

Differential expression of genes involved in oxidative stress, cellular signaling, muscle development and repair, and collagen and connective tissue

Absolute transcript abundance of 19 genes across four biological pathways, i.e., oxidative stress response, cellular signaling, muscle development and repair, and collagen and connective tissue, were quantified in breast and tender muscle samples by ddPCR (Fig. 5). When considering the transcript abundance between the two-body weight groups (i.e., 2.7 and 4.1 kg) within the same myopathic category, significant differences were seen in 22 (28.9 %) out of 76 comparisons (4 myopathic categories × 19 genes). Of the 22 differentially expressed genes, 21 exhibited higher abundance in the light birds. Conversely, only one gene, *COL3A* in severely gaped tenders showed elevated expressions in the heavy birds, due to an outlier. Specifically, regarding the oxidative stress pathway (Fig. 5A), significantly higher expression was observed in light compared to heavy birds for the following genes: *H1F1A* in normal breast fillets ($P = 0.008$), *SOD1* in severe SM ($P = 0.016$) fillets, *SOD2* in severely gaped tenders ($P = 0.004$), *SOD3* in both normal fillets ($P = 0.004$) and severely gaped tenders ($P = 0.005$), and *GSTM2* in severe SM fillets ($P = 0.025$) and severely gaped tenders ($P = 0.007$). For the cellular signaling pathway (Fig. 5B), significant upregulation was observed for *mTOR* in both normal fillets ($P = 0.004$) and severely gaped tenders ($P = 0.004$), and *TGFB* in the severely gaped tenders ($P = 0.016$). *PRKAB2* and *PRKAG3* were both upregulated in the severely gaped tenders in light birds compared to the heavy birds ($P = 0.037$), while no significant differences were noted for *PRKAA1* and *PRKAA2*. An increased *LKB1* abundance was observed in severely gaped tenders in light birds ($P = 0.004$) while the abundance of *CAMKK2* showed no significant differences between the body weight groups. *LITAF* was significantly higher in the normal fillets from the light compared to the heavy birds ($P = 0.025$).

In the muscle development and repair pathway (Fig. 5C), *MSTN* abundance was significantly higher in light birds compared to heavy birds across multiple tissue types: normal breast ($P = 0.025$), severe SM ($P = 0.016$), and severely gaped tender ($P = 0.01$). Similarly, *MYOD1* abundance was significantly higher in light birds' severely gaped tenders ($P = 0.004$). *IGF1* was significantly higher in light birds for both SM ($P = 0.015$) and normal tenders ($P = 0.025$). Regarding collagen and connective tissue pathway (Fig. 5D), *COL3A1* abundance was significantly higher in the normal breast fillets of light birds compared to heavy birds ($P = 0.004$).

When gene abundance was compared among the four myopathy categories within the same age group, significant differences were observed in all four biological pathways. In the oxidative stress response pathway (Fig. 5A), *SOD3* demonstrated significant upregulation in normal tenders compared to severely gaped tenders in heavy birds ($P = 0.005$). In the cellular signaling pathway (Fig. 4B), *mTOR* abundance was significantly higher in severely gaped tenders compared to normal tenders in light birds ($P = 0.021$). In heavy birds, severely gaped tenders exhibited significantly elevated *mTOR* abundance compared to SM breast fillets ($P = 0.002$). *PRKAA2* exhibited increased expression in normal tenders compared to severely gaped tenders in the heavy birds ($P = 0.015$). *LKB1* showed elevated expression in normal breast fillets compared to severely gaped tenders in heavy birds ($P = 0.006$). Also, SM affected breast showed elevated expression compared to severely gaped tenders in heavy birds ($P = 0.039$). *LITAF* demonstrated elevated expression in SM breast fillets compared to normal breast fillets ($P = 0.047$) in heavy birds. In the muscle development and repair category (Fig. 4C), *MYOD* abundance was significantly higher in normal breast fillets compared to SM fillets in heavy birds ($P = 0.04$). Lastly, analysis of collagen and connective tissue formation pathway revealed that *COL3A1* expression was significantly elevated in severely gaped tenders compared to both normal tenders ($P = 0.002$) and normal breast in light birds ($P = 0.041$)

Associations between macroscopic severity of gaping and physical & histological scores

Univariable ordered logistic regression analysis revealed several significant associations between predictor variables gaping severity in tenders (Supplementary Table 2). Muscle area (OR = 1.03, 95 % CI: 1.01 – 1.07, $P = 0.038$), weight (OR = 1.01, 95 % CI: 1.00 – 1.03, $P = 0.036$), and width (OR = 2.56, 95 % CI: 1.54 – 4.25, $P < 0.001$) were all positively associated with increased gaping severity. Additionally, all four histological parameters showed significant associations: myodegeneration (OR = 1.85, 95 % CI: 1.45 – 2.37, $P < 0.001$), perivascular infiltration (OR = 2.24, 95 % CI: 1.26 – 4.00, $P = 0.006$), lipidosis and fibrosis (OR = 1.89, 95 % CI: 1.43 – 2.49, $P < 0.001$), the sum of the histological scores (OR = 1.48, 95 % CI: 1.28 – 1.72, $P < 0.001$). All these were fed into the final model, and subsequent multivariable regression analysis identified two significant predictors: tender width and histology myodegeneration score (Table 3). For each centimeter increase in width of tenders, the odds of the muscle being classified in a more severe gaping category (from normal to moderate, or moderate to severe) increase by a factor of 1.99 (95 % CI: 1.15 – 3.46), holding other variables constant. For each one-unit increase in the myodegeneration score, the odds of the muscle being classified in a more severe gaping category increase by a factor of 1.75 (95 % CI: 1.37 – 2.23), holding other variables constant.

Discussion

This study investigated the prevalence of gaping in commercial broilers from the USA's east coast, one of the nation's top chicken-producing regions, and examined physical and histological

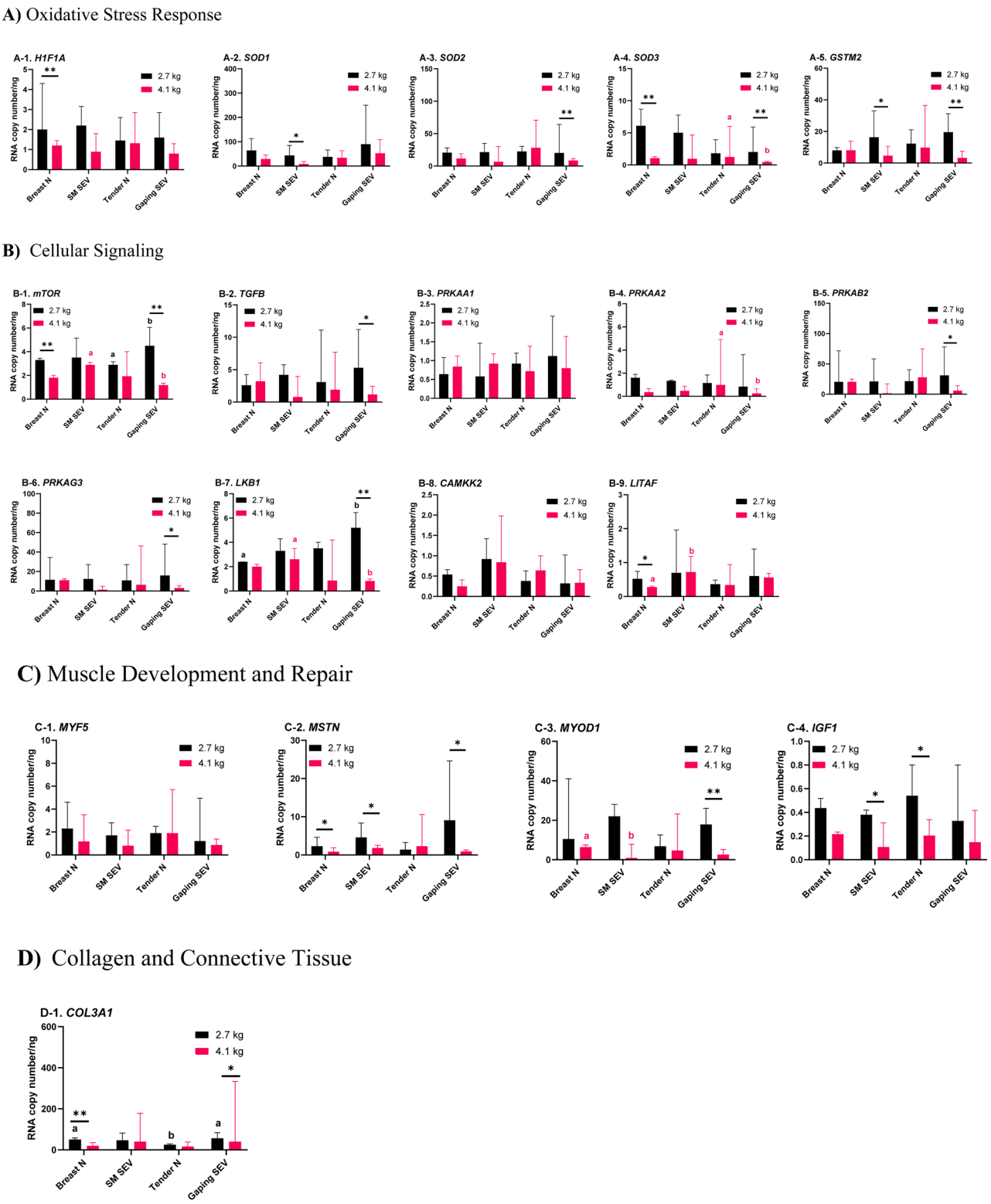

Fig. 5. Differential gene expression (RNA copies / ng of cDNA template) in the *Pectoralis major* (breast) and *Pectoralis minor* (tender) muscles of broiler chickens with two body weights (2.7 kg vs. 4.1 kg) and four myopathy conditions (Breast N: normal breast, SM SEV: severe spaghetti meat, Tender N: normal tender, Gaping SEV: severe gaping). The expression levels of 19 genes were analyzed using ddPCR and categorized into four groups: **(A)** Oxidative Stress Response, **(B)** Cellular Signaling, **(C)** Muscle Development and Repair, and **(D)** Collagen and Connective Tissue. Data are presented as median with interquartile range.
Statistical analysis was performed using Scheirer-Ray-Hare test to evaluate main effects of body weight (2 levels), myopathy condition (4 levels), and their interaction. Asterisks denote significant differences between body weight groups (*$p < 0.05$, **$p < 0.01$). When significant main effects or interactions were detected, Dunn's post-hoc test with Holm's correction was performed for pairwise comparisons. Different letters indicate significant differences between myopathy conditions ($p < 0.05$).

Table 3
Results of a multivariable ordered logistic regression model assessing the relationship the severity (normal, moderate, severe) of gaping in *pectoralis minor* tender muscles as outcome variable with physical parameters and histology score ($n = 120$).

Predictor	Type of variable	Value	Odds Ratio	Standard error	Z score	P value	95 % CI
Width (cm)	Continuous	3.5-6.9	1.99	0.56	2.44	0.015	1.15-3.46
Histology DEG	Continuous	0-24	1.75	0.22	4.48	<0.001	1.37-2.23

Histology DEG: Histology score of myodegeneration.
95 % CI: 95 % Confidence interval.

characteristics of the affected tender muscles. The additional aims included comparing the prevalence of gaping and SM, defining associations between morphological features and the severity of these conditions, and obtaining a better understanding at the molecular level of the pathogenesis through gene expression analysis across both breast and tender muscles.

The present study identifies a notably high prevalence of severe gaping (54.7 %) within the examined poultry population, highlighting its likely substantial impact on US poultry production. This prevalence is markedly higher than that reported among Italian broilers, where severe gaping was observed in only 8 % of cases (Soglia et al., 2019). Such discrepancy may be attributed to differences in genetic strains and processing procedures between the regions. The notable variation in gaping prevalence between US and Italian populations requires further investigation into the multifactorial etiology of this myopathy.

Chicken tenders derived from *P. minor* are currently marketed at higher prices compared to chicken breast fillets, making them a highly profitable segment of the poultry market (USDA AMS, 2024). However, the precise market value of tenders, as well as the economic impact of gaping on this product, has not been estimated in detail. Economic losses of severe gaping are likely a combination of decreased aesthetic appeal and compromised meat quality. Soglia and colleagues reported that severely gaped tenders tend to lose more water during cooking, have softer texture after marination, and present weaker water retention properties compared to normal tenders (Soglia et al., 2019). Considering that economic losses from broiler breast myopathies are estimated to range between 200 million (Kuttappan et al., 2016) and $1 billion annually in the USA (Barbut, 2019), the high prevalence of severe gaping could similarly impose significant financial burdens on the poultry industry. Future studies should focus on examining the magnitude of the economic impact caused by gaping, with a focus on meat quality parameters.

Our findings reveal an inverse relationship between body weight and the prevalence of severe gaping in broiler chickens. Birds with lower body weights (2.7 kg, 50 d) exhibited a higher prevalence of severe gaping compared to heavier birds (4.1 kg, 58 d). This pattern is similar to the prevalence of SM, which peaks at intermediate weights (2.4 - 2.9 kg) before declining as live weight increases further (Mueller et al., 2023). The similarity in these patterns suggests that gaping in the tenders may be analogous to SM, potentially arising from a comparable mechanism of muscle fiber separation.

The prevalence of moderate and severe SM was approximately 30 %, with severe SM at 8.3 % across both plants, indicating its ongoing significance in the poultry industry. These findings align with a previous Canadian study (Che et al., 2022b). Unlike gaping, which exhibited statistically significant differences between the two plants, SM prevalence remained consistent across both facilities. This lack of variation in SM incidence is noteworthy, especially considering that previous studies have reported a peak in SM prevalence at body weights up to 2.9 kg (Mueller et al., 2023). The absence of significant differences in SM prevalence suggests that factors contributing to SM may be more universally present or less influenced by the variables that affect gaping.

Our findings indicate a trend towards higher SM prevalence in the warm season; however, this difference did not reach statistical significance ($P > 0.05$). Previous research has identified elevated ambient temperatures during the grow-out period as a potential risk factor for these conditions (Che et al., 2022b). This discrepancy emphasizes the complex nature of myopathy pathogenesis in broiler chickens, influenced by factors such as genetic predisposition, environmental conditions within poultry housing, nutritional strategies, age and growth rate, and potential subclinical manifestations that may not be captured by our assessment methods.

Our investigation into the effects of PAA treatment on chicken tenders revealed a significant increase in severe gaping post-treatment. PAA is widely used as a disinfectant in food processing due to its strong oxidizing properties, which effectively inactivate microorganisms by disrupting their cell membranes (Kitis, 2004). While PAA is approved for direct food contact in wash water applications (USDA FSIS, 2024), its effects on meat quality, particularly in the context of emerging broiler myopathies, require further investigation, focusing on understanding the mechanisms by which PAA interacts with myopathic muscle tissue and assessing any potential impacts on meat quality and consumer acceptance.

Physical measurements demonstrated that tender muscles with greater width were associated with increased odds of exhibiting the gaping myopathy. This aligns with previous research on tender muscles affected by SM (Che et al., 2022a). It's important to note that while our statistical model demonstrates a correlation, it does not establish causation. The increased width in the affected muscles may be attributed to the characteristic softness of myopathic tissue (Baldi et al., 2021; Wu et al., 2024), resulting from a loss of structural integrity and reduced cohesion between muscle fiber bundles. Consequently, affected muscles tend to flatten and expand laterally.

Histological examination revealed that tender muscles affected by the gaping myopathy exhibit myodegeneration, perivascular inflammatory cell infiltration, lipidosis, and fibrosis. In addition, the myodegeneration severity was positively correlated with the likelihood of gaping myopathy occurrence. These findings are consistent with previous studies on WB, another myopathy affecting *P. major* (Papah et al., 2017; Chen et al., 2019). The histological similarities between gaping myopathy in tender muscles and WB in breast fillets suggest potential common underlying mechanisms, which may inform future research on prevention and treatment strategies for both conditions.

The upregulation of oxidative stress genes (*H1F1A, SOD1, SOD2, SOD3* and *GSTM2*) in light birds compared to heavy birds across myopathy groups suggests the enhanced oxidative stress management in light birds. Higher expression of *SOD* isoforms (*SOD1, SOD2, SOD3*) indicates enhanced antioxidant capacity (Fukai and Ushio-Fukai, 2011). *HIF-1* mediates survival under hypoxic conditions by regulating genes involved in reducing reactive oxygen species production and increasing oxygen availability (Papandreou et al., 2006). Elevated *H1F1A* suggests better adaptation to hypoxic conditions. The concurrent upregulation of *GSTM2*, a critical enzyme regulating cellular antioxidant and detoxification processes (Jin et al., 2022), suggests a coordinated response to oxidative stress. The results suggest that lighter birds possess a more robust and efficient antioxidant defense system, potentially serving as a protective mechanism against oxidative damage. However, the observed decrease in *SOD3* levels in severely gaped tenders in heavy birds suggests a marked downregulation of antioxidant defense mechanisms in this condition, potentially contributing to increased oxidative stress and structural abnormalities.

The expression patterns of cellular signaling genes reveal distinct

regulatory mechanisms between light and heavy birds. In light birds, the upregulation of *mTOR*, a central regulator of cellular metabolism and protein synthesis (Shaw, 2009; Xu and Velleman, 2023), along with energy-sensing kinases *PRKAB2* and *PRKAG3*, suggests enhanced metabolic adaptation and energy homeostasis (Tamargo-Gómez and Mariño, 2018). Additionally, the elevated *TGFB* expression in light birds suggests variations in tissue remodeling and fibrosis control (Frangogiannis, 2020). *LKB1*, crucial for metabolism and cell growth regulation (Compton et al., 2023), showed significantly higher expression in light birds compared to heavy birds, particularly in gaped tenders, suggesting an active metabolic regulation attempt in light birds, while suppressed expression in heavy birds might indicate an impaired regulatory response due to increased mechanical stress from greater muscle mass.

The expression patterns across myopathy groups reveal distinct molecular signatures among groups. In heavy birds, *mTOR* levels were significantly lower in severely gaped tenders compared to SM, while *PRKAA2* showed reduced expression in severely gaped tenders relative to normal tenders. This downregulation of *PRKAA2*, a key regulator of cellular energy homeostasis, may indicate compromised metabolic adaptation in severely gaped tenders.

In heavy birds, *LITAF* RNA copy number was significantly higher in SM compared to normal breast fillets, indicating enhanced inflammation and immune response (Chen et al., 2021). This finding supports previous studies linking chronic inflammation to the development of another myopathy, WB (Petracci et al., 2019; Caldas-Cueva and Owens, 2020; Che et al., 2024) and supports observations of elevated LITAF abundance in fast-growing chickens (Malila et al., 2022).

The increased expression of *MSTN, MYOD1,* and *IGF1* may represent compensatory mechanisms aimed at maintaining muscle structure. *MYOD1* is a myogenic regulatory factor that promotes muscle differentiation and repair (Massenet et al., 2021). Elevated *MYOD1* expression can enhance muscle regeneration by activating satellite cells and promoting myogenic differentiation. However, previous studies have shown conflicting results regarding *MYOD1* expression levels. Some studies found no significant differences in *MYOD1* abundance in *P. major* muscle between fast- and medium-growing chickens (Malila et al., 2022) and between fast- and slow-growing chickens (Praud et al., 2020), suggesting that *MYOD1* expression might not always correlate directly with growth rate. Conversely, increased *MYOD1* expression observed in WB affected muscles (Velleman and Clark, 2015; Malila et al., 2019) indicates that *MYOD1* upregulation might be a response to muscle damage or stress. The upregulation of *MSTN*, a negative regulator of muscle growth (Grade et al., 2019), may counteract the beneficial effects of *MYOD1* by inhibiting myoblast proliferation and differentiation. This interplay between *MYOD1* and *MSTN* could potentially lead to conditions such as muscle gaping in chicken skeletal muscle. *IGF1*, a potent stimulator of muscle growth and regeneration, promotes protein synthesis and inhibits protein degradation (Yoshida and Delafontaine, 2020). Its upregulation, alongside *MYOD1*, could be part of a compensatory mechanism to counteract the growth-inhibitory effects of *MSTN*, aiming to maintain an optimal level of muscle mass and function in light birds.

The upregulation of *COL3A1* expression in severely gaped tendered in light birds indicates enhanced collagen deposition and extracellular matrix remodeling. *COL3A1*, which encodes the pro-alpha1 chains of type III collagen, plays a crucial role in connective tissue structure and function (Kuivaniemi and Tromp, 2019). Previous research demonstrated *COL3A1* upregulation in SM compared to normal breast fillets in 2.23 kg birds (Che et al., 2024). However, our study revealed no significant differences in *COL3A1* expression between normal and SM-affected fillets across bird weights, suggesting that collagen regulation in poultry muscle abnormalities may be weight-dependent and condition-specific. This variation likely depends on factors such as the specific muscle condition, bird weight category, and stage of abnormality development.

Our findings suggest a complex interplay between muscle location, bird weight, and myopathy development in broiler chickens. While SM and gaping may initially present similar pathological features, their molecular signatures, particularly in *mTOR* and *LKB1* expression, indicate divergent progression pathways that are heavily influenced by muscle mass and location. The bird's weight emerges as a critical determinant in how these myopathies manifest, suggesting that the increased muscle mass in modern broilers not only affects the likelihood of developing these conditions but also influences their molecular characteristics. This weight-dependent divergence in pathology suggests that what appears to be similar conditions may actually require different approaches for prevention and treatment, particularly when considering the specific demands placed on different muscle groups in rapidly growing birds. Several factors require further investigation, including the origin of different weight groups from distinct production systems (no antibiotics vs. organic) and the influence of processing conditions between plants on muscle fiber integrity through various physiological and biochemical pathways.

One limitation of this study might be the narrow regional and production-type representation, as samples were collected from flocks derived from the East Coast of the USA and subjected only to either no antibiotics or organic production. Moreover, only one PAA treatment type was investigated, as this was similar between the two plants. This may introduce geographical and production system biases, limiting the overall generalizability. It is recommended that future research incorporate sampling from diverse regions, production systems, and PAA application methods. Additionally, physical measurements were conducted during the warm season due to logistical constraints. Future studies should incorporate year-round sampling to enhance generalizability. Also, the statistical power of gene expression analysis might have been affected by the relatively small sample size and the use of non-parametric tests. Future studies should aim to increase sample size to improve robustness.

In conclusion, this study reveals a high prevalence of severe gaping in US commercial broilers, with significant implications for the poultry industry. Our findings demonstrate a complex relationship between bird weight and myopathy development, where light birds (2.7 kg) exhibited higher gaping prevalence compared to heavy birds (4.1 kg). The study also identified PAA treatment as a significant factor influencing gaping severity, suggesting the need for further investigation into processing methods. The molecular signatures revealed weight-dependent divergence in pathology, indicating that prevention and treatment strategies may need to be tailored based on bird weight and specific muscle groups affected.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psj.2025.104976.

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