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Occurrence and Severity of White Striping in Broilers Until 50d of Age Fed with High and Low-Energy Diets: Body Weight, Histopathological Changes and Meat Quality

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Abstract

White striping (WS) is a condition characterized by the occurrence of white striations parallel to the muscle fibers on breast, thigh, and tender muscle of broilers. This study was aimed to evaluate the occurrence and severity of white striping and histopathological changes in breast fillets from 10 to 50 d broilers disregarding the effect of diet. Birds (n=572) were randomly assigned to a high- (HED) or low-energy (LED) diet (11 replicates of 26 birds/ dietary treatment) and were processed at 10, 20, 30, 40 and 50 d of age to evaluate occurrence of white striping, BW, histological analysis, and meat quality. The results showed that at 10 d of age, 2.27% of the birds showed some degree of WS and at 20 and 30 d of age the occurrence of WS (%) was higher in birds fed HED than birds fed LED diets. There was no differences (P>0.05) in the L*, a*, and b* values for meat or among different degrees of WS as well as between dietary treatments; however, carcass characteristics varied by age or carcass weight. Histologically, the severity of WS causes changes in myofibers, with muscle fibers showing sarcoplasmic reticulum vacuolization, a higher intercapillary distance and a reduced capillary to fiber ratio, suggesting loss of integrity of the cell structure, that was seen in birds fed both diets (low and high nutrient densities) after 30 d of age and lower oxygenation.

Keywords: Breast muscle; Energy; Myopathy; Oxygenation

Introduction

The high productive of poultry as a result of a constant increase in the world demand for white meat with a intensive genetic selection for fast growth rate and high breast yield, have been spurring the broiler industry towards practices that increase its production [1-3], and these factors may induce metabolic stresses that can negatively impact meat quality [4-6]. More recently, high growth rate and high breast yield have been associated with the occurrence of other myopathies affecting pectoralis major and other muscles, i.e., white striping [7,8]. White Striping (WS) is visually characterized by the white lines of intramuscular deposits in raw meat seen parallel to the muscle fibers mainly in breast, tenders, and certain thigh muscles [9]. Histologic reports of WS demonstrated alterations with loss of cross striations between muscle fibers, variability in muscle fiber size, floccular/vacuolar degeneration and lysis of fibers, mild mineralization, mononuclear cell infiltration, lipidosis, instersticial inflammation, and fibrosis [9,10].

Feeding strategies may be used to control de occurrence of myopathies in broiler chickens: low-energy diets reduce both growth rate and the occurrence of white striping [11], however the correlation between the occurrence of this myopathy and growth rate/final live weight and/or breast yield in current broiler genotypes under commercial intensive conditions has been proposed but not fully demonstrated [3]. Bailey et al. [12] also described that the analysis of data from two broiler lines that differed in terms of selection for breast yield showed that there is also a strong non-genetic component for all breast muscle myopathy traits. So, these factors may induce metabolic stresses that can negatively impact meat quality [4-6]. Thus, evaluation of changes in muscle fibers is crucial for elucidation of the pathological basis for white striping [13]. Also, the occurrence of severe degrees of these modern myopathies in broiler breast fillets reduces the quality and acceptance of both raw as well as cooked meat and meat products [14]. The present study was conducted to determine the occurrence and severity of white striping and histopathological changes in breast fillets from 10 to 50 d broilers disregarding the effect of diet.

Materials and Methods

Experimental design

A total of 572 1-d-old Cobb X Cobb 500 male broiler chicks were assigned to two dietary treatments: high (HED; 3,000 to 3,300 kcal ME/kg and 24.19 to 19.56% CP) and low nutrient density diet sequences (LED; 2,900 to 3,050 kcal ME/kg and 20.44 to 18.37% CP). The HED and LED diet sequences were formulated based on the suggested needs for male broilers with high and low performance, respectively [15] using corn, soybean meal and toasted soybeans (Tables 1 and 2). Birds were fed on an *ad libitum* basis in four phases, consisting of pre-starter (1 to 7 d), starter (8 to 21 d), grower (22 to 35 d), and finisher (36 to 50 d). All diets were fortified with complete vitamin and trace mineral mixes obtained from commercial sources. The study was conducted in a completely randomized design having 2 treatments and 11 replicates of 26 birds at the beginning of the study. Birds had *ad libitum* access to feed and water. Temperature in the first week of life was 32°C, and then decreased to 25°C until the end of experiment. Birds were kept on 18 hours of light throughout the study. All pens were checked for sick and dead birds on a daily basis. Two birds per pen were randomly selected from each pen and processing at 10, 20, 30, 40 and 50 d-of-age. All procedures throughout the current study were approved by the Ethics Committee on Animal Use of the Federal University of Rio Grande do Sul.

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Ingredients, g/kg or as noted	Diet nutrient densities[1]							
	High-energy				Low-energy			
	1	2	3	4	1	2	3	4
Corn (7.8% CP)	47.7	54.1	55.7	58.5	62.2	62	65	68
Soybean meal (45% CP)	44.2	37.8	35.6	32.1	33.4	33.7	31	28.23
Soybean oil	4.16	4.46	5.58	6.52	0.22	0.91	1	1.15
Sodium Bicarbonate	0.03	0.05	0.04	0.13	0.41	0.12	0.13	0.19
Hiphos GT	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Dicalcium phosphate	1.23	1.02	0.82	0.64	1.31	1.04	0.85	0.65
Limestone	1.25	1.16	0.94	0.86	1.25	1.16	0.95	0.87
Salt	0.57	0.5	0.46	0.34	0.3	0.45	0.39	0.3
MHA, 84%	0.44	0.39	0.37	0.33	0.35	0.26	0.25	0.21
L-Lysine 78%	0.18	0.2	0.2	0.21	0.22	0.08	0.11	0.11
L-Threonine 98.5%	0.07	0.1	0.09	0.09	0.06	0.02	0.02	0.01
Min. and Vit. Premix[1]	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Choline chloride 60%	0.04	0.06	0.06	0.06	0.08	0.07	0.07	0.08
Monensin sodium[3], ppm	100	100	100	100	100	100	100	100
Flavomycin[4], ppm	25	25	25	25	25	25	25	25

[1]Pre-starter, d 1-7; 2, starter, d 8-21; 3, grower, d 22-35; and 4, finisher, d 36-50 met or exceeded the Nutrient Requirement of Poultry according to Rostagno et al.
[2]Composition per kg of feed: retinyl acetate, 2,400 mg; cholecalciferol, 0.005 mg; alpha-tocopherol acetate 30 mg; menadione, 2 mg; thiamine, 2 mg; riboflavin, 6 mg; pyridoxine, 2.5 mg; cyanocobalamine, 0.012 mg, panthothenic acid, 15 mg; niacin, 35 mg; folic acid, 1 mg; biotin, 0.08 mg; iron, 40 mg; zinc, 80 mg; manganese, 80 mg; copper, 10 mg; iodine, 0.7 mg; selenium, 0.3 mg.
[3]Poucox® 40.
[4]Flavomycin® 80.

Table 1: Formulations of experimental diets.

Energy and nutrients, % or as noted	Diet nutrient densities[1]							
	High-energy				Low-energy			
	1	2	3	4	1	2	3	4
AME_n[2], kcal/kg	3,000	3,100	3,200	3,300	2,900	2,950	3,000	3,050
CP	24.19	21.81	20.95	19.56	20.44	20.38	19.43	18.37
Ca	1.05	0.95	0.82	0.74	1.05	0.95	0.82	0.74
Av. P	0.5	0.45	0.41	0.37	0.5	0.45	0.41	0.37
Na	0.25	0.23	0.21	0.19	0.25	0.23	0.21	0.19
K	0.95	0.85	0.81	0.76	0.79	0.8	0.76	0.71
Cl	0.43	0.4	0.37	0.31	0.29	0.35	0.32	0.27
DEB, mEq/kg	230	205	195	190	230	205	195	190
Dig. Lys[3]	1.37	1.23	1.18	1.1	1.15	1.05	1.01	0.94
Dig. Met	0.7	0.63	0.6	0.56	0.58	0.51	0.49	0.45
Dig. Met+Cys	1.03	0.92	0.89	0.83	0.86	0.79	0.76	0.7
Dig. Thr	0.89	0.84	0.8	0.75	0.75	0.71	0.69	0.64
Dig. Val	1.03	0.92	0.89	0.82	0.86	0.87	0.83	0.78
Dig. Ile	0.96	0.85	0.82	0.76	0.79	0.8	0.75	0.71
Dig. Leu	1.84	1.69	1.64	1.55	1.62	1.63	1.58	1.51

[1]1, pre-starter, d 1-7; 2, starter, d 8-21; 3, grower, d 22-35; 4, and finisher, d 36-50 met or exceeded the Nutrient Requirement of Poultry according to Rostagno et al.
[2]AME, Apparent Metabolizable Energy.
[3]Amino acid: lysine (digestible): Met+Cys 75%, Tre 65%, Val 75%, Ile 70%, Arg 108%, Trp 17%.
[4]Flavomycin® 80.

Table 2: Compositions of experimental diets.

Birds were fasted for 6 h, individually weighted before electrical stunning (45 V for 3 s), bled for 3 min after carotid and jugular veins cut, scalded at 60°C for 45 s, and mechanically defeathered. Carcasses were manually eviscerated and then statically chilled in slush ice for 3 h before processing. Breast fillets were manually deboned from the carcasses and WS evaluations were immediately performed on boneless skinless breast according to Kuttappan et al. [7] as: normal, no striping (NORM) samples, whereas those with striations were moderate (MOD, <1-mm-thick striations) or severe (SEV, >1-mm-thick striations). Carcass yield was expressed as a percentage of live weight and breast yield was expressed as a percentage of eviscerated carcass weight.

Tissue sample processing

All the muscle sections were cut parallel to the direction of muscle fibers and fixed in 10% buffered neutral formalin. Histopathology samples were dehydrated with increasing concentrations of ethanol, cleared, infiltrated, and embedded in paraffin. Five tissue cross sections were prepared from each muscle sample collected. Blocks were cut into serial sections of 3 µm thickness using a microtome (Leica RM 2125RT, Leica Biosystems, Nussloch, Germany) and stained with hematoxylin and eosin (H&E) to evaluate the general morphology of the tissues. Ten slides for each muscle sample were examined under a light microscope (Leica ICC50 HD, Leica Biosystems, Nussloch, Germany) at 10X objective and capillary density (CP) at 1000X. The means of capillary to

fiber ratio (C:F) was calculated by the ratio of the number of capillaries per area divided by the number of fibers in a given area and the intercapillary distances (IcD) were calculated using Krogh's cylinder method, because its radius, $r_k\sqrt{1/(\pi \times \text{capillary density})}$ is half of the mean intercapillary distance [16,17]. Three veterinary pathologists scored histological lesions separately.

Measured responses

Muscle pH at time 0 (pH_0) or 24 h post-mortem (pH_{24}) were measured using a Sensoglass spear tip probe and meter (Lutron-208, Lutron Electronic Enterprise CO., Taiwan), which was inserted in the inner portion of the muscle. The color of the pectoral muscles was assessed in triplicate with a CIELAB* System (L*: lightness, a*: red, and b*: yellow) using a Minolta colorimeter (model 410R, Konica Minolta, Ramsey, NJ). Each breast sample was packed in a heat-resistant Perflex11 Film Viskase plastic bag and heated in water baths at 80°C for 60 min [18] until an internal temperature of 71 ± 2°C was reached [19]. Three cores (1.27 cm diameter) were manually removed from each fillet parallel to the muscle fibers using a punch tool adapted to an electric drill. The shear force of 2.5 cm thick samples was measured using a TAXT-2i texture analyzer coupled with a Warner-Bratzler blade (Warner-Bratzler Shear Force; WBSF, G-R Manufacturing Co., Manhattan, KS). Breast samples were cored and sheared before (SF_{raw}) and after cooking (SF_{cooked}) and the average of six readings for each was used as the final value (expressed in kgF/g), according to the methodology used by Liu [20]. Due the white striations be clearly visualized on the *Pectoralis major* muscle *in nature*, for analysis to SF_{raw}, an adaptation of methodology used by Liu [20] was adopted, why particular technique.

Statistical analyses

Occurrence of white striping: Incidence of white striping on breasts of the two dietary treatments was examined by use of a multinomial baseline logit mixed effects model. Specifically, Y_{ijk} denotes the k^{th} categorical record (k=1, …, n_{ij}) on the i^{th} treatment (i=1, 2) and j^{th} pen (j=1, …, 22) and represents a multinomial sampling model with probabilities $\{\pi_{ijk1}, \pi_{ijk2}, \pi_{ijk3}\}$. The probabilities sum to one and represent the probabilities of being classified in one of the three categories of white striping, i.e., π_{ijk1}=P (Y_{ijk}=Normal), π_{ijk2}=P (Y_{ijk}=Moderate) and π_{ijk3}=P (Y_{ijk}=Severe). The effects of treatment, BW, and pen were modelled as:

$$\log\left(\frac{\pi_{ij}kr}{\pi_{ij}k1}\right)=\beta_{0r}+\beta_{1r}X_{ijk}+\alpha_{ir}+\gamma_{jr},\ r=2, 3.$$

where β_{0r} is the intercept for category r, β_{1r} is the slope for category r describing the change in the log odds with an increment of one kilogram of BW, X_{ijk} is the centered BW weight in kilograms, α_{ir} is the fixed effect of i^{th} dietary treatment on the log odds for category r and γ_{jr} is the random effect associated with the of j^{th} bin on the log odds of category r. It was assumed that random effects are independent and distributed $\gamma_{jr} \sim N(0,\sigma_r^2)$. The model was fitted in a Bayesian framework with the Open BUGS software [21], which was interfaced with the R software through the BRugs package [22]. Minimally informative priors were specified for all parameters and convergence was assessed by specifying two chains with over dispersed initial values and the monitoring of history and autocorrelation plots and the use of the Gelman-Rubin diagnostics [23]. A burn-in period of 10,000 samples was used and the inference was based on additional 500,000 samples thinned by a factor of 50.

Effects of dietary treatments and white striping on physicochemical variables: Effects of dietary treatment and white striping on the measured physicochemical variables were investigated using the following linear mixed effects model:

$$Y_{ijkm}=\mu+\alpha_i+\delta_j+(\alpha\delta)_{ij}+\beta X_{ijkm}+\gamma_k+\varepsilon_{ijkm}$$

where Y_{ijkm} is the m^{th} record (m=1, …, n_{ijk}) of the dependent variable measured on the i^{th} dietary treatment (i=1, 2), j^{th} level of white striping (j=1, 2, 3) and k^{th} pen (k=1, …, 22), μ is the overall mean, α_i is the fixed effect associated with the i^{th} dietary treatment, δ_j is the fixed effect associated with the j^{th} level of white striping, $(\alpha\delta)_{ij}$ is the interaction term, γ_k is the random effect associated with the k^{th} bin, β is the regression coefficient for the relationship between the dependent variable and the animal BW, X_{ijkm} is the centered BW weight and ε_{ijkm} is the error. It was assumed that random effects and errors are independent and distributed as $\gamma_k \sim N(0, \tau)$ and $\varepsilon_{ijkm} \sim N(0, \sigma^2)$. Variance functions of the fitted values were used to model the variance structure of the within-group errors when heteroscedasticity was present as described in Pinheiro and Bates [24]. Models were fitted in the R statistical software [25] with the nlme package [26]. When treatment effects were present, multiple comparisons were conducted with the multcomp package [27]. When no interactions were present, treatments were compared directly, but when interactions were present treatments were compared within each level of the other factor. The P-value of the multiple comparisons was estimated with the single-step method [27].

Results

Occurrence of white striping

The estimated parameters of the multinomial baseline logit mixed effects model are in Table 3. Results suggest that the odds of having MOD rather than NORM white striping for the LED diet were 0.40 times the odds for the HED diet. Similarly, the odds of having SEV rather than NORM white striping for the LED diet were 0.32 times the odds for the HND diet. Moreover, results also suggested that the odds of having MOD rather than NORM white striping was multiplied by

Estimated parameters	Estimate	95% Credible interval
$\beta_{0,r=2}$	2.58	(1.50, 3.82)
$\beta_{0,r=3}$	1.79	(0.63, 3.07)
$\beta_{1,r=2}$	3.01	(2.13, 4.05)
$\beta_{1,r=3}$	3.8	(2.84, 4.90)
$\alpha_{r=2}$	-1.05	(-2.10, -0.05)
$\alpha_{r=3}$	-1.31	(-2.52, -0.18)
$\sigma^2_{r=2}$	0.11	(0.0008, 0.6506)
$\sigma^2_{r=3}$	0.04	(0.0006, 0.3098)
Odds*		
$\theta_{diet,r=2}$	0.4	(0.12, 0.95)
$\theta_{diet,r=3}$	0.32	(0.08, 0.84)
$\theta_{bw,r=2}$	23.01	(8.44, 57.24)
$\theta_{bw,r=3}$	51.15	(17.18, 133.40)

*$\theta_{diet,r}$ represents the change in the odds of having white striping classified as r (r=2 for moderate or r=3 for severe) rather than the baseline category (normal white striping) for the low energy diet in respect to the high energy diet. Similarly, $\theta_{bw,r}$ represent the change in the odds for one kilogram increment in body weight.

Table 3: Estimated parameters and corresponding estimated effects on the odds, with associated 95% Credible Intervals, for the multinomial baseline mixed effects model.

23.01 for each kilogram increase in BW weight. Similarly, the odds of having SEV rather than NORM white striping were multiplied by 51.15 for each kilogram increase in BW. The frequencies of occurrence of each degree of white striping with respect to BW for each diet treatment (LED and HED) are shown in Figure 1.

The graphs in Figure 1 clearly indicate that there was a reduction in the odds of occurrence of NORM samples as BW increased, confirming the report by Kuttappan et al. [11] that the occurrence of white striping was significantly related to carcass weight and yield. This research, the authors showed that the increase in growth rates result in higher incidence of this myopathy, corroborating with our results. In our study, the results showed the progression of the development of WS until 50 d of age, indicating that at 20 d-age the histological changes begin in the birds.

The occurrence date of white striping (%) showed that at 10 d of age only 2.27% of the animals showed some degree of WS, and these animals fed HED diet, the others did not show WS. At 20 d and 30 d of age the occurrence of white striping (%) was higher in birds fed HED (59.09 and 77.27, respectively, $P<0.05$) than birds fed LED diets (13.64 and 68.18, respectively). At 20 days, just animals fed HED showed SEV white striping. However, after 40 d of age, all animals (100%, both diets) showed WS.

The average body weight of birds with 30 d of age showed difference ($P<0.05$) between the two dietary treatments (1840.00 ± 32.66; 1662.27 ± 43.09 g). Birds fed a high-diet-fed (HED) grew more rapidly (higher growth rate) than LED diet-fed birds at 20, 30, 40 and 50 d of age.

These results are in accordance to other authors, that associated that the use of high-energy diets in conjunction with farming systems, allowing a low mobility of the animals and increasing slaughter ages and weights which have been employed by poultry industry may be the important factors involved in this problem [11,28,29].

Effects of dietary treatments and white striping on physicochemical variables

The analysis of variance tables with F-tests and associated P-values of the general linear model hypothesis of dietary treatment and white striping associated effects are shown in Table 4.

There were significant differences in the values of pH_0, pH_{24}, SF_{raw} and SF_{cooked} at different BW and ages (Table 4, $P<0.001$). The values of pH_0 and pH_{24} increased with BW, showing mean values 6.35 and 6.20, ranging from 5.22 and 5.21 to 8.73 and 6.90, respectively. However, the pH_{24} value in breasts of birds fed LED diets tended to be higher in MOD than NORM white striped (6.21 ± 0.27 vs. 5.72 ± 0.29; $P=0.09$).

Kuttappan et al. [8] found no association between the occurrence of white striping and changes in pH values and the pH_{24} values in NORM breast in that study were similar (5.78 ± 0.02) to those found in our study. Low pH_{24} values (<5.7) can be indicative of poor meat quality [30]. Woelfel et al. [31] found mean ultimate pH_{24} values of 5.76 for pale meat and 6.07 for normal meat, values that are similar to the mean values found in the present study, in which the NORM broiler breast samples were in the pH_{24} range of 5.21 to 6.30.

Velleman et al. [32] found that feed restricted birds had decreased BW gain from 1 d post hatch through 28 d of age. Furthermore, the *Pectoralis major* muscle weight was significantly reduced by the feed restriction. In contrast to our study, those authors concluded that for both the body and *Pectoralis major* muscle weights there was no interaction between age and treatment.

There were no differences in the L*, a* and b* values of breast meat samples with different degrees of white striping, as well as of breasts of birds fed different diets. Normal L* values of raw broiler breast

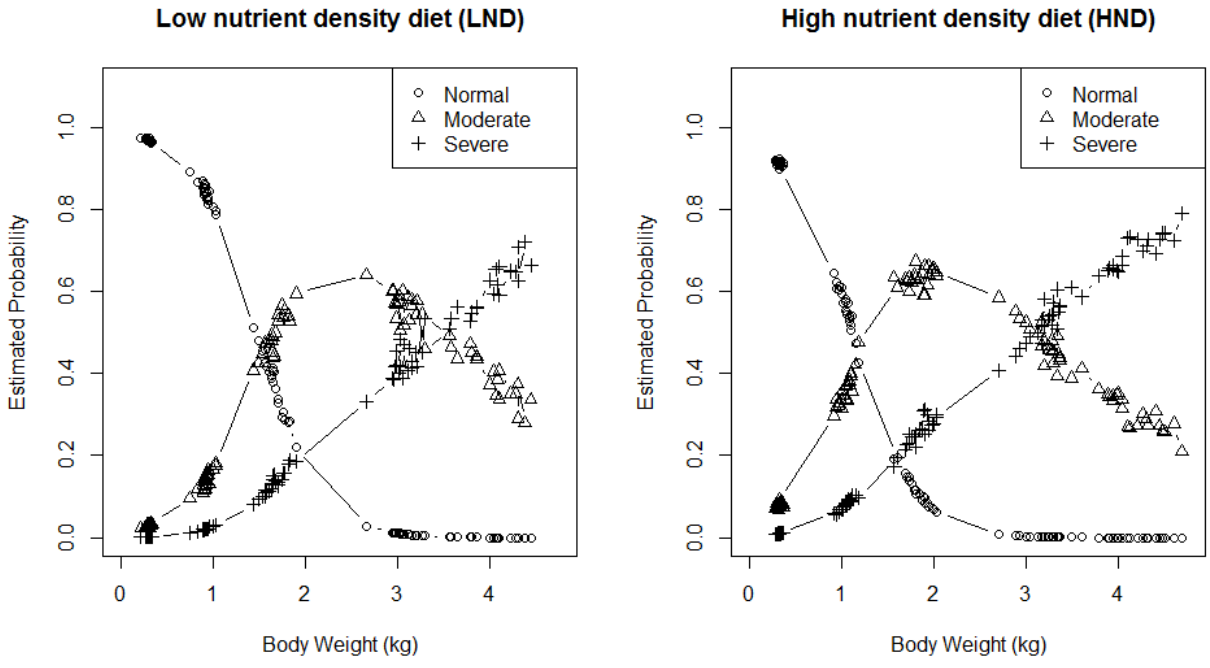

Figure 1: Probability of occurrence of white striping with respect to the body weight. (A) Values were polled in birds fed a low-energy (LED). (B) Values were polled in birds fed high-energy (HED).

	WS degree[1]	Treatments[1]		Mean ± SEM	*P-value***			
		HED	LED		BW	Diet	WS	Diet X WS
pH_0	NORM	6.08 ± 0.36	6.20 ± 0.59	6.12 ± 0.51[a]	<0.001	0.19	0.002	0.21
	MOD	6.49 ± 0.42	6.67 ± 0.64	6.57 ± 0.53[b]				
	SEV	6.57 ± 0.24	6.52 ± 0.20	6.55 ± 0.22[ab]				
pH_{24}	NORM	5.81 ± 0.28	5.72 ± 0.29[a]	5.76 ± 0.29	<0.001	0.32	0.38	0.04
	MOD	6.06 ± 0.36	6.21 ± 0.27[b]	6.13 ± 0.32				
	SEV	6.26 ± 0.36	6.36 ± 0.13[ab]	6.30 ± 0.30				
$Shear\ force_{raw}$	NORM	2.58 ± 0.77	2.69 ± 0.73	2.68 ± 0.70	<0.001	0.62	0.31	0.08
	MOD	2.62 ± 0.66	2.79 ± 0.65	2.71 ± 0.66				
	SEV	2.90 ± 0.66	2.82 ± 0.61	2.86 ± 0.64				
$Shear\ force_{cooked}$	NORM	3.65 ± 0.87	3.57 ± 0.79	3.61 ± 0.95	<0.001	0.97	0.95	0.03
	MOD	3.41 ± 0.93	3.41 ± 0.91	3.41 ± 0.91				
	SEV	3.44 ± 0.81	3.34 ± 0.83	3.39 ± 0.82				
L*	NORM	56.72 ± 4.23	57.89 ± 4.56	57.68 ± 4.03	0.14	0.89	0.56	0.71
	MOD	57.68 ± 4.18	57.45 ± 4.24	57.56 ± 4.15				
	SEV	57.93 ± 3.83	58.24 ± 4.37	58.07 ± 4.06				
a*	NORM	1.33 ± 0.25	1.31 ± 0.22	1.35 ± 0.23	0.8	0.19	0.49	0.56
	MOD	1.38 ± 0.22	1.34 ± 0.15	1.36 ± 0.19				
	SEV	1.37 ± 0.18	1.29 ± 0.20	1.33 ± 0.19				
b*	NORM	1.15 ± 0.24	1.17 ± 0.29	1.13 ± 0.21	0.81	0.68	0.92	0.81
	MOD	1.06 ± 0.27	1.05 ± 0.26	1.06 ± 0.26				
	SEV	1.10 ± 0.18	1.07 ± 0.19	1.08 ± 0.18				

* Multiple comparisons among dietary and white striping effects. When no interactions were present, treatments were compared directly whereas, when interactions were present, treatments were compared within each level of the other factor. The P-value of the multiple comparisons was adjusted with the single-step method (Hothorn et al.), where: [A-B] Means within a row followed by different superscript letters differ significantly ($P \leq 0.05$) and [a-b] Means within a column followed by different superscript letters differ significantly ($P \leq 0.10$).

[1] In this notation, NORM, MOD and SEV denote normal, moderate and severe white striping degrees (Kuttappan et al., 2012a) and HED and LED denote the high and low nutrient dietary treatments. pH_0 and pH_{24} denote muscle pH at time 0 and 24 h post-mortem.

** Analysis of variance table with P-values of the general linear hypotheses of treatment and white striping effects. For each variable, the P values of each row are associated with one the four hypotheses H_0: $\beta=0$, H_0: $\alpha_i=0$, for all i, H_0: $\delta_j=0$ for all j and H_0: $(\alpha\delta)_{ij}=0$ for all i and j.

Table 4: Mean and SEM values of meat quality attributes of broiler breast meat classified as 3 degrees of white striping from birds feed two dietary treatments (HED and LED)*.

meat lie between 48 and 51, it indicated that the average luminosity of our breast muscles was higher than normal for all groups, however no significant difference (P=0.56) was found between degrees of white striping. Kuttappan et al. [8], Petracci et al. [33] and Trocino et al. [34] also observed no differences in L* values in breast meat samples with different degrees of white striping. These results are also in the same numerical range from those found by Qiao et al. [35] with values above 53 and below 46 indicate meat that is lighter and darker than normal, respectively.

Previous studies demonstrated that fillets affected by WS are more yellow than non-affected ones, probably due to the strong fibrotic response [8,36,37] however, Trocino et al. [34] found that the presence of WS decreased a* and b* indexes.

In general, white-striped breasts exhibit some minor differences in final pH and color indexes, not always consistent among studies [34,36,38,39].

Also, there were significant differences in the values SF_{raw} and SF_{cooked} at different BW and ages (P<0.001). but no significant difference between degrees of white striping and diets with regard to both SF_{raw} and SF_{cooked}. Shear force on raw meat is manly reflecting background or collagen toughness, whereas shear force on cooked meat may be consider a measurement of myofibrillar toughness. In WS meat, the overall connective tissue increases to the detriment of the protein amount, which was found to be lower than normal meat [29].

In a study conducted by Kuttappan et al. [40] consumers indicated that fillets with severe WS look fattier and have a marbled appearance. The occurrence of WS is negatively impacting the poultry processing industry, and the industry is facing great economic losses due to customer complaints about fillets affected by this myopathy [41]. Meat quality traits such as texture, color and pH can affect consumer preference for meat [42]. According to Kuttappan et al. [40], consumers are more likely to buy normal fillets without any white striations and over 50% of consumers indicated that they will probably not or definitely not buy moderate or severely white striped fillets.

Histological results

Figure 2 compares the histological lesions observed in breast muscles of broilers fed low or high-nutrient diets, and compares breasts with NORM, MOD, and SEV degrees of white striping. At 10 d of age, the breast muscles of all birds fed the LED diets had no histopathological alterations (Figure 2A), however some birds fed the HED diets had flocculate and hyaline necrosis of moderate multifocal myofibers, macrophage and heterophil infiltration, residual myofiber phagocytosis and some regenerating myofibers (Figure 2B). In this study, 20 d-old birds from both dietary treatments (LED and HED) had breast muscles with white striping, but only birds fed the HED diets had breast muscles with SEV white striping and myopathy (Figure 2C), indicating that growth rate was correlated with the severity of myopathy at the cellular level.

Histologically, in breast muscle of animals at 20 d of age, we observed hyaline and floccular scattered necrosis in myofibers, some with calcification, infiltration of macrophages, lymphocytes and a few heterophils, phagocytosis of residual myofibers, regenerating myocytes in some cases and sarcoplasmic vacuolization (Figure 2D). At 30 days

of age, birds fed both diets showed all degrees of white striping severity, including normal breasts. So, in some skeletal muscles we found no histopathological changes (Figures 2E and 2F). At 40 and 50 d-old, we found no NORM breasts (no apparent striations), so that by 40 d of age, regardless of diet nutrient density or growth rate, all broiler breasts showed MOD or SEV white striping. This shows that at any age or stage of development, regardless of growth rate, the muscles presented histological changes that compromised the cellular structures, including myocytes with sarcoplasmic vacuolization (Figures 2G and 2H).

Similarly to our histological results, Kutappan et al. [9] reported an increase in mean scores for degenerative or necrotic lesions, fibrosis, and lipidosis as the degree of white striping increased from NORM to SEV. Velleman et al. [32] found that until 42 d of age the morphological structure of the *Pectoralis major* muscle in growth-restricted birds was not well organized into muscle fiber bundles with distinct individual muscle fibers as observed in unrestricted birds. The muscle fibers of growth-restricted birds showed increased necrosis and larger and more extensive fat cell depots beginning at 28 d post-hatch (Figure 3).

Our results indicated that growth rate was correlated with the severity of myopathy at the cellular level. This correlation also was presented by Wilson et al. [43] that observed positive correlations between age, growth rate and plasma creatine kinase (CK) concentrations and the incidence of damaged muscle in birds. Those authors concluded that broiler muscles outgrow their life support systems, which negatively affects myocellular integrity. Furthermore, our results in breast muscle of animals at 20 d of age are in accordance to Wilson et al. [43], who found degenerative changes that included scattered and focal necrosis and infiltration of necrotic areas by mononuclear cells in damaged skeletal muscle of birds.

In another study, Kuttappan et al. [8] concluded that the occurrence of MOD and SEV degrees of white striping was histologically characterized by chronic myopathic lesions like loss of cross striations, mild mineralization, lipidosis, and fibrosis. Moreover, similar to our results, Kuttappan et al. [11] found floccular and vacuolar degeneration, mononuclear cell infiltration, and interstitial inflammation. The changes at any age or stage of development observed in our study indicated the impairment of cellular structures such as sarcoplasmic reticulum vacuolization that are also characteristic of exercise-induced muscle injury [44], supporting the notion that the muscles exceeded their support systems.

According to Table 5, in relation to capillary density (CD), capillary to fiber ratio (C:F) and intercapillary distance (IcD) there was no statistical difference to CD between the degrees of WS on 20, 30 and 40-days of age. The values of C:F was lower in SEV compared to NORM breasts at 40 d, however higher values were observed on SEV birds to IcD at 20, 30 and 40 d, suggesting a lower capillary supply and oxygenation on the breast fillets affected with this myopathy.

This myopathy has been associated with an increased muscle hypertrophy of fast-growing chickens, witch brings about a reduced capillary supply that could result in a decreased source of nutrients and oxygen and slower removal lactic acid from breast muscles, which ultimately may lead to muscle damage [2,45].

Conclusions

The results of this study revealed that at 10 d of age, 2.27% of the birds showed some degree of WS and at 20 and 30 d of age the occurrence of WS was higher in birds fed HED than birds fed LED diets. The odds of having moderate rather than normal or severe

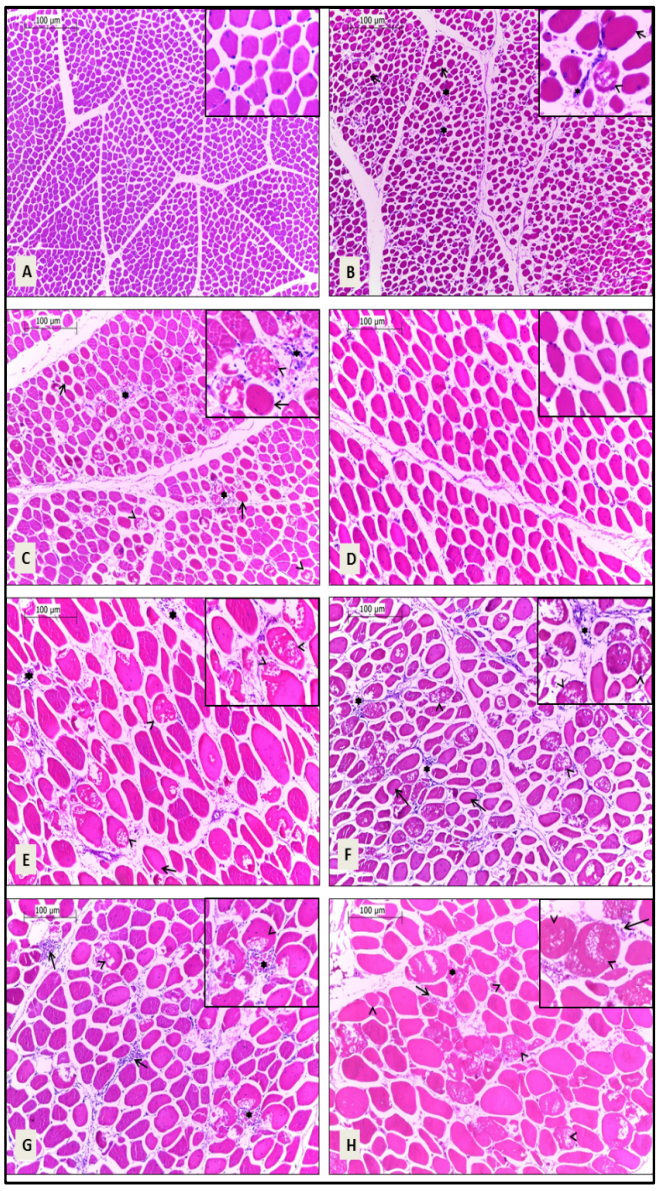

Figure 2: Morphological structure of muscle fibers in broilers: (A) at 10 d-old fed LED-diet classified as normal white striping (WS) (without myopathy on macroscopic observation) and (B) HED-diet (B) classified as moderate WS: flocculate necrosis (arrowhead) and hyaline necrosis of myofiber (arrow) moderate multifocal, discrete infiltration of macrophages and a few heterophils (asterisk). (C) at 20 d-old fed HED-diet classified as severe WS: flocculate (arrowhead) and hyaline (arrow) necrosis of diffuse myofibers, diffuse infiltration of macrophages around myofibers necrotics, lymphocytes and few heterophils (asterisk), myocytes with sarcoplasmic vacuolization. *Inset* (40X): flocculate necrosis (arrowhead) and hyaline necrosis of myofiber (arrow) moderate multifocal, infiltration of macrophages around myofibers necrotics, lymphocytes and few heterophils (asterisk). (D) at 30 d-old fed HED-diet classified as normal WS, without histological change. (E, F) at 40 d-old fed HED-diet (E) or LED-diet (F) classified as severe WS: flocculate (arrowhead) and hyaline (arrow) necrosis of myofibers diffuse, and infiltration of macrophages (asterisk), phagocytosis of residual myofibers. *Inset* (40X): flocculate (arrowhead) and hyaline necrosis of myofibers diffuse, and infiltration of macrophages (asterisk), phagocytosis of residual myofibers. (G, H) at 50 d-olf fed HED-diet classified as moderate (G) and severe (H) WS. (G) Flocculate and hyaline moderate-multifocal necrosis (arrowhead) with interstitial infiltration of macrophages (arrow) and phagocytosis of necrotics myofibers (asterisk). (H) Flocculate and hyaline necrosis of diffuse myofibers (arrowhead) with infiltration of macrophages (asterisk). HE.

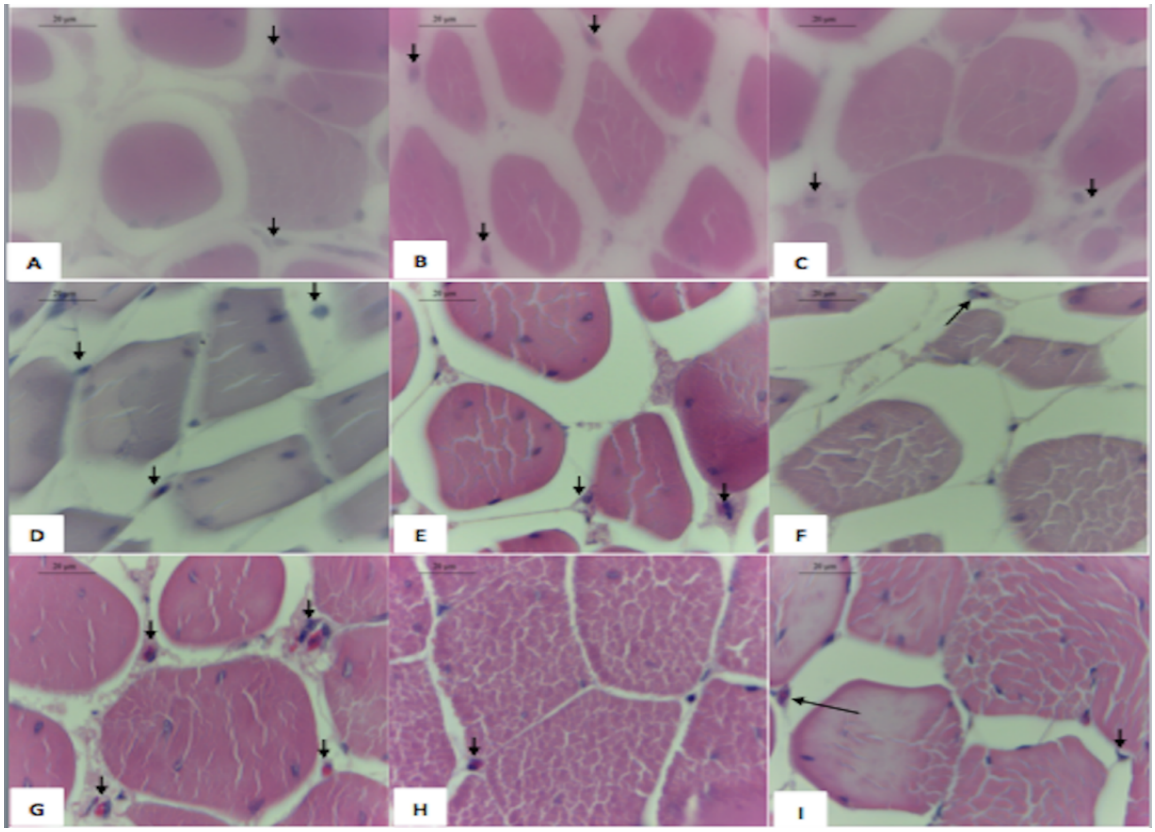

Figure 3: Histological evaluation of muscle fibers in broilers with capillaries (black arrows): (A) 20 d-old classified as normal white striping (WS); (B) 20 d-old classified as moderate WS; (C) 20 d-old classified as severe WS; (D) 30 d-old classified as normal white striping (WS); (E) 30 d-old classified as moderate WS; (F) 30 d-old classified as severe WS; (G) 40 d-old classified as normal white striping (WS); (H) 40 d-old classified as moderate WS; (I) 40 d-old classified as severe WS. HE. 1000x.

Age	WS degree[1]	CD (mm²)	IcD (mm)	C:F
20	NORM	12,667	0,300^{c}	0,414
	MOD	12,333	0,320^{b}	0,412
	SEV	11,333	0,340^{a}	0,382
	p-value	0,888	<0,0001	0,902
30	NORM	10,000	0,360^{b}	0,303
	MOD	10,333	0,360^{b}	0,309
	SEV	7,333	0,420^{a}	0,340
	p-value	0,069	<0,0001	0,818
40	NORM	10,333	0,360^{b}	0,446^{a}
	MOD	11,000	0,340^{c}	0,390^{ab}
	SEV	7,333	0,420^{a}	0,254^{b}
	p-value	0,306	<0,0001	0,009

[1]In this notation, NORM, MOD and SEV denote normal, moderate and severe white striping degrees (Kuttappan et al).
[a-c] Means followed by different letters in the same column are significantly different (P<0.05).

Table 5: Values of capillary density (CD), intercapillary density (IcD) and capillary to fiber ratio (C:F) of broiler breast fillets affected by different degrees of white striping and processed at 20, 30 and 40 d.

white striping were multiplied by 23X and 51X, respectively, for each kg increase in BW and the odds of having moderate white striping rather than normal white striping for the low diet was 0.40 times the odds for the high diet. There was no differences (P>0.05) in the L*, a*, and b* values for meat or among different degrees of WS as well as between dietary treatments; however, carcass characteristics varied by age or carcass weight. Overall, white striping was more prevalent in broilers fed high as compared to low energy diets, and involved histopathological changes in myofibers, with muscle fibers showing sarcoplasmic reticulum vacuolization, a higher intercapillary distance and a reduced capillary to fiber ratio, suggesting loss of integrity of the cell structure, that was seen in birds fed both diets (low and high nutrient densities) after 30 d of age and lower oxygenation.

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