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# The effects of supplemental dietary chitosan on broiler performance and myopathic features of white striping

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**ABSTRACT** White striping (WS) is a common myopathy seen in fast-growing broilers. Studies have demonstrated that chitosan is effective as an antioxidant and has antiobesity and fat-absorption reduction properties. We hypothesized that the dietary supplementation of chitosan would have similar effects when fed to fast-growing broilers and would thus lower WS incidence and improve meat quality. One hundred twenty-six broilers were fed corn-soy diets. The grower and finisher diets contained either 0, 0.2, or 0.4% chitosan. After a 6 wk growth period, birds were euthanized, and then WS and gross pathology scores were assessed. Pectoralis major tissues were collected to evaluate cook loss, drip loss, histopathology scores, and the gene

expression of *CCR7*, *LECT2*, *CD36*, *PPARG*, and *PTGS2*. There were no significant differences between the broiler weights, thus chitosan did not appear to compromise the overall growth of the broilers. Female broilers fed 0.4% chitosan had the lowest WS incidence, while male broiler fed 0.4% chitosan had the least cook loss. However, gene expression analyses did not offer insight into any grossly or histologically visualized differences in the muscles. Thus, while we can postulate that chitosan could have some positive effect in reducing WS incidence and improving meat quality, further studies are required to better scrutinize the mechanisms by which chitosan affects WS and other such myopathies in fast-growing broilers.

**Key words:** broiler, white striping, chitosan, pathology

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## INTRODUCTION

White striping (WS) has become one of the most common myopathies in commercial broiler chickens. This myopathy is characterized by the appearance of white striations throughout the pectoralis major muscle that reduce palatability, meat quality, and consumer acceptance (Kuttappan et al., 2012; Brambila et al., 2016; Lee et al., 2021). WS is highly heritable, likely an outcome of genetic selection for fast growth with a strong genetic correlation to the wooden breast myopathy (Alnahhas et al., 2016; Lake et al., 2021). Causes of WS have been recently reviewed by Lee and Mienaltowski (Lee and Mienaltowski, 2023). Briefly, WS is associated with myovascular inflammation within broiler breast muscle that is growing faster than the vasculature's ability to maintain metabolic demands. Lipid accumulation in the muscle and vascular walls leads to macrophage migration to the region; macrophages intake lipids and become fat-laden foam cells.

Dietary intervention and genetic selection strategies to mitigate this myopathy have already been pursued, but few have successfully decreased WS without compromising growth (Lee and Mienaltowski, 2023).

The current study investigated the application of chitosan as a feed additive approach to reduce WS. Chitosan is a polysaccharide derived from the deacetylation of chitin, an abundant natural polymer with annual production over 1 billion tons (Ogawa et al., 2004; Dhillon et al., 2013). In a previous broiler study, an inclusion of 3% chitosan in the total diet decreased ileal fat digestibility and plasma concentrations of triacylglycerols and cholesterol (Razdan and Petterson, 1994). Chitosan has also been shown to have hypoglycemic and antiobesity effects in diabetic rats (Hsieh et al., 2012). Such effects could be of great benefit to reducing myopathies as altered lipid metabolism in broilers can lead to lipid accumulation in the muscle. Accumulation of fat in the pectoralis major muscle can lead to glucose toxicity since glucose uptake into muscle is increased despite the downregulation of glycolysis and glycogenesis, much like what is seen with smooth and cardiac muscle in Type 2 diabetics (Lake and Abasht, 2020). The presence of ectopic extracellular lipids in muscle can also initiate pathological changes as early as 1 wk posthatching (Papah et al., 2017). Inflammatory cells infiltrate

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myofibers to form lipogranulomatous lesions and fat-laden foam cells to contain ectopic fat, and inflammation consequently contributes to worsened meat quality by initiating fibrosis and myoregeneration as muscle tissue is being degraded (Papah et al., 2017). Chitosan's properties may offset glucolipotoxicity and pathology associated with WS; the effect of supplemental dietary chitosan on broiler myopathies has not yet been explored. We hypothesized that supplementation of dietary chitosan could reduce WS and improve meat quality. To test this hypothesis, broilers were fed 0, 0.2, and 0.4% chitosan in the grower and finisher phases. At market weight, the effect of dietary chitosan on WS, drip loss, cook loss, gross pathology, histopathology, and gene expression of the pectoralis major muscles was assessed.

## MATERIALS AND METHODS

### Experimental Design, Diets, and Animal Housing

The current study was approved by the University of California, Davis (UC Davis) Institutional Animal Care and Use Committee. A total of 126 one-day-old

Cobb-500 broilers were received from Foster Farms and housed in the Hopkins Avian Facility at UC Davis. Upon arrival, broilers were weighed and sorted randomly into 18 pens (4 ft × 4 ft), with 7 birds per pen for 3 treatment groups with 6 pens per treatment group. Temperature was set at 30°C during the first 3 d and decreased by 2°C to 3°C weekly until maintained at 20°C. Feed and water were provided ad libitum throughout the experiment. Broilers were fed a starter diet until d 10, a grower diet from d 11 to 21, and a finisher diet from d 22 to 42 that met or exceeded NRC recommendations (Table 1). Treatment groups were based upon the grower and finisher diets; control received no chitosan, and for the 2 other groups corn was replaced with food grade chitosan oligosaccharide (3,000 Da, Matexcel) at 0.2 or 0.4% chitosan. Broilers were weighed weekly and sexed before being culled via CO<sub>2</sub> inhalation at 6 wk.

### Necropsy

Euthanized birds were laid dorsally with 70% ethanol sprayed on the skin and feathers to wet and disinfect the surface. The skin was elevated and incised vertically along the midline to expose the full pectoralis major muscle for inspection, WS scoring, and photographing

**Table 1.** Control diet ingredients.

Ingredients as fed (kg) or percent composition	Starter (d 0–10) 298 g/bird	Grower (d 11–22) 1,011 g/bird	Finisher (d 23–45) 3,477 g/bird
Organic corn, yellow (kg)	48.13	55.11	58.29
Organic soybean meal (kg)	46.40	39.34	35.86
Organic soybean oil (kg)	1.8	2.11	2.72
Dicalcium phosphate (kg)	1.68	1.49	1.38
Limestone, ground (kg)	1.03	1.07	0.91
Salt (kg)	0.45	0.4	0.38
DL-methionine 99% (kg)	0.26	0.23	0.19
NRC vitamins/minerals (kg)	0.25	0.25	0.25
Calculated nutrients			
% Dry matter	90.8	90.6	90.3
% Acid detergent fiber	5.1	4.8	4.4
% Total nitrogen	4.7	3.8	3.5
% Protein	29.2	23.9	21.6
% Total digestible nutrients	70.7	70.9	71.2
% Crude fat	6.8	7.1	7.6
% Ash	7.9	7	6.7
% Cellulose	4.6	4.3	4
% Hemicellulose	7.4	7.1	7.2
% Asx	2.27	3.13	1.98
% Thr	0.78	1.06	0.7
% Ser	0.97	1.28	0.84
% Glx	3.8	5.06	3.34
% Pro	1.11	1.38	0.98
% Gly	0.89	1.05	0.73
% Ala	0.86	1.17	0.84
% Val	1.66	1.23	0.83
% Ile	0.71	1.21	0.78
% Leu	1.66	2.15	1.48
% Tyr	0.71	0.96	0.58
% Phe	1.06	1.46	0.94
% His	0.56	0.75	0.51
% Lys	1.21	1.69	1.09
% Arg	1.5	2.11	1.31
% Cys	0.36	0.44	0.31
% Met	0.5	0.47	0.46
% SAA	0.86	0.91	0.77

Asx, asparagine or aspartate; Glx, glutamate or glutamine; SAA, sulfur amino acids methionine and cysteine. 0.2% chitosan and 0.4% chitosan were added at grower and finisher phases for the 0.2 and 0.4% chitosan groups.

gross pathology of breast muscles. Then samples were collected.

### White Striping Analysis

The pectoralis major muscles of all of the broilers were exposed, and WS analysis of the pectoralis major muscles was performed. WS scores of 0, 1, and 2 were given, corresponding to normal (absent), moderate (<1 mm thick), or severe (>1 mm thick), based on the extent of WS (Russo et al., 2015).

### Gross Pathology Analysis

During necropsy, photographs of the pectoralis major muscles for all broilers were taken for gross pathology analysis. Images were blinded and examined for muscle pathology scores of 0 to 4, which were given based on the extent of muscle damage: 0, no presence of WS; 1, presence of WS only; 2, presence of surface hemorrhaging near sternal apex; 3, presence of intramuscular hemorrhaging near sternal apex; and 4, ischemia (Figure S1A–D) (Griffin et al., 2018; Vanhatalo et al., 2021).

### Sample Collection

Following WS scoring and photography for gross pathology analysis, two 1 cm × 2 cm × 0.5 cm portions were incised from the left anterior pectoralis major muscles for all birds. For each broiler, one such sample was snap frozen using liquid nitrogen and stored at –80°C for RNA isolation, and the other sample was stored in 10% neutral buffered formalin at 22°C to be processed for histological analyses. The right anterior pectoralis major muscles for selected broilers were dissected out, divided into 2 equal-sized pieces, weighed, vacuum sealed, and placed on ice for subsequent drip loss and cook loss analyses.

### Drip Loss and Cook Loss

Two approximately equal-sized pieces from the right anterior pectoralis major muscles were isolated with a 5 cm diameter circular mold, weighed, vacuum sealed in a polyethylene food storage bag, and placed on ice for transport to the laboratory. Samples from 1 sample set were wrapped in cotton meat netting and stored in an inflated plastic bag at 4°C for 7 d. Drip loss was calculated based on the following equation: drip loss (%) = (raw weight – stored weight)/raw weight × 100 (Chang et al., 2020). Samples from the second sample set were used for cook loss, where the samples were kept at –20°C for 7 d, then thawed overnight at 4°C and cooked at 80°C for 20 min, cooled on ice for 20 min, blotted dry, and weighed. Cook loss values were calculated based on the following equation: cooking loss (%) = (raw weight – cooked weight)/raw weight × 100 (Chang et al., 2020).

### Histopathology

Anterior pectoralis major muscle was fixed in 10% neutral buffered formalin, trimmed, processed through Sakura Tissue-Tek VIP 5, and embedded in paraffin. Tissue sections (4–5 μm) were stained with hematoxylin and eosin (H&E) and Masson's Trichrome stains using an adapted chicken breast muscle specific protocol (Vanhatalo et al., 2021). Images were captured using a BX43F microscope fitted with a DP80 digital and cellSens Dimension software v.1.12 (Olympus). Muscle histopathology scores were based on macrophage infiltration, tissue damage, adipose cell and collagen presence as follows: Mild (some macrophage infiltration), Moderate (few macrophage phagocytosis and appearance of fibrotic tissue), and Severe (high levels of macrophage infiltration with complete muscle destruction) (Figure S1E–L) (Kuttappan et al., 2013).

### Gene Expression

Total RNA was isolated from powdered pectoralis major tissue with an adapted protocol using TRIzol tri-reagent and a QIAGEN Micro RNeasy Kit (Sachs et al., 2019). A NanoDrop microvolume UV spectrophotometer (ThermoFisher Scientific) was used to determine the concentrations and purities of the total RNA samples. DNA-free total RNA (1 μg) was reverse-transcribed into cDNA using a High Capacity cDNA Reverse Transcription Kit (Life Technologies). For each sample, one-hundredth of the cDNA template was added to reactions with Fast Advanced TaqMan Master Mix (Life Technologies) for RT-qPCR analysis in a StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA). Chicken specific Taqman primer probe sets were used for *RER1* (*Retention in Endoplasmic Reticulum 1*, normalizing gene), *CCR7* (*C-C Chemokine Receptor 7*, chemokine receptor leukocyte migration), *LECT2* (*Leukocyte Cell-Derived Chemotaxin 2*, leukocyte-derived chemotaxin), *PPARG* (*Peroxisome Proliferator-Activated Receptor-Gamma*, fat marker), *PTGS2* (*Prostaglandin-Endoperoxide Synthase 2*, inflammation), and *CD36* (*Cluster of Differentiation 36*, foam cell marker) (Sachs et al., 2019; Malila et al., 2020). RT-qPCR reactions were performed in duplicate and gene specific efficiencies were calculated using LinRegPCR v7.5 software for each qPCR plate with relative expression found for each replicate (Ramakers et al., 2003). Briefly, mean relative expression was determined for each gene for each sample by calculating the efficiency of the RT-qPCR reaction for each gene with LinReg PCR v7.5; with the mean efficiency of reactions for each gene on each plate the reported  $C_T$  for each gene for each sample, a ratio of expression of the gene of interest vs. expression of the normalizing gene was calculated (Ramakers et al., 2003; Schefe et al., 2006; Sachs et al., 2019). GraphPad Prism software was used to analyze gene expression.

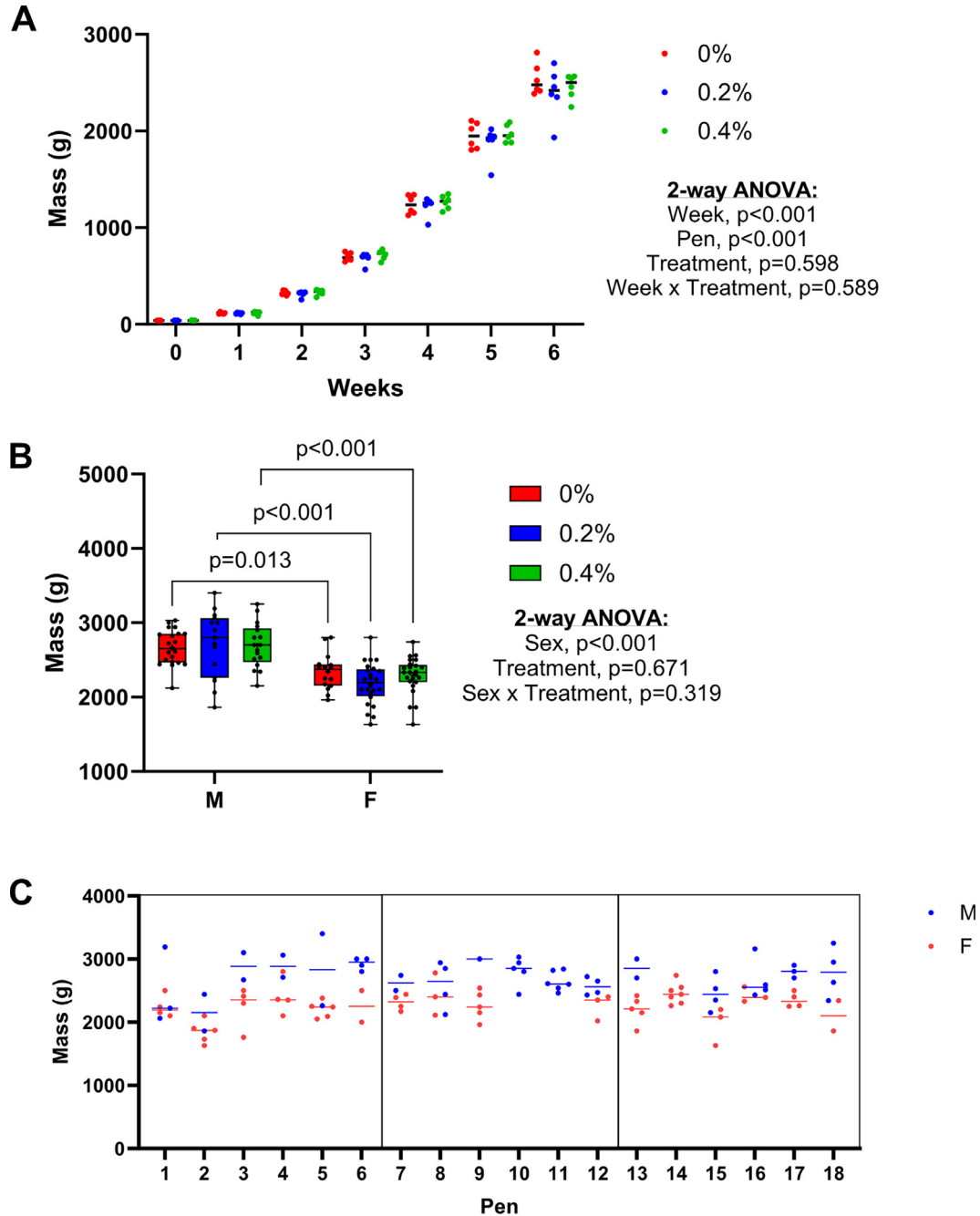
## Statistical Analyses

Weights of culled broilers, drip loss, and cook loss were each analyzed using 2-way ANOVA analyses, with Dunnett's multiple-comparison test for weight, and Tukey's multiple-comparison tests for drip loss and cook loss, by sex and chitosan group. WS scores, pathology ranks, histopathology scores, and RT-qPCR measurements were analyzed by 2-way ANOVA analyses with Tukey's multiple-comparison tests performed by sex and chitosan group. GraphPad Prism (GraphPad Software, La Jolla, CA) was used to perform these statistical

analyses and nonparametric analyses were calculated manually in Excel (Microsoft).

## Weights

Dietary chitosan fed at 0.2 and 0.4% did not alter broiler weights throughout the course of the study (Figure 1A). However, by wk 6, females in each treatment group had significantly decreased body weight in comparison to males in their respective groups (Figure 1B). Pen and sex differences were delineated



**Figure 1.** Broiler weights. Weights for each broiler were tracked each week for birds supplemented with 0, 0.2, and 0.4% dietary chitosan starting at d 11. A dot plot provides mean bird weights by pen (A) with  $n = 6$  pens per group. A box plot gives the weights of broilers by supplementation group and sex, providing the median, first and third quartiles, with whiskers representing range (B);  $n = 16$  to 24 birds/group. A dot plot providing wk 6 weights of each bird individually by pen is provided to show the differences between sex in each pen (C). Individual birds were represented as dots in Panels B and C. No significant differences were found between treatments overtime. A 2-way ANOVA with Tukey-multiple comparison test revealed significant differences by sex within each group of chitosan-supplemented birds.

when examining differences all broiler weights individually by sex and pen at 6 wk posthatching (Figure 1C).

### Drip Loss and Cook Loss

No differences in drip loss were detected among treatment groups or sex (Table 2). However, differences in cook loss were detected with significance overall for differences by treatment ( $P = 0.030$ ), sex ( $P = 0.050$ ), and the treatment-sex interaction ( $P = 0.017$ ). Males fed 0.4% chitosan had significantly less cook loss than males fed 0.2% chitosan and all females regardless of treatment.

### White Striping Scores

Overall, there was no significant difference for WS scores between treatment groups, though there was a sex effect ( $P < 0.001$ ) as female broilers had lower WS score overall (Table 2). Multiple test comparisons demonstrated that female broilers supplemented 0.4% chitosan had lower WS scores than male broilers fed 0, 0.2, 0.4% chitosan ( $P = 0.002$ ,  $P = 0.008$ , and  $P = 0.032$ ), respectively (Table 2).

### Gross Pathology Ranks

The mean gross pathology scores for 0, 0.2, 0.4% chitosan supplemented birds were 1.95, 1.87, and 1.70, respectively. There were significant treatment ( $P = 0.016$ ) and sex effects ( $P = 0.016$ ) (Table 2). However, the only significant interaction was between male broilers supplemented with 0.4% chitosan and 0% chitosan supplemented females ( $P = 0.008$ ).

### Histopathology

For both male and female broilers, the histopathology scores tended to decrease with chitosan supplementation. However, there were no statistically significant differences in histopathology scores for market weight broilers by treatment nor by sex (Table 2).

### Gene Expression

We examined the gene expression of *CCR7* (chemokine receptor leukocyte migration), *CD36* (foam cell marker), *LECT2* (leukocyte-derived chemotaxin), *PPARG* (fat marker), and *PTGS2* (inflammation marker) by treatment and sex. There were no significant differences in expression among treatment groups for *CCR7*, *LECT2*, and *PTGS2* (Figure 2A, C, E). However, treatment differences were noted for *CD36* and *PPARG*, likely because of elevated expression for both genes for 0.2% chitosan supplemented broilers (Figure 2B, D). Expression patterns for *CCR7*, *CD36*, *PPARG*, and *PTGS2* did not differ between males and females (Figure 2A, B, D, E). Transcript abundance of *LECT2* was lower in female broilers fed chitosan ( $P = 0.045$ ) (Figure 2C).

## DISCUSSION

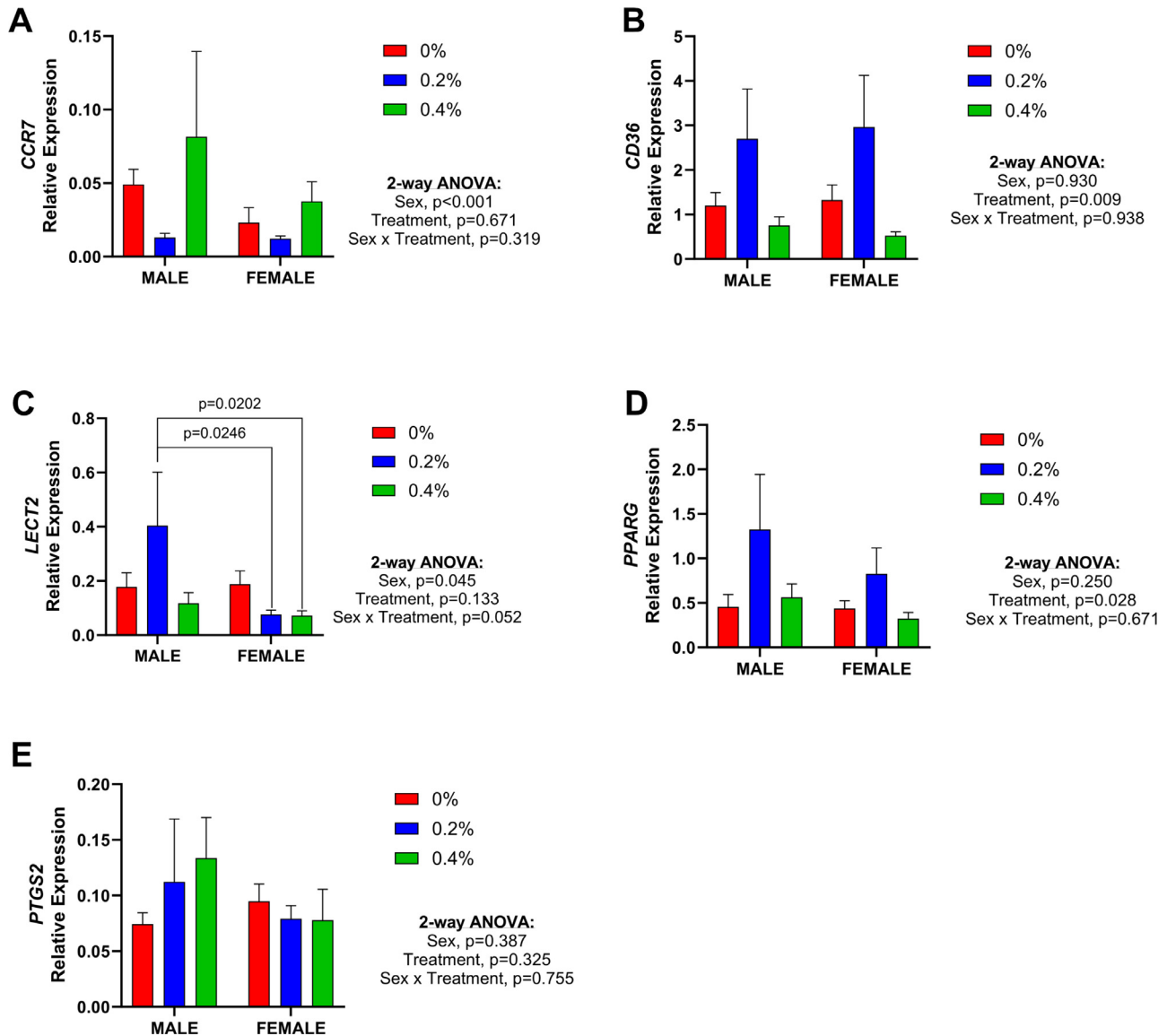
Due to increasing demands for poultry products, broilers have been selected to grow faster despite risks of compromising meat quality. In this study, we investigated how implementation of chitosan as a feed additive could affect broiler performance, meat quality, and WS. As anticipated, male broilers were significantly heavier at 6 wk posthatching (Howlender and Rose, 1992; England et al., 2023). However, no significant differences in body weight were observed between treatment groups for the duration of the study. The consistency in performance indicates that the level of dietary chitosan fed did not compromise weight gain, unlike weight losses previously seen for a study feeding broilers 3% dietary chitosan (Razdan and Pettersson, 1994).

Drip loss and cook loss are important features of meat quality. The capacity for meat to hold water, ions, minerals like iron, and proteins is essential for palatability (Ponsuksili et al., 2008). In indigenous yellow-feathered chickens, drip loss decreased when broilers were fed 0.6% chitosan for 8 wk (Wang et al., 2022). Contrarily, no differences in drip loss were observed in the present study, which may be attributed to the supplementation of lower levels of chitosan. However, studies have also shown that muscle degeneration and myopathy severity

**Table 2.** Meat quality and muscle pathology parameters, as mean  $\pm$  standard deviation.

Groups (Sex $\times$ Treatment)	Drip loss percent	Cook loss percent	White striping score (0–2)	Gross pathology score (0–4)	Histopathology score (0–3)
Male					
0%	7.99 $\pm$ 2.03	23.01 $\pm$ 5.77 <sup>ab</sup>	1.26 $\pm$ 0.45 <sup>a</sup>	2.00 $\pm$ 0.95	1.14 $\pm$ 1.04
0.2%	8.47 $\pm$ 2.46	24.00 $\pm$ 3.81 <sup>a</sup>	1.27 $\pm$ 0.46 <sup>a</sup>	1.73 $\pm$ 0.70	0.75 $\pm$ 1.06
0.4%	6.54 $\pm$ 2.7	14.83 $\pm$ 9.97 <sup>b</sup>	1.18 $\pm$ 0.53 <sup>a</sup>	1.41 $\pm$ 0.62	0.73 $\pm$ 0.80
Female					
0%	8.80 $\pm$ 1.18	23.26 $\pm$ 2.89 <sup>a</sup>	1.00 $\pm$ 0.39 <sup>ab</sup>	2.50 $\pm$ 1.10	0.88 $\pm$ 0.88
0.2%	8.73 $\pm$ 1.61	23.35 $\pm$ 2.66 <sup>a</sup>	1.04 $\pm$ 0.20 <sup>ab</sup>	1.96 $\pm$ 1.08	0.60 $\pm$ 0.75
0.4%	8.13 $\pm$ 3.82	23.67 $\pm$ 4.76 <sup>a</sup>	0.71 $\pm$ 0.69 <sup>b</sup>	1.92 $\pm$ 0.72	0.50 $\pm$ 0.69
<i>n</i>	4–12	5–13	15–24	14–24	12–22
<i>P</i> value					
Sex	0.237	0.050	<0.001	0.016	0.965
Chitosan	0.374	0.030	0.112	0.016	0.220
Sex $\times$ Chitosan	0.775	0.017	0.491	0.740	0.133

Means without a common superscript are significantly different ( $P < 0.05$ ).



**Figure 2.** Analysis of differentiation markers in pectoralis major muscles by treatment and sex. RT-qPCR results for (A) *CCR7*, (B) *CD36*, (C) *LECT2*, (D) *PPARG*, and (E) *PTGS2* genes, relative to housekeeping gene *RER1*, in pectoralis major muscles for the control (0%) and chitosan diet groups (0.2 and 0.4%). Values given as mean  $\pm$  SEM;  $n = 15$  to 24 broilers/group (treatment  $\times$  sex); comparisons were analyzed by 2-way ANOVA with Tukey multiple comparison tests between diet groups and sex. Significant differences are depicted in panels.

may not modify raw meat qualities, such as drip loss (Mazzoni et al., 2015). Cook loss also signifies a reduction in water-holding capacity. Thus, with more cook loss, there is more water loss, increased shrinkage of collagen and muscle fibers, and ultimately tougher meat (Weston et al., 2002). Significant differences in cook loss were observed by sex, level of dietary chitosan supplemented, and in sex  $\times$  chitosan interactions as breasts from the 0.4% chitosan supplemented males exhibited the lowest cook loss. Improvements in gross pathology with chitosan supplementation were not reflected in either histopathology or WS scores; thus, it is difficult to conclude that differences seen in cook loss come from any reductions in myopathy-associated muscle degeneration in the present study.

Female broilers fed 0.4% dietary chitosan demonstrated lower WS scores compared to male broilers supplemented 0, 0.2, and 0.4% chitosan. Thus, only a

significant sex effect was seen in our assessment of WS. As inflammation is an important contributor to the mechanisms of myopathies like WS and Wooden Breast Disease (WBD) (Papah et al., 2018; Lake and Abasht, 2020; Soglia et al., 2021), we used expression profiling to assess inflammatory genes in the breast muscles. While no specific interaction was detected for *CCR7*, expression of *LECT2* was reduced in female broilers fed 0.2 and 0.4% chitosan relative to male broilers fed 0.2% chitosan. This could be associated with decreased severity of WS experienced by females, or it could also be a function of the female broilers overall smaller size. Sex effects were not seen for other markers like *PTGS2*, *PPARG*, and *CD36*. Even though treatment effects were seen for *PPARG* and *CD36*, multiple comparison tests revealed no significant differences between any treatment groups. Thus, while WS was reduced in female broilers, particularly those supplemented 0.4%, expression profiles that

inform mechanisms for myopathies did not help us to conclude that the reductions in WS were due to treatment, but instead were more likely due to sex.

Overall, in this study, one finding led us to consider that there was some benefit to supplementing at least 0.4% dietary chitosan to Cobb500 broilers. Namely, the 0.4% chitosan supplemented males had better cook loss values. Several follow-up studies would help advance our understanding of how dietary supplementation of chitosan might improve broiler performance and mitigate myopathies like WS. Hypoxia within the rapidly growing muscle due to a lack of oxygen from a restricted vascular supply leads to the generation of reactive oxygen species (Sihvo et al., 2018). Since chitosan is an antioxidant and regulator of antioxidant enzyme activity, follow-up studies could further address meat quality by examining levels of reactive oxygen species, antioxidant enzyme activity, and levels of fat and muscle oxidation in and around the breast muscles of broilers fed chitosan (Ivanova and Yaneva, 2020). Moreover, another proposed etiology of WS and WBD includes pathogenesis arising from broilers being reared in a state of glucolipotoxicity, where dysregulation of lipid metabolism in the muscle advances the myopathies. Briefly, lipid dysregulation leads to fat accumulation with subsequent foam cell formation, inflammation, and degeneration of muscle tissue, much like the pathogenesis of atherosclerosis in vascular smooth muscle (Bobryshev et al., 2016; Lake and Abasht, 2020; Vanhatalo et al., 2021). Follow-up studies could associate pathological changes with breast fillet yield and composition, as well as lipid content in muscle, liver, and plasma metabolites. Finally, to discern the extent that supplementation alters fat absorption, studies should examine feed intake, feed conversion ratio, and fecal lipid levels.

## CONCLUSIONS

In conclusion, the present study indicates that adding at least 0.4% low molecular weight dietary chitosan supplementation in the grower and finisher phases could help reduce the incidence of myopathies and improve cook loss. More experiments could be done in the future to better discern broiler metabolic and muscle physiologic mechanisms that contribute to broiler performance when dietary chitosan is supplemented.

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## DISCLOSURES

All authors have no competing interests to declare.

## SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at [doi:10.1016/j.psj.2023.103396](https://doi.org/10.1016/j.psj.2023.103396).

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