

Progressive in vivo detection of wooden breast in broilers as affected by dietary energy and protein

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ABSTRACT Wooden breast (WB) myopathy was investigated in broilers fed varying energy and protein at early ages. Correlation analyses were conducted between echogenicity of ultrasound images (US) of breast muscle from live birds and WB after slaughter. A total of 1,000 Cobb 500 one-day-old male chicks were fed on five dietary programs with eight replicates of 25 birds each, in a completely randomized design. Control feeds (commercially used ME and ideally balanced amino acids) or low-density feeds (low EP, with reductions of 50 kcal/kg ME and 0.20% dig. Lys compared to the control) were formulated. Feeds were provided in different periods: 1 to 7 d, 8 to 14 d, 15 to 21 d or 22 to 28 d. All broilers were fed a common basal diet thereafter until 49 d. Images using US were obtained once a week from all individuals and WB scored from one slaughtered bird per replication (0, normal; 1, mild hardening in the upper breast muscle; 2; moderate hardening

in the upper and/or lower breast muscle; 3, severe hardening; 4, severe hardening with hemorrhagic lesions and yellow fluid). Blood was collected for enzyme investigation from the weekly slaughtered bird. Broilers had lower BWG and higher FCR when fed low EP feeds, regardless of the period fed when compared to the control ($P < 0.001$). Growth compensation, however, occurred afterwards such that all birds presented similar performance at the end. At 14, 21, and 28 d, broilers previously fed low EP feeds had lower WB scores ($P < 0.001$) compared to birds fed the control; however, both groups presented increased WB scores after 28 d. Wooden breast was positively correlated with breast echogenicity at 21 d ($r = 0.31$), 28 d ($r = 0.43$), 35 d ($r = 0.21$) and 42 d ($r = 0.39$). In conclusion, dietary energy and protein affected the development of WB scores in broilers and breast US images can be used as an early predictor of WB.

Key words: compensatory growth, echogenicity, feed restriction, ultrasound, wooden breast

2021 Poultry Science 100:101120

<https://doi.org/10.1016/j.psj.2021.101120>

INTRODUCTION

Wooden breast (WB) as well as other breast myopathies are positively related to fast growth rate and high breast meat yields in broiler chickens (Kuttappan et al., 2012; Sihvo et al., 2014; Cemin et al., 2018; Kawasaki et al., 2018). Wooden breast causes important economic losses due to changes in breast meat visual appearance as well as to negative meat quality characteristics, such as lower protein content and higher cooking losses when compared to non-affected meat (Mudalal et al., 2014; Trocino et al., 2015; Tijare et al.,

2016; Bowker et al., 2019; Petracci et al., 2019). Breast muscle tissue affected by WB is characterized by accumulation of interstitial connective tissue (Sihvo et al., 2014), variability in fiber size, immune cell infiltration and extensive collagen deposition (Velleman and Clark, 2015; Sihvo et al., 2017).

Ultrasound (US) is a fast and consolidated non-invasive diagnosis method that allows the observation of living animal tissues and, therefore, the identification of deviations from normality. Images obtained by US have been successfully used in genetic selection to estimate in vivo carcass and breast muscle weight and yields with broilers and turkeys (Silva et al., 2006; Oviedo-Rondón et al., 2007; Case et al., 2012). Muscle dystrophies have been well-diagnosed using US images of breast muscle of live broilers (Pillen et al., 2009; Simões et al., 2020). Echogenicity is a light intensity measurement calculated on a grayscale, which presents high correlation with the amount of interstitial fibrous tissue and fat and, therefore, became a

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Received November 9, 2020.

Accepted February 26, 2021.

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reliable method to identify muscle damage as well as the severity of lesions in breast muscles (Pillen et al., 2009). Ultrasound images of breast muscle infiltrated with inflammatory fluids, as it occurs in WB, are lighter in color than nonaffected ones (Simões et al., 2020).

Occurrence of WB in modern fast-growing broilers is accentuated as broilers grown to heavy body weights (Kuttappan et al., 2016; Livingston et al., 2018; Petracci et al., 2019). On the other hand, feed restriction programs have been shown to reduce myopathies (as well as other metabolic diseases related to growth), either by limiting the daily feed allowances or by feeding low energy and low protein density feeds (De Jong et al., 2012; Trocino et al., 2015; Meloche et al., 2018a,b; Simões et al., 2020). Growing broiler chickens have demonstrated the capability of reaching acceptable final body weight as well as live performance when early feed restriction was applied attempting to reduce body fat content of broilers (Leeson et al., 1992). Therefore, broilers fed diets that limit early growth may have competitive life performance paralleling lower WB incidences and reduced WB scores.

The objective of the present study was to determine the occurrence of WB in broilers fed in early dietary programs having lower metabolizable energy (ME) and ideal protein (IP) densities in comparison to those popularly used by broiler meat producers. Feeding programs were applied at different week periods until 28 d. Echogenicity from US images and visual WB scores were weekly obtained and were until 49 d-of-age.

MATERIALS AND METHODS

All procedures used in this study were approved by the Ethics and Research Committee of the Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil.

Bird Husbandry

A total of 1,000 one-day-old, slow feathering Cobb x Cobb 500 male chicks, vaccinated for Marek's disease at the hatchery (BRF, Lajeado, RS, Brazil) averaging 46 ± 1.1 g were randomly distributed into 40 floor pens (1.65×1.65 m; 9.2 birds/m²). Birds were individually weighed, neck tagged with individual numbers and allocated to five treatments with 8 replications of 25 birds each in a completely randomized design. Bedding material was new rice hulls and individual pens were equipped with a 15 kg capacity tube feeder and three nipple drinkers. The average temperature was 32°C at placement being reduced by 1°C every 2 d until 23°C to provide comfort throughout the study with the use of thermostatically controlled heaters, fans and foggers. Lighting was continuous until 7 d-of-age, with a 16 L:8 D cycle used afterwards. Birds had free access to water and pelleted feeds.

Experimental Feeds

Broilers were fed pelleted feeds originated from a commercial integrator in a 5-phase feeding program,

comprising a pre-starter (1-7 d), a starter (8-21 d), a grower I (22-28 d), a grower II (29-42 d) and a finisher (43-49 d) diet. Birds were fed a control feeding program having standard ME and IP or low-density feeds (**low EP**) having reductions of 50 kcal/kg ME and 0.20% digestible lysine (**dig. Lys**) for one week in the corresponding feed (1-7 d, 8-14 d, 15-21 d, or 22-28 d-of-age). From 29 to 49 d, all broilers had free access to common feeds.

The control feeds had pre-starter, starter, and grower I feeds with 3,030, 3,120 and 3,200 kcal/kg ME and 1.31, 1.20, and 1.12% dig. Lys, respectively. The other 4 groups were fed low EP prestarter, starter, and grower I feeds with 2,980, 3,070, and 3,150 kcal/kg and 1.11, 1.00, and 0.92% dig. Lys. Feed compositions are presented in Table 1.

Growth Performance and Carcass Yield

Body weight gain (**BWG**), feed intake (**FI**), and feed conversion ratio corrected for the weight of dead birds (**FCR**) were weekly determined throughout the study. Processing at 49 d was done with 5 birds randomly selected from each pen for carcass and commercial cuts evaluation. Broilers were fasted for 8 h and individually weighed prior to slaughter. Birds were humanely rendered insensible using electrical stunning (45 V for 3 s), then bled through a jugular vein cut for 3 min, scalded at 60°C for 45 s, and lastly defeathered. Evisceration was manually performed, and carcasses were statically chilled in ice for approximately 3 h. Eviscerated carcasses (without feet and neck) were hung for 3 min to remove excess water prior to weighing. Commercial cuts were performed by a crew of industry-trained personnel into bone-in drumsticks, thighs, and wings as well as deboned breast fillets and tenders. Abdominal fat was manually removed from carcasses and weighed separately. Carcass yield was expressed relative to live weight whereas commercial cuts and abdominal fat were expressed as percentages of eviscerated carcass.

Ultrasonography, Wooden Breast Scoring and Blood Sera

One bird was randomly taken every week from each pen ($n = 72$) to obtain echogenicity using US from 7 to 49 d. A Logiq E equipment (GE Healthcare, Little Chalfont, United Kingdom) with a probe (L8-18i-RS) was placed on the skin surface parallel to the keel as well as cranial and caudal to the ribcage (Figure 1). Image frequency was 18 MHz with 64% brightness. Mean grey values were calculated from the obtained histogram (black to white scale from 0 to 255, respectively) using Adobe Photoshop (Adobe Systems Inc, San Jose, CA, USA) (Pillen et al., 2009; Simões et al., 2020). Breast depth was measured using the same US images with the Image ProPlus software (Media Cybernetics Inc, Bethesda, MD, USA) as described by Simões et al. (2020).

Table 1. Ingredient and nutrient composition of experimental feeds varying in energy and protein (as-is basis).

Item	Pre-starter(1 to 7 d)		Starter(8 to 21 d)		Grower I(22 to 28 d)		Common	
	Control	Low EP ¹	Control	Low EP	Control	Low EP	Grower II(29 to 42 d)	Finisher (43 to 49 d)
Ingredients, %								
Corn	52.61	62.93	57.26	68.78	61.59	72.65	63.64	65.93
Soybean meal	40.10	30.20	35.10	25.80	31.00	22.50	29.20	26.91
Soybean oil	2.99	1.00	3.60	1.00	3.90	1.29	3.85	4.30
Dicalcium phosphate	1.20	1.30	1.10	1.10	0.90	1.00	0.80	0.70
Limestone	1.55	1.56	1.40	1.41	1.25	1.25	1.20	1.00
Salt	0.47	0.55	0.50	0.50	0.40	0.40	0.40	0.40
DL-Met, 89%	0.36	0.26	0.31	0.22	0.28	0.19	0.29	0.25
L-Lys HCl, 70%	0.23	0.28	0.25	0.30	0.25	0.30	0.26	0.27
L-Thr, 98.5%	0.03	0.03	0.03	0.02	0.04	0.02	0.04	0.04
Choline chloride, 75%	0.11	0.14	0.10	0.12	0.08	0.09	0.08	0.01
Vit. and min. mix ²	0.33	0.33	0.33	0.33	0.29	0.29	0.22	0.17
Kaolin	0.00	1.40	0.00	0.40	0.00	0.00	0.00	0.00
Enzymes ³	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Energy and nutrient composition, % or as shown ⁴								
ME, kcal/kg	3,030	2,980	3,120	3,070	3,200	3,150	3,220	3,220
CP	23.85 (23.7)	19.95 (19.9)	21.92 (21.8)	18.37 (18.5)	20.33 (20.4)	17.11 (17.5)	19.67(19.7)	18.70 (18.5)
Ca	1.05 (1.08)	1.05 (1.09)	0.96 (0.99)	0.95 (0.97)	0.86 (0.85)	0.86 (0.86)	0.79 (0.80)	0.74 (0.75)
Total P	0.58 (0.57)	0.57 (0.56)	0.54 (0.53)	0.51 (0.52)	0.49 (0.48)	0.48 (0.48)	0.46 (0.45)	0.43 (0.44)
Available P	0.50	0.51	0.47	0.46	0.42	0.43	0.40	0.38
Na	0.24	0.24	0.22	0.22	0.18	0.18	0.18	0.18
Cl	0.39	0.39	0.35	0.35	0.29	0.29	0.29	0.28
Dig. Lys	1.31	1.11	1.20	1.00	1.12	0.92	1.07	1.02
Dig. Met +Cys	0.98	0.81	0.89	0.74	0.84	0.69	0.81	0.78
Dig. Thr	0.85	0.71	0.78	0.65	0.73	0.61	0.71	0.71
Dig. Trp	0.24	0.19	0.22	0.17	0.20	0.16	0.19	0.19
Dig. Arg	1.49	1.20	1.35	1.08	1.23	0.99	1.18	1.18
Dig. Val	0.98	0.82	0.90	0.75	0.84	0.70	0.81	0.81
Dig. Ile	0.71	0.77	0.86	0.70	0.78	0.64	0.75	0.75

¹Low EP = low-density feed formulated with 50 kcal/kg ME and 0.2% digestible Lys reductions in the control feed.

²Composition per kg of premix: vitamin A, 9,000 UI; vitamin D₃, 2,500 UI; vitamin E, 20,000 UI; vitamin K₃, 2,500 mg; thiamine, 2,000 mg; riboflavin, 6,000 mg; pyridoxine, 3,000 mg; cyanocobalamin, 0.015 mg; pantothenic acid, 12 g; niacin, 35 g; folic acid, 1,500 mg; biotin, 100 mg; iron, 100 g; zinc, 130 g; manganese, 130 g; copper, 20 g; iodine, 2 g.; selenium, 250 mg.

³Phytase at 20,000 fungal phytase units (FTU)/L (AB Vista, Plantation, FL, US) and 700 xylanase units (AXC)/mL + 1,000 β -glucanase units (AGL)/mL (Adisseo, Alpharetta, GA, US)

⁴Analyzed crude protein, calcium and phosphorus from one pooled sample from each feed mix batch.

After obtaining echogenicity using US, birds were slaughtered to evaluate WB occurrence. Deboned and skinless breasts fillets were submitted to a 4-subject panel evaluation to provide scores according to Simões et al. (2020) from 7 to 49 d-of-age. Scores included the absence of WB (normal breast-score 0); mild hardening in the upper (score 1); moderate hardening in the upper and/or lower part of the fillet (score 2); severe hardening (score 3); and severe hardening with hemorrhagic lesions, increased volume and presence of yellow fluid (score 4).

Blood collection was done from 21 to 49 d immediately after a jugular vein cut being then centrifuged (2,500 RPM for 10 min) and the obtained serum was used to estimate activities of creatine kinase (**CK**; Randox, Crumlin, County Antrim, UK), alanine aminotransferase (**ALT**) and lactate dehydrogenase (**LDH**) (Sigma Aldrich, Poole, Dorset, UK), as well as aspartate aminotransaminase (**AST**; Thermo Electron Corporation, Runcorn, Warrington, UK). Biochemical tests were performed on thawed samples in duplicate. A high performance reading spectrophotometer with automatic calibration (CM 200; Wiener lab, Rosario, Argentina) was used at a wavelength of 340 nm (Konelab 20i; ThermoElectro Corporation, Espoo, Finland) by means of commercially diagnostic kits.

Statistical Analysis

Data was analyzed for homoscedasticity (Shapiro and Wilk, 1965) and those normally distributed were submitted to the analysis of variance using the PROC MIXED of SAS (SAS, 2012). Significance was accepted at 5%. Dietary treatments were used as independent variables and results were separated according to broiler age. Pearson's correlation analysis was conducted among WB and echogenicity, depth or blood measurements using the CORR procedure (SAS, 2012).

RESULTS

There were no effects of dietary treatments on mortality (grand mean = 0.49%). Effects of feeding programs on BWG, FCR, and FI are presented in Tables 2 and 3. Decreases in BWG of broilers were observed in the concurrently low EP fed weeks compared to the control feed ($P < 0.001$). From 29 to 49 d, broilers previously feed restricted demonstrated compensatory BWG and all birds presented similar performance at the end ($P > 0.05$). Cumulative data from 1 to 49 d showed that broilers fed the low EP feeds presenting similar ($P > 0.05$) BWG compared to the other groups. Feed intake

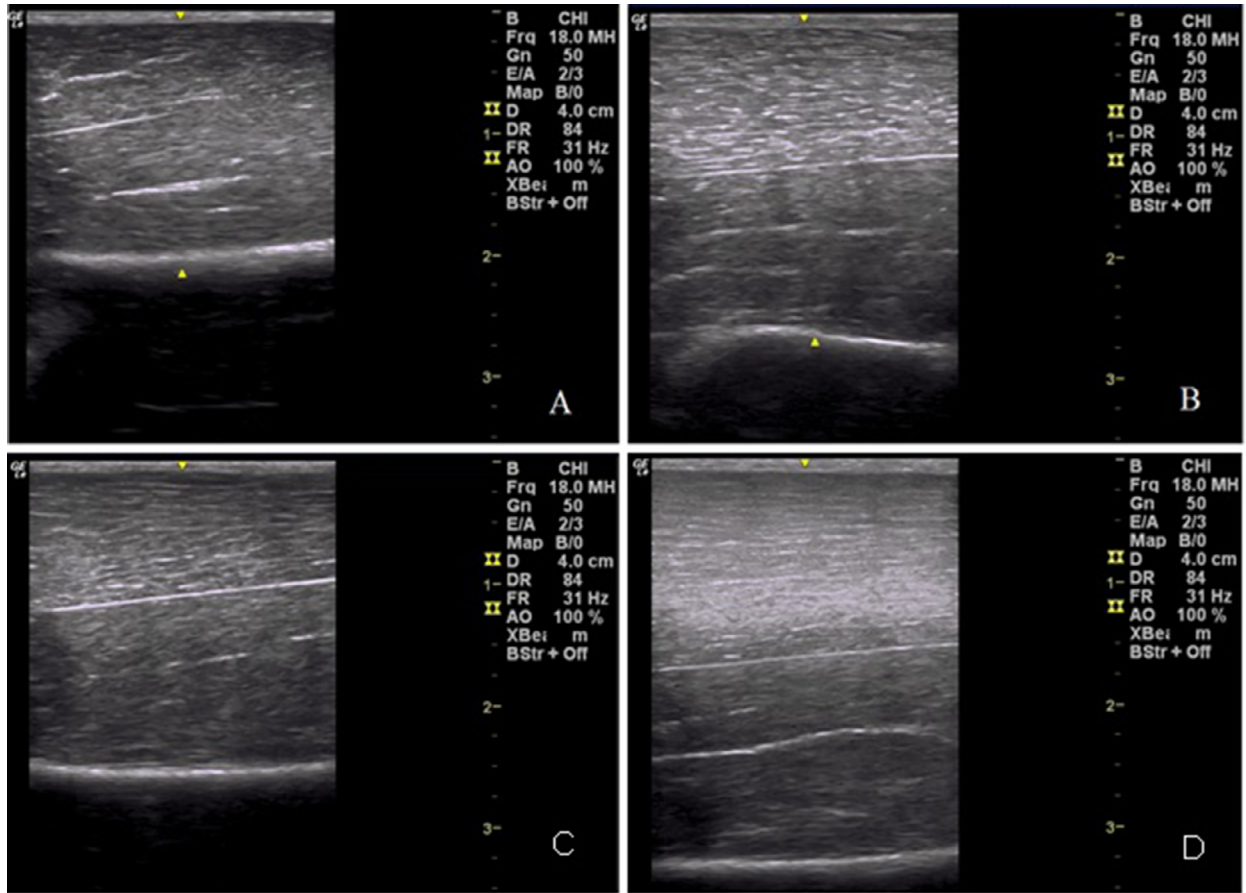


Figure 1. Cross-sectional view of the breast muscle of broilers at 42 and 49 d captured using ultrasound equipment. Mean grey values were calculated from the obtained histogram (black to white scale from 0 to 255, respectively) to determine echogenicity. (A) Wooden breast score 1 at 42 d. (B) Wooden breast score 4 at 42 d. (C) Wooden breast score 1 at 49 d. (D) Wooden breast score 4 at 49 d.

was lower when broilers were fed the low EP feed in comparison to the other treatments ($P < 0.01$); however, from 29 to 49 d no differences were observed on FI ($P > 0.05$). In the overall period, broilers fed the low EP feed from 1 to 7 d had lower FI than broilers fed the control ($P < 0.05$).

There were no differences on FCR among treatments from 1 to 7 d. However, in the following periods, broilers fed the low EP feeds had higher FCR than broilers fed the control ($P < 0.01$). Feed conversion from 1 to 49 d

was lower in broilers fed the low EP diet from 1 to 7 d compared to the other groups ($P < 0.01$).

No effects of dietary treatments were observed on carcass as well as in the yields of commercial cuts at 49 d (Table 4). The WB scores were affected by feeding programs at different ages (Table 5.) At 14 d, broilers fed the low EP feeds from 1 to 7 d or 8 to 14 d had lower ($P < 0.0001$) WB scores when compared to broilers fed the control. At 21 d, broilers fed the low EP from 15 to 21 d presented the lowest score of WB ($P < 0.05$). The

Table 2. Effect of early feeding restriction programs varying in energy and protein on weekly growth performance of broilers.

Feeding program	Body weight gain, g				Feed conversion ratio				Feed intake, g			
	1 to 7 d	8 to 14 d	15 to 21 d	22 to 28 d	1 to 7 d	8 to 14 d	15 to 21 d	22 to 28 d	1 to 7 d	8 to 14 d	15 to 21 d	22 to 28 d
Control	125 ^a	332 ^a	528 ^a	759 ^a	1.316	1.255 ^b	1.451 ^b	1.509 ^b	164 ^a	417 ^a	765 ^a	1,146 ^a
Low EP ¹ 1-7 d	74 ^b	240 ^b	490 ^{ab}	702 ^a	1.249	1.372 ^b	1.305 ^c	1.430 ^{bc}	92 ^b	329 ^b	640 ^b	1,003 ^b
Low EP 8-14 d	124 ^a	135 ^c	467 ^b	664 ^a	1.372	1.568 ^a	1.227 ^c	1.470 ^{bc}	170 ^a	318 ^b	572 ^c	976 ^b
Low EP 15-21 d	121 ^a	322 ^a	408 ^c	690 ^a	1.355	1.228 ^b	1.677 ^a	1.384 ^c	164 ^a	394 ^a	680 ^b	955 ^b
Low EP 22-28 d	124 ^a	320 ^a	506 ^{ab}	529 ^b	1.351	1.246 ^b	1.448 ^b	1.928 ^a	168 ^a	399 ^a	733 ^a	1,020 ^{ab}
SEM	3.3	12.4	8.3	16.9	0.016	0.072	0.026	0.033	5.07	7.33	12.19	18.32
P-value	0.0001	0.0001	0.0001	0.0001	0.1123	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0031

^{a-c}Means with different superscript letter differ ($P < 0.05$) based on Tukey's honestly significant difference test.

¹Low EP = low-density feed formulated with 50 kcal/kg ME and 0.2% digestible Lys reductions in the control feed. The low EP diet was provided only for one treatment in each period and all other groups were fed the control.

Table 3. Effect of early feeding restriction programs varying on energy and protein on cumulative growth performance of broilers.

Feeding program	Body weight gain, g		Feed conversion ratio		Feed intake, g	
	29 to 49 d	1 to 49 d	29 to 49 d	1 to 49 d	29 to 49 d	1 to 49 d
Control	2,208	3,963	1.841 ^a	1.656 ^a	4,065	6,563 ^a
Low EP ¹ 1-7 d	2,290	3,797	1.791 ^{ab}	1.582 ^b	4,101	6,007 ^b
Low EP 8-14 d	2,361	3,750	1.771 ^{ab}	1.633 ^{ab}	4,181	6,125 ^b
Low EP 15-21 d	2,392	3,948	1.750 ^{ab}	1.620 ^{ab}	4,186	6,396 ^{ab}
Low EP 22-28 d	2,342	3,822	1.741 ^b	1.650 ^a	4,077	6,306 ^b
SEM	35.9	43.3	0.011	0.007	47.9	53.6
<i>P</i> -value	0.4088	0.1589	0.0345	0.0018	0.6250	0.0030

^{a-c}Means with different superscript letter differ ($P < 0.05$) based on Tukey's honestly significant difference test.

¹Low EP = low-density feed formulated with 50 kcal/kg ME and 0.2% digestible Lys reductions in the control feed. The low EP diet was provided only for one treatment in each period and all other groups were fed the control.

Table 4. Effect of early feeding restriction programs varying on energy and protein on carcass and commercial cuts yield of broilers at 49 d, %.¹

Feeding program	Carcass ²	Breast fillet ³	Tenders	Thighs	Drumsticks	Wings	Abdominal fat
Control	82.6	31.3	5.26	18.0	11.6	9.3	1.57
Low EP ⁴ 1-7 d	82.9	30.4	5.06	18.3	12.1	9.1	1.71
Low EP 8-14 d	83.0	30.5	5.27	18.2	12.1	9.2	1.68
Low EP 15-21 d	82.6	30.2	5.28	18.0	11.7	9.2	1.73
Low EP 22-28 d	83.5	30.5	5.35	18.1	11.7	9.2	1.72
SEM	0.19	0.22	0.059	0.06	0.07	0.03	0.057
<i>P</i> -value	0.5691	0.5544	0.2061	0.5314	0.3670	0.4907	0.8302

¹Means of 8 replicates of 5 birds randomly sampled per pen.

²Eviscerated carcass as a percentage of body weight, whereas cuts are proportions of the carcass.

³Skinless boneless *Pectoralis major* as a proportion of carcass.

⁴Low EP = low-density feed formulated with 50 kcal/kg ME and 0.2% digestible Lys reductions in the control feed. The low EP diet was provided only for one treatment in each period and all other groups were fed the control.

same response was observed at 28 d, where broilers fed the low EP diet from 22 to 28 d presented the lowest WB occurrence ($P < 0.01$). After 28 d, all broilers presented similar means of WB even those birds previously fed with low EP feeds ($P > 0.05$).

The occurrence of WB from 14 to 49 d is presented in the [Figure 2](#). Nonaffected breasts (score 0) were

observed only until 21 d, whereas the WB score 4 was firstly identified at 28 d. Broilers fed the low EP feeds from 1 to 7 d or 8 to 14 d had lower WB occurrence at 14 d compared to broilers fed the control. At 21 d, broilers fed the low EP from 15 to 21 d presented higher presence of normal breast compared to broilers fed the low EP feeds from 1 to 7 or 8 to 14 d and the control. After 28 d, the WB occurrence was similar among dietary treatments, and at 42 d or 49 d all treatments presented occurrences of WB scores 3 and 4.

Echogenicity of the *Pectoralis major* in live broilers as well its depth from 7 to 49 d-of-age are shown in the [Table 6](#). At 21 d, echogenicity decreased ($P < 0.01$) when broilers were fed the low EP feed from 15 to 21 d ($P < 0.01$). However, the echogenicity and breast depth were not different among treatments at 35, 42 and 49 d. Breast depth decreased ($P < 0.01$) when broilers were fed the low EP feed from 1 to 7 d, 8 to 14 d, 15 to 21d or from 22 to 28 d whereas broilers fed the control diet during these periods presented the greatest breast depth ($P < 0.01$).

The ALT serum concentration was not affected by the feeding programs during the whole period ([Table 7](#)); however, the concentration of AST and CK at 21 d decreased ($P < 0.05$) when broilers were fed the low EP diet from 15 to 21 d compared broilers fed the control.

Table 5. Effect of early feeding restriction programs varying on energy and protein on wooden breast scores of broilers at different ages.¹

Feeding program	14 d	21 d	28 d	35 d	42 d	49 d
Control	0.87 ^a	1.62 ^a	2.62 ^a	2.57	2.62	2.75
Low EP ² 1-7 d	0.12 ^b	1.59 ^{ab}	2.50 ^a	2.37	2.62	2.62
Low EP 8-14 d	0.12 ^b	1.43 ^{ab}	2.00 ^{ab}	2.38	2.75	2.50
Low EP 15-21 d	0.87 ^a	1.12 ^b	2.00 ^{ab}	2.42	2.50	2.75
Low EP 22-28 d	1.00 ^a	1.75 ^a	1.87 ^b	2.37	2.50	2.62
SEM	0.089	0.093	0.105	0.081	0.112	0.116
<i>P</i> -value	0.0001	0.0356	0.0068	0.9414	0.9575	0.7493

^{a-b}Means with different superscript letter differ ($P < 0.05$) based on Tukey's honestly significant difference test.

¹Average of wooden breast scores of broilers according to the feeding programs. Scores ranged from 0 (absence) to 4 (severe hardening with hemorrhagic lesions and yellow fluid). All birds evaluated at 7 d presented score 0 of WB.

²Low EP = low-density feed formulated with 50 kcal/kg ME and 0.2% digestible Lys reductions in the control feed. The low EP diet was provided only for one treatment in each period and all other groups were fed the control.

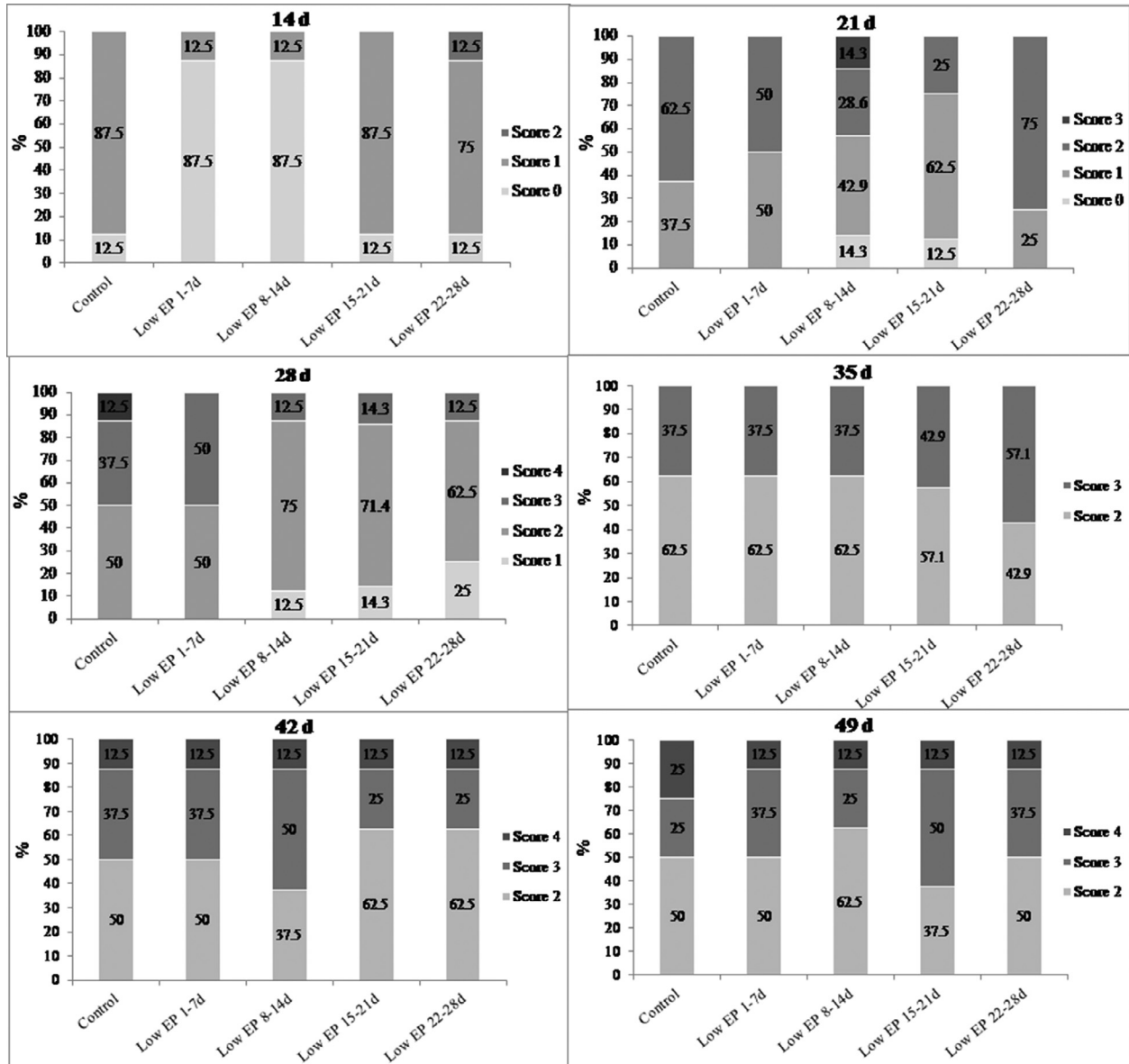


Figure 2. Wooden breast occurrence in breast fillets of broilers from 14 to 49 d-of-age. Wooden breast scores were normal breast (score 0), mild hardening in the upper (score 1), moderate hardening in the upper and/or lower part of the fillet (score 2), severe hardening (score 3), and severe hardening with hemorrhagic lesions, increased volume, and presence of yellow fluid (score 4).

The LDH concentration at 21 d was lower when broilers were fed low EP diet from 15 to 21 d compared to birds fed the control feed or fed low EP feed from 1 to 7 d ($P < 0.05$). After 28 d, all broilers presented similar serum enzymes concentration.

Correlations among WB scores with significant responses are presented in the [Table 8](#). Wooden breast was positively correlated ($P < 0.01$) with echogenicity at 21 d ($r = 0.31$), 28 d ($r = 0.43$), 35 d ($r = 0.11$) and 42 d ($r = 0.39$) as well as with breast depth, where values were $r = 0.51$, $r = 0.33$, $r = 0.36$, and $r = 0.18$ at 21, 28, 35, and 42 d, respectively. Positive correlations were also observed between WB score and serum CK concentration at 28 d ($r = 0.46$), 35 d ($r = 0.74$), 42 d ($r = 0.37$), and 49 d ($r = 0.53$) as well as with serum AST at 42 d ($r = 0.37$) and 49 d ($r = 0.63$).

DISCUSSION

The current study used low-density feeds in programs aiming to reduce growth rate of broilers at early ages when compared to those usually fed by broiler chicken integrators in order to reduce WB occurrence and severity. In studies conducted recently, positive correlations between growth rate and WB has been demonstrated; however, the strategy used in the present study was unique ([Kuttappan et al., 2016](#); [Huang and Ahn, 2018](#); [Livingston et al., 2018](#)).

Research recently conducted have evaluated characteristics and occurrence of WB in broilers using dietary energy and nutrient intakes that impacted broiler ability to mitigate myopathy occurrence and severity ([Cruz et al., 2017](#); [Bodle et al., 2018](#); [Gratta et al., 2019](#); [Wang et al., 2020](#)). When feed was restricted at 80% of

Table 6. Effect of early feeding restriction programs varying on energy and protein on echogenicity and breast depth of broilers at different ages.

Age, d	Item	Feeding program					SEM	P-value
		Control	Low EP ¹ 1-7 d	Low EP 8-14 d	Low EP 15-21 d	Low EP 22-28 d		
7	Echogenicity ²	71.8	70.1	66.4	68.4	72.4	1.05	0.3232
	Depth, mm	7.5 ^a	5.9 ^b	6.6 ^{ab}	7.1 ^{ab}	7.6 ^a	0.17	0.0032
14	Echogenicity	78.6 ^a	73.3 ^{ab}	68.0 ^b	77.0 ^a	76.8 ^a	1.25	0.0498
	Depth, mm	11.8 ^a	10.4 ^b	9.3 ^c	11.7 ^a	11.9 ^a	0.18	0.0001
21	Echogenicity	83.7 ^a	85.4 ^a	79.3 ^{ab}	72.1 ^b	83.7 ^a	1.09	0.0001
	Depth, mm	20.1 ^a	19.3 ^a	17.0 ^b	18.6 ^{ab}	20.5 ^a	0.31	0.0006
28	Echogenicity	96.1	96.6	93.4	92.2	91.6	1.13	0.5380
	Depth, mm	21.6 ^a	20.3 ^{ab}	19.9 ^{ab}	19.8 ^{ab}	18.4 ^b	0.31	0.0100
35	Echogenicity	100.7	102.6	103.3	106.0	105.2	1.13	0.6500
	Depth, mm	23.3	24.7	23.1	24.6	25.7	0.35	0.1056
42	Echogenicity	108.6	105.8	106.6	106.4	109.1	0.84	0.6721
	Depth, mm	29.8	28.8	27.4	29.1	28.1	0.30	0.2608
49	Echogenicity	106.3	108.7	102.3	104.0	104.5	1.16	0.5414
	Depth, mm	30.6	29.9	30.0	29.8	29.7	0.58	0.9904

^{a-c}Means with different superscript letter differ ($P < 0.05$) based on Tukey's honestly significant difference test.

¹Low EP = low-density feed formulated with 50 kcal/kg ME and 0.2% digestible Lys reductions in the control feed. The low EP feed was provided only for one treatment in each period and all other groups were fed the control.

²Echogenicity mean grey values were calculated from the obtained histogram (black to white scale from 0 to 255, respectively) of *Pectoralis major* muscle in live broilers.

ad libitum from 13 to 21 d, Trocino et al. (2015) showed birds compensating growth afterwards, which allowed to reduce differences in final body weight while improving FCR. Cruz et al. (2017) demonstrated that growth rate and WB severity were reduced by feeding Lys deficient diets. These authors also observed that maximum responses for BWG, carcass weight, and breast weight of broilers at 42 d were obtained using 0.99%, 0.98%, and 0.98% of dig. Lys, respectively.

The current study demonstrated a decrease in BWG when broilers were fed reduced ME and dig. Lys compared to broilers fed on standard amino acids

and energy. Nevertheless, after feeding broilers with low EP, birds also demonstrated to compensate their BWG and presented similar WB severity at 49 d. The FI followed BWG results and it decreased when broilers were fed low EP feeds; however, no differences on FI and BWG were observed after 28 d when all groups received common feeds. Compensatory gain was also observed in the study conducted by Cruz et al. (2017), when broilers fed 1.01% dig. Lys from 12 to 28 d presented similar carcass and breast weights at 35 and 42 d compared to 1.17% dig. Lys.

Table 7. Effect of early feeding restriction programs varying on energy and protein on serum enzymes from broilers at different ages, U/L.

Age, d	Serum enzymes ¹	Feeding program					SEM	P-value
		Control	Low EP ² 1-7 d	Low EP 8-14 d	Low EP 15-21 d	Low EP 22-28 d		
21	ALT	0.85	0.50	1.87	1.25	0.55	0.349	0.4429
	AST	243 ^a	230 ^{ab}	224 ^{ab}	191 ^b	223 ^{ab}	5.6	0.0496
	CK	8,122 ^a	7,860 ^{ab}	5,379 ^b	5,688 ^{ab}	7,414 ^{ab}	326.8	0.0075
	LDH	3,303 ^a	3,298 ^a	3,113 ^{ab}	2,629 ^b	3,160 ^{ab}	81.6	0.0490
28	ALT	2.25	5.14	3.25	3.85	5.50	1.137	0.6964
	AST	282 ^{ab}	345 ^a	241 ^b	302 ^{ab}	243 ^b	11.6	0.0201
	CK	9,942 ^a	8,222 ^{ab}	6,046 ^b	6,920 ^b	7,154 ^b	341.4	0.0010
	LDH	3,506	3,921	2,931	3,873	2,730	164.7	0.0689
35	ALT	0.88	1.25	1.00	0.71	0.87	0.226	0.9667
	AST	445	470	426	603	502	23.1	0.1018
	CK	14,324	14,625	13,804	13,744	14,935	684.1	0.9826
	LDH	4,621	5,331	4,152	5,513	4,851	272.6	0.5500
42	ALT	3.14	1.50	2.28	1.12	0.62	0.381	0.2638
	AST	547	591	548	534	500	22.3	0.8165
	CK	20,040	20,224	20,797	18,235	17,526	645.7	0.4589
	LDH	6,374	5,782	5,238	5,454	4,731	238.8	0.2386
49	ALT ³	2.15	3.85	4.71	5.14	4.66	0.469	0.2660
	AST ⁴	849	842	727	929	904	49.1	0.7474
	CK ⁵	30,412	28,586	26,899	31,273	26,756	1,565	0.8701
	LDH ⁶	7,023	7,011	6,261	8,593	6,219	390.8	0.3229

^{a-b}Means with different superscript letter differ ($P < 0.05$) based on Tukey's honestly significant difference test.

¹Serum enzymes were evaluated weekly from 21 to 49 d: ALT: alanine aminotransferase; AST: aspartate aminotransferase; CK: creatine kinase; LDH: lactate dehydrogenase.

²Low EP = low-density feed formulated with 50 kcal/kg ME and 0.2% digestible Lys reductions in the control feed. The low EP feed was provided only for one treatment in each period and all other groups were fed the control.

Table 8. Pearson correlation among wooden breast scores and evaluated responses as broilers aged after being fed on feed restriction programs varying on energy and protein.

Age, d	Echogenicity ¹	Depth ²	AST ³	CK ⁴
21	0.31*	0.51**	NS	NS
28	0.43**	0.33*	NS	0.46**
35	0.11*	0.36*	NS	0.74**
42	0.39**	0.18*	0.37*	0.37*
49	NS	NS	0.63**	0.53*

¹Echogenicity, mean grey values were calculated from the obtained histogram (black to white scale from 0 to 255, respectively) of *Pectoralis major* muscle in live broilers.

²Depth of breast fillets using US images.

³Aspartate aminotransferase serum concentration.

⁴Creatine kinase serum concentration.

** $P \leq 0.01$; *** $P \leq 0.001$; NS $P > 0.05$.

Results in the present study were in parallel with those from [Meloche et al. \(2018a\)](#) when evaluating dietary amino acid and ME intake with resulting WB. These authors observed that reducing amino acid density and ME to 90 and 95% from 8 to 14 d led to growth compensation in the re-feeding period. [Radaelli et al. \(2017\)](#) evaluated 80% feed restriction in broilers from 13 to 21 d and found a successful reduction on WB with also the subsequent re-feeding period resulting in compensated growth at 46 d.

[Griffin et al. \(2018\)](#) reported that WB score 4 was firstly observed at 30 d-of-age. In the present study this finding was similar since the WB score 4 was firstly observed at 28 d. Major breast muscle affected with severe WB are characterized by variable degrees of hardness, showing bulging and pale expansive areas, often accompanied with white stripping ([Sihvo et al., 2014](#); [Sihvo et al., 2017](#); [Bowker et al., 2019](#)). Both myopathies showed myodegeneration with interstitial connective tissue accumulation ([Kuttappan et al., 2013, 2016](#); [Sihvo et al., 2014](#); [Tasoniero et al., 2016](#)). [Velleman et al. \(2018\)](#) also reported that the primary feature of WB is the muscle fibrosis where extracellular matrix proteins, such as collagen replaced muscle fibers.

Since WB could be correlated to echogenicity obtained using US ([Simões et al., 2020](#)), the present study also attempted to detect WB at early stages and follow its evolution when birds were fed on varying energy and amino acids. [Pillen et al. \(2009\)](#) evaluated muscular dystrophy in dogs using US images and reported a positive correlation between fibrous tissue and interstitial fat with echogenicity. [Simões et al. \(2020\)](#) concluded that the use of breast echogenicity could be an additional tool to early detect alterations related to WB in live broilers. Similarly, in the present study, broilers with higher BWG presenting higher WB scores also had higher breast muscle echogenicity. Broilers fed the control feed presented higher BWG and WB scores than broilers fed low EP feed. Additionally, breast echogenicity at 14 d and 21 d as well as the breast depth at 14, 21, and 28 d were higher when increased WB severity was observed, confirming that US images can be adequately utilized to assess the WB of broilers in vivo.

The application of real-time US had already been successfully used in broilers to estimate in vivo carcass and breast muscle weight and yields on broilers and turkeys, being characterized as a fast, easy and non-destructive technique ([Silva et al., 2006](#); [Oviedo-Rondón et al., 2007](#); [Case et al., 2012](#)). Hence, this technique seems to be a valuable tool for assessing breast meat yield development in broilers. The increase on muscle echogenicity observed in the present study probably can be caused by the disruption of a normal muscle architecture by the infiltration of fat and fibrous tissue as already described ([Heckmatt et al., 1982](#); [Walker et al., 2004](#); [Pillen et al., 2009](#)). According to [Serrano and Muñoz-Canóves \(2010\)](#), the accumulation of fibrous connective tissue due to muscle damage eventually evolves to necrosis with the replacement of muscle fibers with connective tissue.

Serum clinical biochemistry is a common tool used to diagnose disease conditions in animals and humans. Intracellular enzymes are reliable markers of muscle damage and can be used in association with other methods to support the diagnosis of breast myopathies in live chickens ([Macrae et al., 2006](#)). The muscle damage observed in WB and other muscular diseases can be adequately diagnosed by observing serum enzyme profiles from affected birds. It was reported by [Meloche et al. \(2018b\)](#) that broilers fed *ad libitum* or at 95%, 90%, or 85% of *ad libitum* feed intake had CK and LDH decreasing linearly in parallel to the reduction of WB occurrence. The WB-affected muscle typically shows disrupted cells, resulting in increasing levels of serum enzymes such as CK, ALT, AST, and LDH ([Lumeij, 2008](#); [Kawasaki et al., 2018](#)). In the present study, decreased serum CK, AST, and LDH concentrations were observed when broilers were fed low EP feeds, probably because the WB occurrence also decreased. The WB scores presented a positive correlation with CK and AST concentration. [Simões et al. \(2020\)](#) also observed a positive correlation between WB severity and CK serum concentration in broilers submitted to feed restrictions from 10 to 50% of the *ad libitum* feed intake.

The main purpose of the present study was to evaluate if the capacity of growth compensation of early feed restricted birds could lead to competitive live performance of broilers that would have lower losses due to WB. It was concluded that low-density feeds provided at early ages reduced growth while being fed and led to lower WB occurrence and severity. Using US to early detect WB is a useful non-destructive tool for the prediction of this myopathy.

ACKNOWLEDGMENTS

The authors wish to thank Conselho Nacional de Pesquisa (CNPq – Brasilia, DF, Brazil), and Cobb-Vantress for funding supporting the research.

DISCLOSURES

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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