Internal organ and skeletal muscle development in commercial broilers with woody breast myopathy

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ABSTRACT Increasing growth rate, body weight, and breast muscle yield have been linked to broiler muscle problems such as woody breast (**WB**). The aim of this study was to investigate the internal organ and skeletal muscle development of broilers with WB myopathy under dietary and *Eimeria* challenge treatments. A 3 diet (control, antibiotic, or probiotic) × 2 challenge (control or *Eimeria*) × 2 sex factorial arrangement of treatments was used in a randomized complete block design. Ross × Ross 708 chicks were randomly assigned to 96 floor pens with 12 treatment combinations (8 replicates per treatment). Internal organs were sampled on d 13 and 41. Skeletal muscles were sampled on d 41. Internal organ and skeletal muscle weights were analyzed using a 3-way analysis of variance (**ANOVA**). Relationships between WB and internal organ and skeletal muscle weights were analyzed using one-way ANOVA as all treatments were pooled together and regrouped according to WB scores. On d 41, absolute and relative heart weights were greater in males when they were averaged over diet and challenge treatments ($P < 0.001$ and $P = 0.026$, respectively). The birds with WB score 3 had greater absolute heart ($P = 0.0002$) and spleen weights ($P = 0.016$), but there was no difference in relative spleen weight ($P > 0.05$). When averaged over diet and challenge treatments, males have greater absolute duodenum, jejunum, and ileum weights (for all $P < 0.0001$). Compared with birds with normal breasts, the birds with WB scores 1, 2, and 3 had a greater live weight (for all $P < 0.0001$) and absolute and relative breast weights (for all $P < 0.0001$). The birds with WB score 1, 2, and 3 had greater ($P < 0.0001$) absolute but lower ($P < 0.0001$) relative drumstick, thigh, and wing weights. Results indicated that broilers with WB had lower relative proventriculus and gizzard weights and greater relative breast meat weight with lower relative drumstick, thigh, and wing muscle weights.

Key words: organ development, processing yield, skeletal muscle, woody breast, broiler

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INTRODUCTION

Genetic selection in commercial broilers has dramatically improved meat yield and growth performance, and the growth rate of intensively reared conventional broilers has accelerated 400% in the past 50 yr (Zuidhof et al., 2014). Compared to slower growing broilers, modern commercial broilers have a 1.2 kg heavier body weight (Torrey et al., 2021) and twice the amount of breast muscle (Havenstein et al., 2003; Schmidt et al., 2009; Zuidhof et al., 2014). However, rapid growth and high-breast yield in broilers lead to some side effects on broiler breast muscle (*pectoralis major*) development, such as woody breast (**WB**) (Caldas-Cueva and Owens, 2020; Zhang et al., 2021a). Increased WB cases have affected producers since 0.8% of breast meat can be downgraded or frequently condemned, leading to losses of marginal economic return (Zanetti et al., 2018). Furthermore, the animal welfare concerns regarding to increased body weight and breast yield have arisen (Kuttappan et al., 2016; Norring et al., 2018), and consumers and supply chains are willing to pay more for higher welfare broiler products (Mulder and Zomer, 2017). The prevalence of these problems indicates that the physiological capabilities of broilers may constrain further increases in broiler meat yield because skeletal, metabolism, and digestive systems are

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reaching their functional limits (Tickle et al., 2014), thus causing welfare issues (Norring et al., 2018).

Characterizing changes in the internal organs and skeletal muscle size of broilers with WB myopathy are crucial for understanding broiler physiology and WB development. Physical observation and scaling rules determine the condition of changes in the mass and proportions of the internal organs which affect organismal form (Schmidt-Nielsen and Knut, 1984). Observing the size of a bird's immune and digestive organs and skeletal muscles is important, because the information can increase the understanding of physical orders that may be present. The liver play essential roles in fat and protein metabolism (Bosc-Bierne et al., 1984; Zaefarian et al., 2019), and the functional anatomical properties of the broiler small intestine tract are crucial to the effectiveness of feed conversion (de Verdal et al., 2010).

Multiple farm management factors such as feed additives, coccidiosis infection, and bird husbandry affect organ and muscle growth and the health of male and female commercial broilers differently. Male broilers are more susceptible to WB due to their biological characteristics related to higher body weight, fat metabolism, and oxidative stress response (Brothers et al., 2019). Antibiotics (bacitracin), probiotics (*Bacillus subtilis*), and cocci challenges influence gut health conditions, which can affect internal organ development (Wang et al., 2018; Poudel et al., 2021). Dietary antibiotics can modify organ size and function, thus improving growth performance (Castanon, 2007; Crisol-Martinez et al., 2017; Manafi et al., 2019). Probiotics improve the birds' immune functions and growth performance (Teo and Tan, 2007; Molnár et al., 2011; Jayaraman et al., 2017). Coccidiosis is a common reason for growth and organ development disorders (Sharma and Fernando, 1975; Su et al., 2014; Rochell et al., 2016). However, limited studies have been conducted to examine the effect of these management practices on WB development. Internal organ status reflects the health and welfare condition of fast-growing broilers with WB. Given the rise of WB incidence in the broiler industry, it is essential to explore the relationship between internal organ and skeletal muscle development of birds with the WB myopathy. Therefore, the current study examines how organ and muscle growth varies under different dietary and disease conditions in male and female commercial broilers with the WB myopathy.

MATERIALS AND METHODS

This experiment was conducted on the Mississippi State University Poultry Research Farm. All rearing and sampling procedures that were used in this study were reviewed and approved by the Institutional Animal Care and Use Committee with protocol number 16-542 at Mississippi State University.

Experimental Design and Birds Management

Treatment assignment, basal diet formulation, and bird management have been previously described (Jia et al., 2022). Briefly, A 3 (Diet) × 2 (Challenge) × 2 (Sex) factorial arrangements of treatments was used in a randomized complete block design. A total of 96 floor pens with commercially used litter bedding and top-dressed with pine shavings were divided into 8 blocks according to the location. A total of 672 male and 672 female Ross × Ross 708 chicks were randomly allotted to 96 floor pens which preassigned into 12 treatment combinations with 14 birds per pen and 8 replicates per treatment. The experimental diets were a control diet (corn-soybean meal basal diet), an antibiotic diet (basal diet + 6.075 mg bacitracin /kg feed), and a probiotic diet (basal diet + 2.2×10^8 CFU *Bacillus subtilis* PB6 /kg feed). Diet formulation followed the recommendation of Ross 708 as-hatched broilers (Aviagen, 2019). On d 14 birds were either challenged with a 20 × the live cocci vaccine to mimic the poultry farm *Eimeria* infection or received same amount of distilled water. Either males or females were included in each treatment combination. Broiler husbandry followed Ross broiler management handbook (Aviagen, 2018).

Internal Organ Measurement

For internal organ sampling, one bird per pen was randomly selected at d 13, which served as a baseline control prior to the development of the WB myopathy. On d 41, live birds were evaluated for WB myopathy by manual palpation using a 0 to 3 WB scoring system (Zhang et al., 2021a). One bird with a normal breast (score 0) and one bird with a WB (score 1, 2, or 3) in each pen were randomly selected on d 41 for internal organ sampling. Sampled birds were tagged, and live weights of birds were taken before they were humanely euthanized using CO_2 asphyxiation. Body weight (**BW**) and weights of the internal organs including the proventriculus, gizzard, spleen, liver, heart, and bursa were recorded. Their absolute weight (g) and relative weight (g/g BW (%)) were measured and calculated from each sampled bird. The absolute weight (g), relative weight (g/g BW (%)), and absolute length (cm) of the small intestine (duodenum, jejunum, and ileum) were measured and calculated from each sampled bird.

Processing

Processing procedure has been previously described (Jia et al., 2022). Briefly, 5 birds from each pen were randomly selected for processing on d 44. A total of 480 birds were weighed and tagged, processed at the Mississippi State University Poultry Processing Facility, and deboned manually. After deboning, breast muscle was palpated and scored using the WB scoring system described in the internal organ measurement section. Carcass, wing, drumstick, thigh, boneless and skinless breast, and tender were weighed after deboning. Relative weights (g/g Carcass weight (%)) were calculated for further data analysis.

Table 1. Effects of antibiotic and probiotic on internal organ absolute weights (g) and relative weights (%) of male and female broilers on d 13.

		Heart		Liver		Bursa		Spleen	
Diet[1]	Sex	AW[2] (g)	RW[3] (%)	AW (g)	RW (%)	AW (g)	RW (%)	AW (g)	RW (%)
Control	Male	2.54	0.672	16.1[ab]	4.25	0.736	0.194	0.416	0.110
	Female	2.37	0.626	16.1[ab]	4.27	0.704	0.187	0.368	0.098
Antibiotic	Male	2.60	0.683	15.4[ab]	4.07	0.705	0.188	0.363	0.095
	Female	2.44	0.632	17.4[a]	4.41	0.730	0.193	0.364	0.095
Probiotic	Male	2.59	0.698	14.7[b]	4.01	0.662	0.180	0.362	0.101
	Female	2.42	0.652	15.3[ab]	4.13	0.660	0.173	0.325	0.089
	SEM[4]	0.082	0.019	0.549	0.110	0.047	0.012	0.020	0.006
	P-value	0.992	0.985	0.216	0.315	0.831	0.849	0.459	0.450
P-value									
Diet		0.714	0.363	0.024	0.166	0.374	0.410	0.068	0.172
Sex		0.014	0.003	0.061	0.081	0.939	0.775	0.096	0.074

[a-b]Means in a column not sharing a common superscript were different ($P < 0.05$).
[1]Experiment diets included a control diet (corn and soybean-meal basal diet), an antibiotic (basal diet + 6.075 mg bacitracin /kg feed), and a probiotic diet (basal diet + 2.2×10^8 CFU *Bacillus subtilis* PB6 /kg feed).
[2]AW, absolute weight of internal organs.
[3]RW, relative weight, was calculated by dividing absolute weight by body weight of each sampled bird.
[4]SEM, standard error of the mean for n = 16.

Data Analyses

One bird with normal breast and one bird with WB from each pen were sampled for internal organ measurements. However, the population of birds with normal breast and birds with WB in each pen was not equaled (unbalanced). To avoid sampling error, weighted average of each internal organ was calculated by multiplying either normal breast percentage or WB incidence before the ANOVA analysis. Internal organ weights on d 13 were analyzed using 2-way ANOVA to test for the diet and sex, and their interactive effect. Internal organ weights on d 41 were analyzed using 3-way ANOVA to test for the diet, challenge, and sex, as well as their interactive effects. The pen was considered the experimental unit, and data were analyzed using the PROC GLM procedure of SAS 9.4 (SAS version 9.4, SAS Institute, 2013). An adjusted Tukey test was used to determine multiple comparisons between treatments. Level of significance was determined at $P \leq 0.05$. Differences of the measured variables of the internal organs and skeletal muscles among different woody breast score (0, 1, 2, and 3) groups were analyzed using one-way ANOVA analysis followed by pairwise *t* tests using R and visualized in boxplots that were created in R environment. For processing weights and yields, data were included for analysis after removing major outliers (3.0 × IQR [interquartile range] outside the central box) based on the carcass percentage. The significance levels were indicated as * if $P < 0.05$, ** if $P < 0.01$, and *** if $P < 0.001$ in figures.

RESULTS

Baseline Data Prior to the Onset of Woody Breast

To evaluate the baseline of organ development prior to the onset of WB formation, samples were collected at d 13. It was confirmed that no WB was observed at that time point. Absolute and relative weights of the internal organs and the length of the small intestine were

Table 2. Effects of antibiotic and probiotic on intestinal weight and length of male and female broilers on d 13.

Treatments		Duodenum			Jejunum			Ileum		
Diet[1]	Challenge	AW[2] (g)	RW[3] (%)	Length (cm)	AW (g)	RW (%)	Length (cm)	AW (g)	RW (%)	Length (cm)
Control	Male	6.33[ab]	1.67[a]	22.8	10.35	2.74[a]	53.7	6.80[a]	1.81[a]	51.5
	Female	5.82[ab]	1.55[ab]	22.2	9.41	2.50[ab]	51.8	5.62[b]	1.49[c]	48.9
Antibiotic	Male	6.41[a]	1.70[a]	23.7	10.13	2.67[ab]	54.3	7.24[a]	1.94[a]	54.0
	Female	5.76[ab]	1.49[ab]	21.8	9.28	2.37[b]	54.1	5.83[b]	1.51[c]	52.2
Probiotic	Male	5.80[ab]	1.60[ab]	23.2	9.70	2.70[a]	53.8	6.51[ab]	1.78[ab]	53.2
	Female	5.50[b]	1.44[b]	23.1	9.18	2.43[ab]	52.1	5.73[b]	1.52[bc]	50.0
	SEM[4]	0.203	0.053	0.518	0.292	0.079	1.294	0.219	0.063	1.265
	P-value	0.687	0.709	0.191	0.763	0.924	0.782	0.353	0.360	0.849
P-value										
Diet		0.055	0.213	0.497	0.324	0.458	0.480	0.142	0.381	0.080
Sex		0.005	0.001	0.035	0.002	<0.0001	0.240	<0.0001	<0.0001	0.016

[a-c]Means in a column not sharing a common superscript were different ($P < 0.05$).
[1]Experiment diets included a control diet (corn and soybean-meal basal diet), an antibiotic (basal diet + 6.075 mg bacitracin /kg feed), and a probiotic diet (basal diet + 2.2×10^8 CFU *Bacillus subtilis* PB6 /kg feed).
[2]AW, absolute weight of internal organs.
[3]RW, relative weight, was calculated by dividing absolute weight by body weight of each sampled bird.
[4]SEM, standard error of the mean for n = 16.

Table 3. Effects of antibiotic and probiotic and *Eimeria* spp. challenge on internal organ absolute weights (g) and relative weights (%) of male and female broilers on d 41.

Treatments			Heart		Liver		Bursa		Spleen	
Diet[1]	Challenge[2]	Sex	AW[3] (g)	RW[4] (%)	AW (g)	RW (%)	AW (g)	RW (%)	AW (g)	RW (%)
Control	Challenge	Male	14.7^{a}	0.515	77.9^{a}	2.72	3.969	0.139ab	3.04	0.106
		Female	11.3^{bcd}	0.479	65.5^{ab}	2.78	2.977	0.127ab	2.96	0.126
	Non-challenge	Male	12.6^{abcd}	0.486	69.6^{ab}	2.69	3.716	0.143^{ab}	3.18	0.122
		Female	11.7^{bcd}	0.479	64.2^{b}	2.64	3.522	0.145^{ab}	3.32	0.136
Antibiotic	Challenge	Male	13.6^{abc}	0.498	72.5^{ab}	2.68	3.881	0.145^{ab}	3.45	0.126
		Female	12.2^{bcd}	0.514	68.5^{ab}	2.87	3.648	0.157^{ab}	2.71	0.116
	Non-challenge	Male	14.0^{ab}	0.513	72.2^{ab}	2.66	3.453	0.127^{ab}	3.28	0.121
		Female	11.2^{cd}	0.464	65.3^{b}	2.70	3.886	0.162^{a}	2.75	0.115
Probiotic	Challenge	Male	13.6^{abc}	0.521	70.6^{ab}	2.68	3.143	0.120^{b}	3.15	0.121
		Female	11.4^{bcd}	0.484	64.2^{b}	2.73	3.570	0.151^{ab}	2.65	0.113
	Non-challenge	Male	13.3^{abc}	0.495	73.3^{ab}	2.72	3.554	0.134^{ab}	3.28	0.121
		Female	10.4^{d}	0.457	65.2^{b}	2.85	3.453	0.152^{ab}	2.96	0.128
		SEM[5]	0.528	0.019	2.605	0.073	0.218	0.009	0.223	0.008
		P-value	0.027	0.209	0.351	0.506	0.062	0.352	0.996	0.704
P-value										
Diet			0.347	0.798	0.764	0.779	0.190	0.300	0.759	0.872
Challenge			0.044	0.084	0.291	0.432	0.602	0.455	0.306	0.229
Sex			<0.0001	0.026	<0.0001	0.101	0.385	0.007	0.011	0.604
Diet × Challenge			0.752	0.888	0.200	0.155	0.683	0.354	0.558	0.371
Diet × sex			0.824	0.708	0.648	0.521	0.027	0.035	0.104	0.081
Challenge × sex			0.788	0.575	0.801	0.525	0.219	0.431	0.433	0.633

[a-d]Means in a column not sharing a common superscript were different ($P < 0.05$).

[1]Experiment diets included a control diet (corn and soybean-meal basal diet), an antibiotic (basal diet + 6.075 mg bacitracin /kg feed), and a probiotic diet (basal diet + 2.2×10^8 CFU *Bacillus subtilis* PB6 /kg feed).

[2]The birds were either challenged with 1 mL 20 × cocci vaccine (COCCIVAC-B52, containing *E. acervulina, E. maxima, E. maxima MFP, E. mivati*, and *E. tenella*) or gavaged the same amount of distilled water on d 14.

[3]AW, absolute weight of internal organs.

[4]RW, relative weight, was calculated by dividing absolute weight by body weight of each sampled bird.

[5]SEM, standard error of the mean for n = 8.

Table 4. Effects of antibiotic and probiotic and *Eimeria* spp. challenge on intestinal absolute and relative weight and length of male and female broilers on d 41.

Treatments			Duodenum			Jejunum			Ileum		
Diet[1]	Challenge[2]	Sex	AW[3] (g)	RW[4] (%)	Length (cm)	AW (g)	RW (%)	Length (cm)	AW (g)	RW (%)	Length (cm)
Control	Challenge	Male	18.5^{a}	0.646	35.8^{a}	31.9^{ab}	1.12	85.2	21.4^{abc}	0.752	90.5^{a}
		Female	15.2^{ab}	0.644	34.4^{ab}	27.3^{b}	1.16	83.8	19.6^{abc}	0.831	84.0^{ab}
	Non-challenge	Male	16.8^{ab}	0.648	34.5^{ab}	31.1^{ab}	1.18	84.1	22.4^{ab}	0.854	86.1^{ab}
		Female	15.4^{ab}	0.633	34.0^{ab}	30.5^{ab}	1.27	81.1	19.3^{bc}	0.792	77.8^{b}
Antibiotic	Challenge	Male	16.7^{ab}	0.615	33.1^{ab}	30.9^{ab}	1.13	82.9	21.3^{abc}	0.784	87.6^{ab}
		Female	15.3^{ab}	0.641	33.6^{ab}	29.6^{ab}	1.25	83.8	20.2^{abc}	0.850	84.7^{ab}
	Non-challenge	Male	16.0^{ab}	0.592	33.6^{ab}	33.1^{ab}	1.22	87.3	21.7^{abc}	0.803	88.1^{ab}
		Female	14.2^{b}	0.592	32.0^{b}	28.8^{b}	1.20	83.5	19.1^{bc}	0.794	81.2^{ab}
Probiotic	Challenge	Male	15.7^{ab}	0.595	34.1^{ab}	30.6^{ab}	1.16	82.8	20.7^{abc}	0.788	86.8^{ab}
		Female	14.2^{b}	0.605	32.9^{ab}	28.3^{b}	1.20	83.9	18.8^{bc}	0.798	81.8^{ab}
	Non-challenge	Male	17.4^{ab}	0.651	34.7^{ab}	34.7^{a}	1.30	87.6	23.5^{a}	0.878	87.3^{ab}
		Female	14.0^{b}	0.612	33.6^{ab}	28.6^{b}	1.25	83.6	17.8^{c}	0.778	85.1^{ab}
		SEM[5]	0.728	0.026	0.707	1.136	0.045	2.164	0.827	0.030	2.243
		P-value	0.160	0.878	0.327	0.036	0.339	0.831	0.511	0.749	0.531
P-value											
Diet			0.065	0.166	0.009	0.874	0.315	0.801	0.688	0.985	0.870
Challenge			0.475	0.847	0.559	0.044	0.012	0.521	0.558	0.365	0.208
Sex			<0.0001	0.815	0.032	<0.0001	0.193	0.188	<0.0001	0.874	0.0001
Diet × Challenge			0.219	0.184	0.291	0.641	0.415	0.320	0.551	0.361	0.086
Diet × sex			0.678	0.727	0.833	0.555	0.557	0.965	0.213	0.200	0.484
Challenge × sex			0.896	0.326	0.632	0.474	0.217	0.136	0.027	0.003	0.702

[a-c]Means in a column not sharing a common superscript were different ($P < 0.05$).

[1]Experiment diets included a control diet (corn and soybean-meal basal diet), an antibiotic (basal diet + 6.075 mg bacitracin /kg feed), and a probiotic diet (basal diet + 2.2×10^8 CFU *Bacillus subtilis* PB6 /kg feed).

[2]The birds were either challenged with 1 mL 20 £ cocci vaccine (COCCIVAC-B52, containing *E. acervulina, E. maxima, E. maxima MFP, E. mivati*, and *E. tenella*) or gavaged the same amount of distilled water on d 14.

[3]AW, absolute weight of internal organs.

[4]RW, relative weight, was calculated by dividing absolute weight by body weight of each sampled bird.

[5]SEM, standard error of the mean for n = 8.

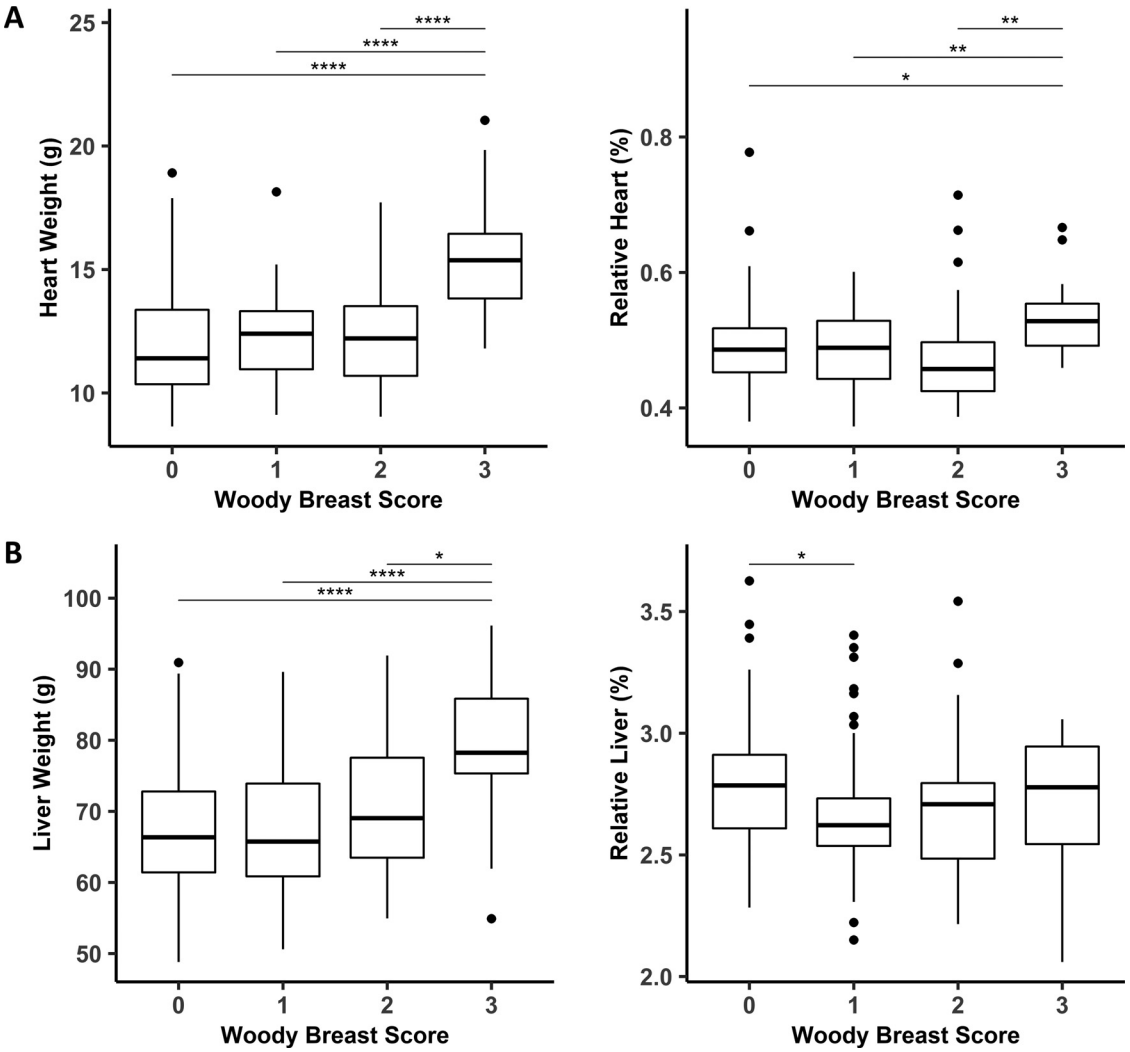

Figure 1. Comparison of birds with normal and woody breast on the absolute and relative heart (A) and liver (B) weight on d 41. Woody breast score 0 (n = 67), 1 (n = 66), 2 (n = 38), and 3 (n = 21) were obtained by palpation. Statistical significance was determined by pairwise *t* test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

measured to examine the effect of diet and sex on internal organ development before the cocci challenge (Tables 1 and 2). There was no diet × sex interaction for any of the variables measured (for all $P > 0.05$). Males had greater absolute and relative heart weights than females ($P = 0.014$ and $P = 0.003$, Table 1). Birds fed the antibiotic diet had heavier absolute liver weights than those fed the probiotic diet ($P = 0.024$, Table 1). Males had heavier absolute and relative duodenum, jejunum, and ileum weights than females (for all $P < 0.05$, Table 2), and males had greater duodenum and ileum lengths than females ($P = 0.035$ and $P = 0.016$).

Treatment Effects on Internal Organs

Treatment effects on the absolute and relative weights and the lengths of the internal organs on d 41 are shown in Tables 3 and 4. There was a significant diet × challenge × sex interaction for absolute heart weight on d 41 ($P = 0.027$, Table 3). The absolute weights of the heart were heavier in challenged birds when averaged across diet and sex ($P = 0.044$). Furthermore, males had greater absolute and relative heart weights than females across diet and challenge ($P < 0.0001$ and $P = 0.026$). When averaged across challenge, males fed the antibiotic diet had heavier absolute and relative bursa weights compared to males fed the control diet ($P = 0.027$ and $P = 0.035$). When averaged across diet and challenge, males had greater absolute duodenum, jejunum, and ileum weights than females (for all $P < 0.0001$, Table 4). When averaged across diet and sex, birds that received a cocci challenge had lighter absolute and relative jejunum weights than those that were unchallenged ($P = 0.044$ and $P = 0.012$).

Relationship Between Woody Breast and Heart, Liver, and Immune Organs

Birds from all the treatment groups were pooled together and regrouped by their WB scores for one-way ANOVA analysis of their intestinal organ, body, and muscle weights (Figures 1–7). Higher absolute heart and liver weights were observed in birds with WB score 3 when compared to those having WB scores 0, 1, and 2

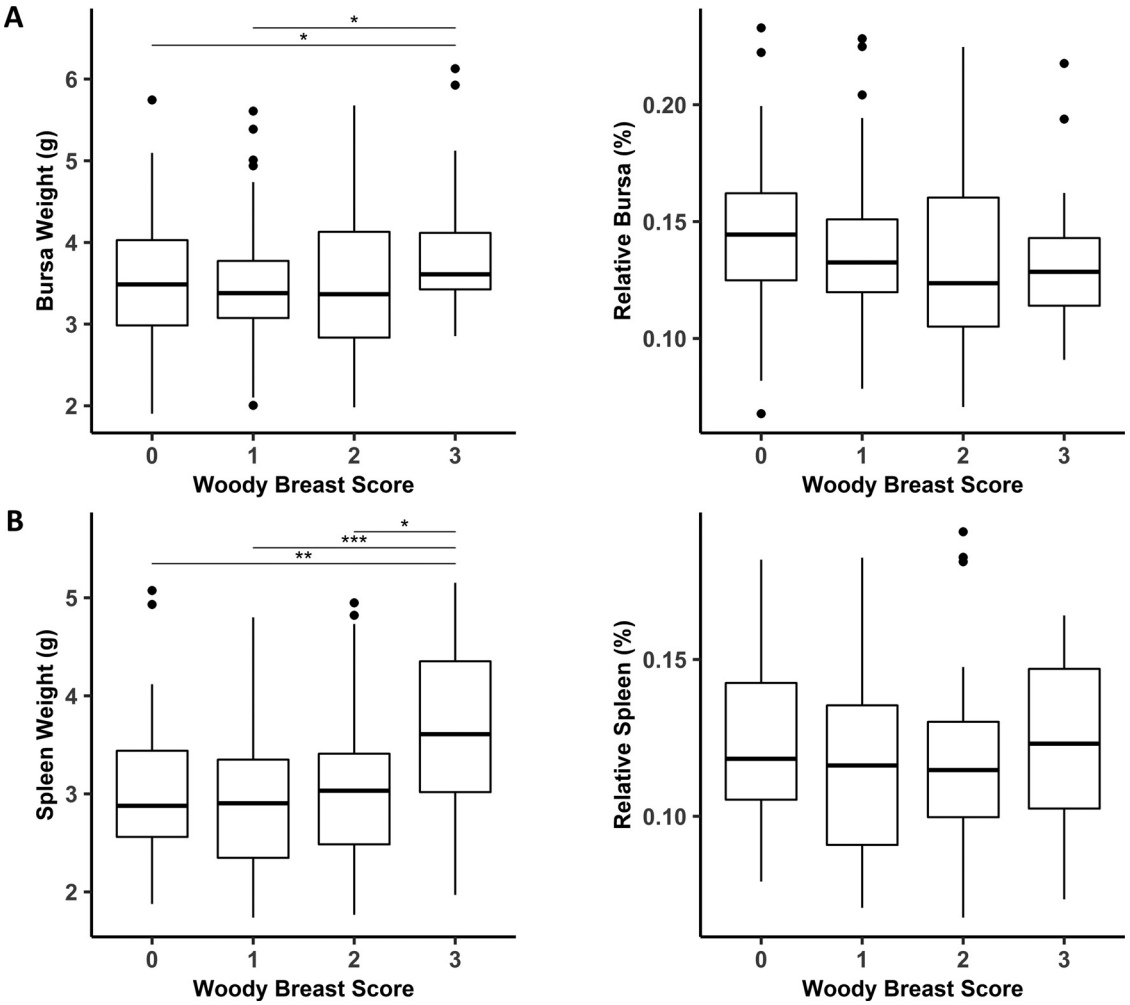

Figure 2. Comparison of birds with normal and woody breast on the absolute and relative bursa (A) and spleen (B) weight on d 41. Woody breast score 0 (n = 67), 1 (n = 66), 2 (n = 38), and 3 (n = 21) were obtained by palpation. Statistical significance was determined by pairwise *t* test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

(for all $P < 0.05$). Relative heart weight of birds with WB score 3 was also higher when compared to those having WB scores 0, 1, and 2 (for all $P < 0.05$). However, relative liver weights were not different between four scores except birds with WB score 1 have lower relative liver weights when compared to those having WB score 0 ($P > 0.05$, Figure 1). Higher absolute bursa and spleen weights were observed in birds with WB score 3 when compared to those having WB scores 0 and 1 (for all $P < 0.05$). However, there was no difference in relative bursa and spleen weights between birds with different WB scores (for all $P > 0.05$, Figure 2).

Relationship Between Woody Breast and Gastrointestinal Organs

Birds with WB score 3 had a heavier proventriculus when compared to birds with normal breast; have a heavier gizzard when compared to birds with WB scores 0, 1, and 2. However, birds with WB had a relatively small proventriculus (score 3) and gizzard (score 2 and 3) (Figure 3). Birds with WB had greater absolute duodenum, jejunum, and ileum weights than those of birds with normal breasts (Figure 4). However, relative duodenum, jejunum, and ileum weights were not different between 4 scores except birds with WB score 2 have lower relative duodenum weights when compared to those having WB score 0 ($P > 0.05$). Longer duodenum, jejunum, and ileum were observed in birds with WB score 3 when compared to those having WB scores 0, 1, or 2 (for all $P < 0.05$).

Relationship Between Woody Breast and Skeletal Muscle

Birds with WB score 1, 2, and 3 exhibited a heavier live ($P < 0.0001$), carcass ($P < 0.0001$) weights, and relative carcass ($P < 0.01$) weights compared to those birds with WB score 0 (Figure 5).

Similar to internal organs, processing yield data from the different treatments were pooled together and regrouped by WB condition for one-way ANOVA analysis of the skeletal muscle absolute and relative weights (Figures 6 and 7). Birds with WB exhibited greater absolute and relative breast weights and heavier absolute tender weights than those with normal breasts. Birds with WB had greater absolute drumstick, thigh,

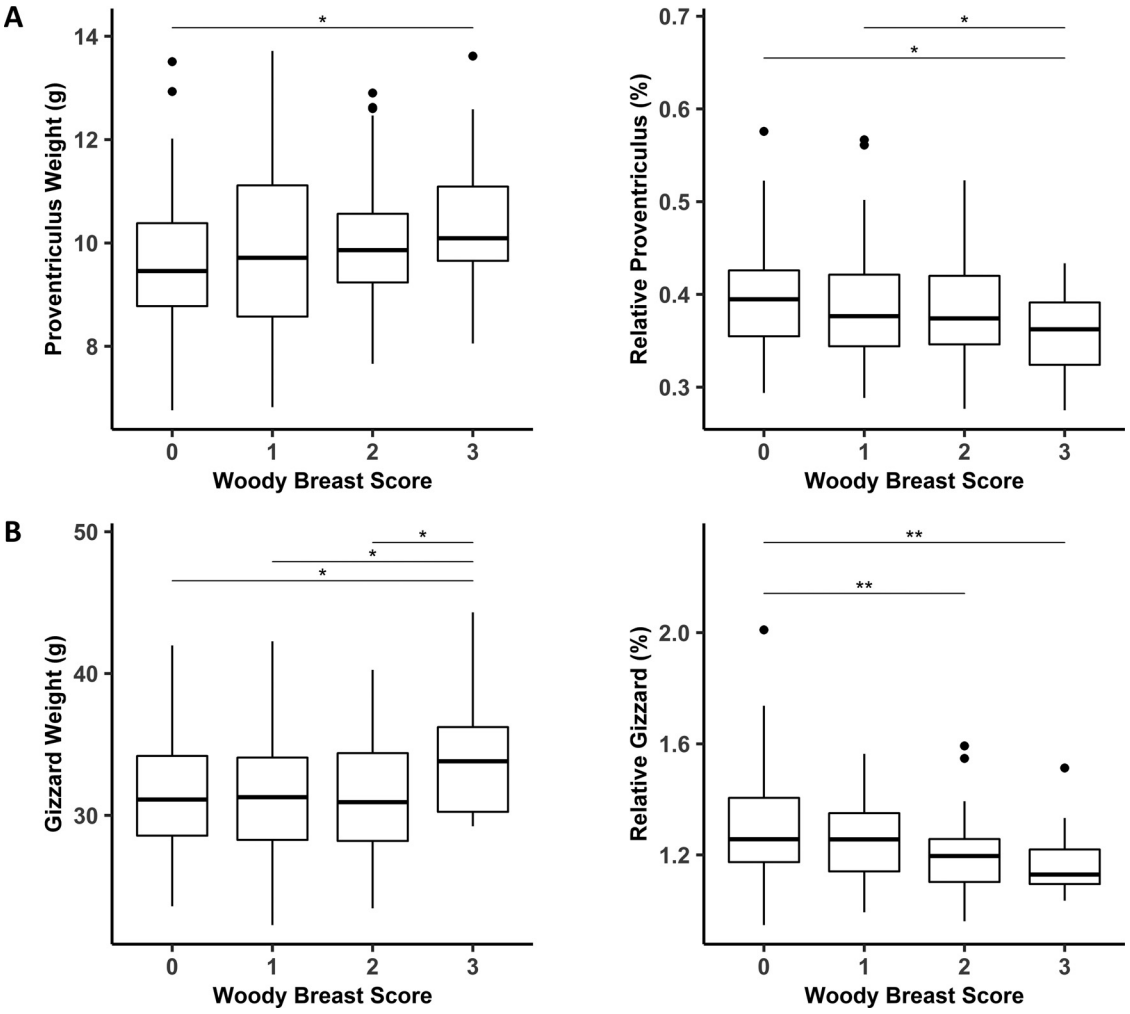

Figure 3. Comparison of birds with normal and woody breast on the absolute and relative proventriculus (A) and gizzard (B) weight on d 41. Woody breast score 0 (n = 67), 1 (n = 66), 2 (n = 38), and 3 (n = 21) were obtained by palpation. Statistical significance was determined by by pairwise *t* test. $^{*}P < 0.05$, $^{**}P < 0.01$.

and wing weights but lighter relative drumstick, thigh, and wing weights than those with normal breasts.

DISCUSSION

Birds with WB myopathy were hypothesized to have poor internal organ and skeletal muscle development. For this purpose, the WB score was measured and compared to a variety of internal organ and skeletal muscle development characteristics to assess the health conditions of WB birds. The results of this study showed differences in internal organ and skeletal muscle weights among different WB score groups. Determinations of internal organ and skeletal muscle differences in birds with WB myopathy will help to reveal potential factors contributing to WB development and potential methods for preventing WB development.

The first objective of this study was to determine the internal organ development of birds with WB. In comparison to females, higher relative and absolute heart weights were observed in males on d 41 (Table 3). This finding is consistent with that of van der Klein et al. (2017) who also found that male broilers had higher heart weights than females on d 35 (van der Klein et al., 2017). In comparison to birds with normal breasts, those with WB score 3 had higher absolute and relative heart weights (Figure 1). The higher absolute heart weights in WB birds may be due to the fact that the higher rate of hypertrophic muscle growth of WB birds results in an increased basal metabolic rate (Kuttappan et al., 2021). Therefore, birds with WB have high demands on their cardiovascular systems to provide sufficient oxygen delivery to their vascularity and for the efficient removal of metabolic products (Scanes, 2015). In addition, relative heart weight also increased by breast conditions, so it is possible that the WB birds need larger heart to maintain the function. The liver is a primary metabolic organ for poultry that has numerous functions such as digestion, metabolism, biosynthesis, waste product removal, and detoxification (Zaefarian et al., 2019; Xing et al., 2021). Birds with WB score 3 had greater absolute but not relative liver weights in comparison to those with normal breasts (Figure 1). Two recent studies have found that birds with the WB myopathy have higher hepatic oxidative stress, apoptosis, and inflammation compared with those with normal breasts (Xing et al., 2021; Zhang et al., 2021b). These chronic inflammatory

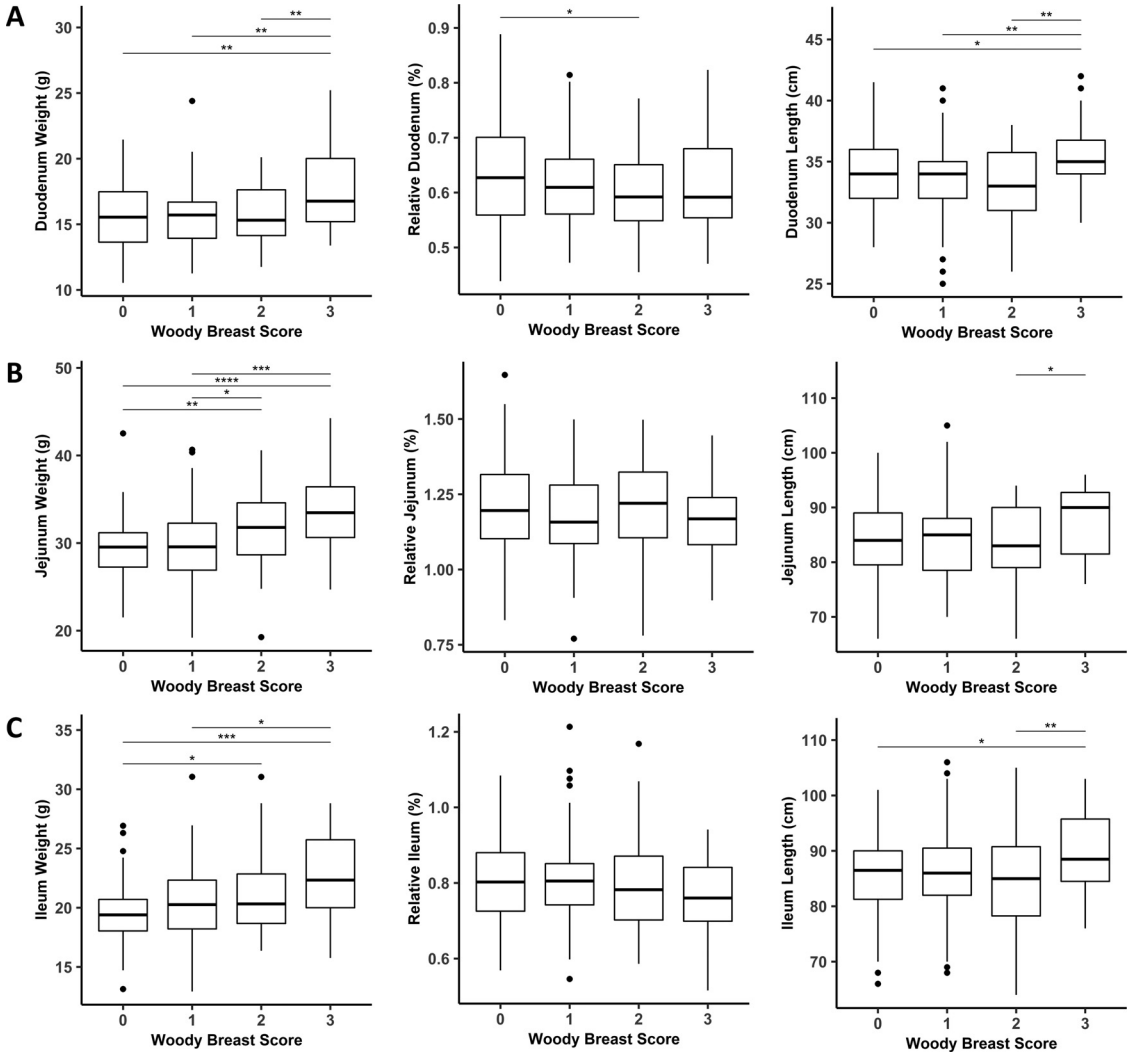

Figure 4. Comparison of birds with normal and woody breast on the absolute and relative duodenum (A), jejunum (B) and ileum (C) weight and length on d 41. Woody breast score 0 (n = 67), 1 (n = 66), 2 (n = 38), and 3 (n = 21) were obtained by palpation. Statistical significance was determined by pairwise *t* test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

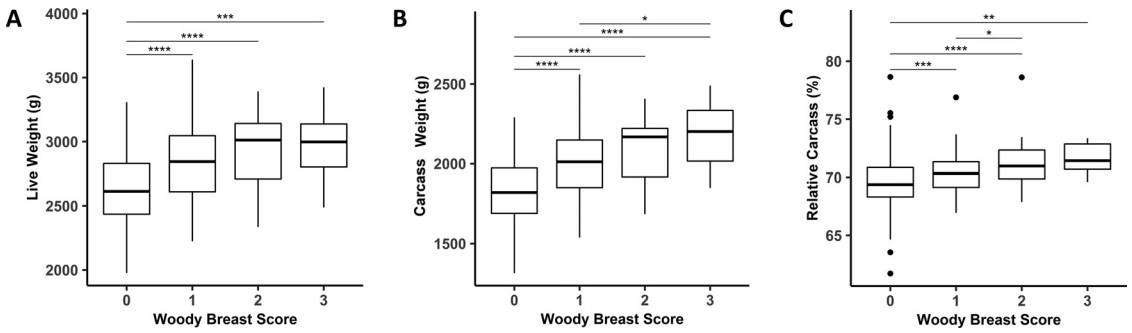

Figure 5. Comparison of birds with normal and woody breast on the live weight (A), carcass weight (B), and relative carcass weight (C) on d 44. Woody breast score 0 (n = 338), 1 (n = 101), 2 (n = 25), and 3 (n = 8) were obtained by palpation. Statistical significance was determined by pairwise *t* test. $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

responses may impair liver morphology and cause a higher absolute liver weight in birds affected by the WB myopathy.

Lymphoid organ weight is commonly used for reflecting the immune status of birds (Heckert et al., 2002). The immune status of animals can be predicted by determining their relative lymphoid organ weights, and immune-compromised animals have reduced lymphoid organ weights (Rose and Hesketh, 1979; Fan et al., 2013). A minimum bursa-to-body weight ratio of 0.11 is proposed to occur in standard commercial healthy flocks of broilers from 7 to 42 d of age (Cazaban et al., 2015). Because the relative bursa weights of the birds in this study were not lower than this minimum value, it is suggested they have normal and healthy bursa. Overall, there was no difference in the relative bursa and spleen weights of birds with woody and normal breasts on d 41 (Figures 2A and 2B). It was found that birds with WB

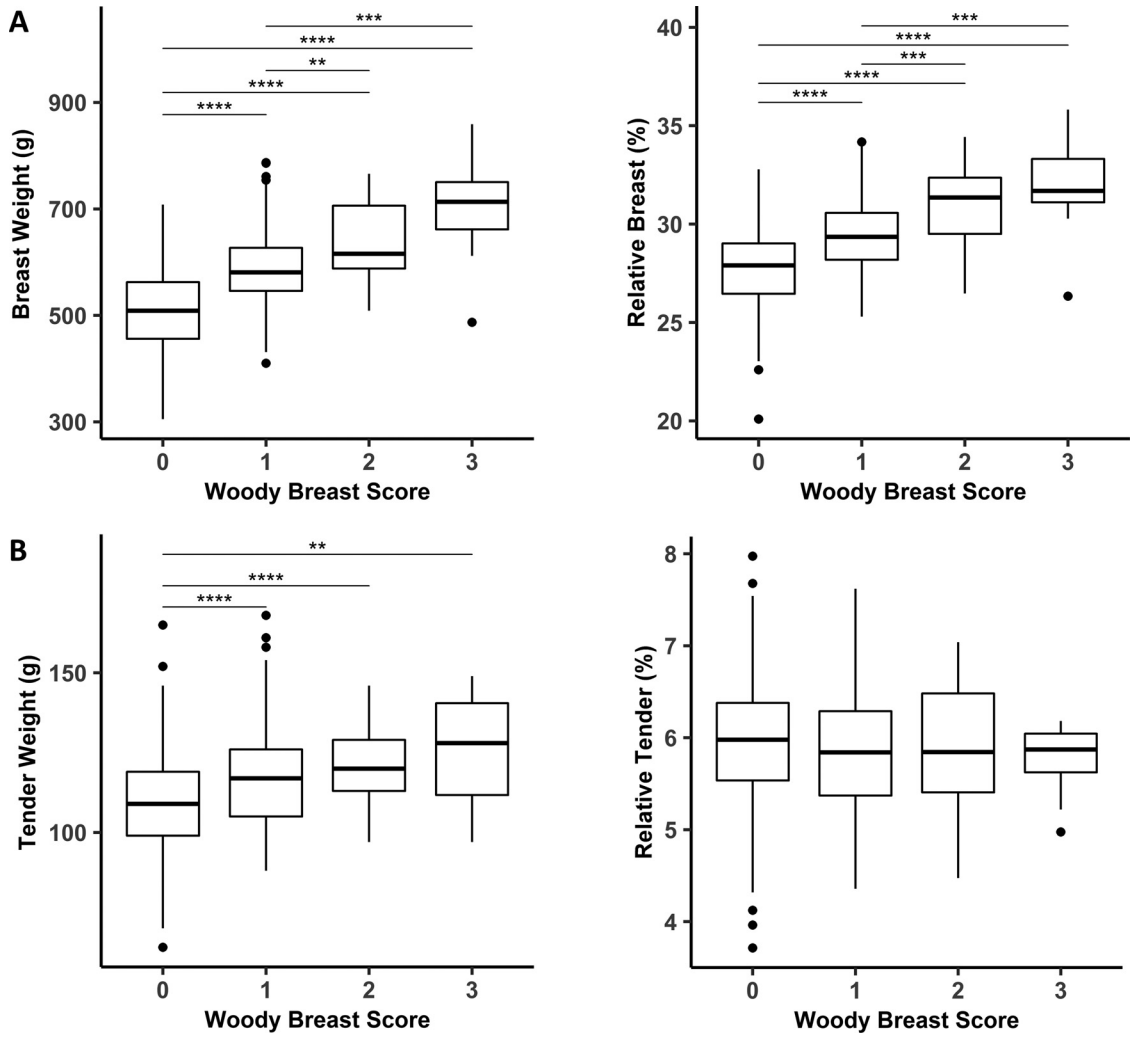

Figure 6. Comparison of birds with normal and woody breast on the absolute and relative breast (A) and tender (B) weight on d 44. Woody breast score 0 (n = 338), 1 (n = 101), 2 (n = 25), and 3 (n = 8) were obtained by palpation. Statistical significance was determined by pairwise *t* test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

score 3 exhibited a higher absolute bursa and spleen weight (Figures 2A and 2B), which may be due to heavier body weight of broilers with WB score 3.

The gastrointestinal (**GI**) tract of chickens includes the proventriculus, gizzard, and intestines, and the GI tract is responsible for nutrient digestion and absorption (Svihus, 2014). Interestingly, it was observed in this study that decreased relative proventriculus and gizzard weight occurred in birds with WB score 3 (Figure 3), suggesting that the birds with WB may have an upper GI tract digestion function that differs from that of birds with normal breasts. However, the reason why WB birds tend to have smaller proventriculi and gizzards is still unknown. Further research should focus on the feed intake and digestive function of birds with WB. It was found that birds with WB score 3 have higher absolute but not relative weights of the duodenum, jejunum, and ileum (Figure 4). Birds with WB have a greater body mass in comparison to birds with normal breasts. Thus, WB birds need to eat more feed to maintain a basal metabolism. Higher absolute duodenum and ileum weights facilitate higher levels of feed digestion and energy intake and subsequently lead to a greater body mass. However, there were no differences in the relative jejunum and ileum weights between birds with or without WB, which would indicate that birds with or without WB have similar small intestine functions and can, therefore, maintain basic digestion and absorption functions.

The second objective was to determine the skeletal muscle development of birds with WB. As expected, birds with WB scores 1, 2, and 3 had higher live and carcass weights than birds with normal breasts (Figure 5). It was found that birds with WB score 1, 2, and 3 exhibited heavier absolute breast and tender weights (Figure 6), which agrees with a previous study that reported that WB incidence is positively associated with breast weight (Zhang et al., 2021b). Birds with WB also exhibited a greater relative breast weight (Figure 6). This increased proportion of breast may reduce the walking ability of birds (Norring et al., 2018). Thus, a reduction in the movement of WB birds may decrease the growth of their legs and wings at the expense of heavier breast muscles. The findings in this study confirmed the hypothesis that lower relative wing, drumstick, and thigh of weights in birds are associated with WB (Figure 7).

The purpose of this study was to observe internal organ and skeletal muscle development in birds with WB

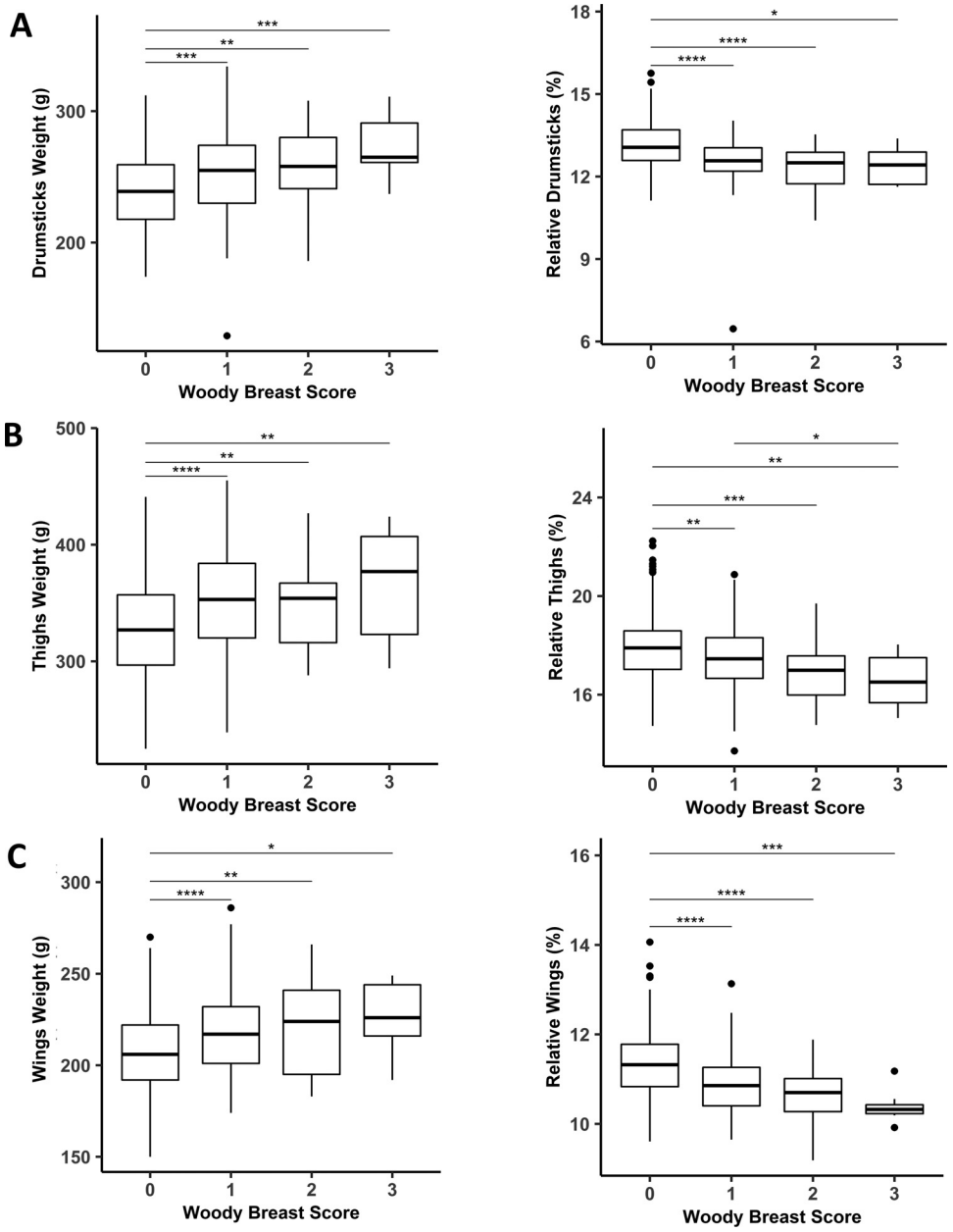

Figure 7. Comparison of birds with normal and woody breast on the absolute and relative drumsticks (A), thighs (B), and wings (C) weight on d 44. Woody breast score 0 (n = 344), 1 (n = 101), 2 (n = 25), and 3 (n = 8) were obtained by palpation. Statistical significance was determined by pairwise *t* test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

and normal breasts. Due to the sampling size, all the treatments were pooled together and regrouped birds by their WB scores for data analyses between an internal organ and skeletal muscle development and woody breast scores. This way, the effect of treatments on the internal organ and skeletal muscle development of broilers were compromised. To remove the noise of treatments, larger sampling size was suggested for future study.

In conclusion, WB myopathy is related to the weight of the digestive organs. Birds with WB have relatively smaller proventriculus and gizzard weights. Also, birds with WB myopathy have a relatively high breast meat weight, but a relatively low drumstick, thigh, and wing muscle weights, which are associated with movement, are relatively smaller in WB birds. These findings suggest that WB myopathy may have the unintended consequence of negatively influencing broiler physiology by interfering with the ability of the internal organs and skeletal muscles to develop.

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DISCLOSURES

The authors have declared no conflict of interest.

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