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Performance, nutrient digestibility, and muscular evaluation of female broiler chickens fed different dietary protein levels and slaughtered at 38 or 46 days

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ABSTRACT - This study evaluated the effects of two protein levels and types of formulation on performance, nutrient metabolization, and myopathies of 390 female broilers from 21 to 46 days old distributed in a completely randomized design, with five treatments and six replicates of 13 broilers each. Treatments were two levels of protein (19.0 and 21.0%) in diets using only commercially available ingredients and three experimental formulations designed to keep similar the main nutrients that could influence nutritional performance (starch, fiber, ether extract), using unusual ingredients, with 19.0% (19E) or 21.0% (21E) crude protein and the third with 19.0% crude protein with amino acid levels similar to treatment 21P (19E+Aa). We studied broiler performance, nutrient digestibility, macroscopic muscular evaluation, and histological muscular evaluation. The design for the performance and myopathy assessment was a 5 × 2 factorial scheme, five diets × two ages (38 or 46 days). The worst feed conversion ratio was observed for the 19P treatment. Neither diet nor age had an effect on myopathies, however, older slaughter age increased the intensity of microscopic lesions. The diet 19P should be avoided and increased inclusion of lipids in diets improves nutrient utilization and, consequently, performance.

Keywords: alternative ingredients, amino acid, digestibility, myopathy

1. Introduction

The possibility and benefits of reducing protein levels in broiler chicken diets have been discussed in other studies, although with controversial results (Attia et al., 2018; Soares et al., 2020). Reduced feed protein levels also bring a reduction in the inclusion of soybean meal, the main source of protein in field diets (Soares et al., 2020), which increases the inclusion of synthetic amino acids and corn and reduces the inclusion of soybean oil or another lipid source. However, the reduced inclusion of a lipid source may impair poultry performance mainly due to the extra-caloric effects of oils and fats.

Soares et al. (2020) found worse performance and higher caloric increment for male broilers in the growing phase with protein reduction from 22 to 16%, which can be attributed to protein content, but also to the lower percentage of soybean oil in the 16% protein diet. This suggests that in addition to nutritional levels, the concentrations of ingredients also contributed to the result, thus highlighting the extra-caloric effects of lipid sources.

Besides providing energy, the addition of lipids in diet leads to changes in gastrointestinal physiology, such as reduced peristaltic movements and improved nutrient digestibility due to increased cholecystokinin production, which stimulates gallbladder contraction and secretion of pancreatic enzymes (Ravindran et al., 2016). Thus, nutrients in the diet are under the action of enzymes and digestive juices for a longer period of time and, thus, can be better assimilated in the gastrointestinal tract. In addition, there is a reduction in caloric increase resulting from lipid metabolism (Baião and Lara, 2005). Thus, the manipulation of protein in association with lipid benefits can be explored to improve performance and carcass characteristics of broiler chickens because proteins and amino acids are closely related to muscle development (Baião and Lara, 2005; Attia et al., 2018).

In addition to diet, broiler sex is also an important factor, as higher growth rates and carcass yield are found in male broilers (Marcato et al., 2010). To meet the demand of the poultry industry for heavier carcasses, nutritional strategies and older slaughtering age have been adopted for female broilers. On the other hand, heavier carcasses are associated with increased occurrence of myopathies in chicken meat, as these changes are correlated with high carcass yield, sex, and diet, beyond chicken handling at slaughter line (Bailey et al., 2015).

The most frequent myopathies are white striping (Kuttappan et al., 2012) and wooden breast (Sihvo et al., 2014) observed in the *pectoralis major* muscle. Green breast or deep pectoral myopathy have also been observed in the *pectoralis minor* (Kijowski et al., 2009), in addition to dorsal muscle myopathy in the *latissimus dorsi*. These myopathies are not associated with infectious agents, yet they impair the appearance, texture, and quality of meat. Prior to the macroscopic appearance of myopathies, some histology changes, such as muscular fiber fragmentation, hyalinization, tissue necrosis, and macrophage infiltration, can be observed in tissues (Zimermann et al., 2012).

In view of the above, our objective was to evaluate the effects of two feed protein levels and types of formulation to female broilers in the growing phase on performance, metabolism, and myopathies. In addition, the effects of slaughter age on performance and myopathies were also evaluated.

2. Material and Methods

The experiment was conducted in Belo Horizonte, MG, Brazil (19°55' S and 43°56' W). The current experimental protocol was approved by the Ethical Principles in Animal Experimentation Committee (case number 179/2018).

2.1. Animals, diets, and experimental design

A total of 390 female Cobb500® broiler chickens were distributed in a completely randomized design, with five treatments and six replicates of 13 broilers each for the metabolism assay. The design for the performance and myopathy assessment was a 5 × 2 factorial scheme (five diets × two ages). One-day-old chicks were purchased from a commercial hatchery where they received vaccination against Marek's and Gumboro diseases and were reared in a thermoneutral-climate room. Broilers were reared under the same conditions from 1 to 21 d receiving water and starter diet (Cobb, 2018). The light program used was six hours of dark and 18 hours of light/day throughout the rearing phase.

At 21 days, all broilers were weighed (average weight of 0.927 kg/broiler) and homogeneously distributed in metabolic cages. Treatments (Table 1) were two levels of protein (19.0 and 21.0%) in diets using only commercially available ingredients, in comparison to experimental diets. The two treatments were practical formulations with minimal cost, increased protein, and other changes with 19.0 (19P) or 21.0% (21P) crude protein (CP). Three experimental treatments were used: experimental formulations designed to keep similar the main nutrients that could influence nutritional performance (starch, fiber, ether extract [EE]), using unusual ingredients (Table 2), with 19.0 (19E) or 21.0% (21E) CP, and the third with 19.0% CP with amino acid levels similar to treatment 21P (19E+Aa). Thus, diets that the broilers received during the growth phase (22 to 46 d) were defined as treatments (Table 2).

Table 1 - Description of treatments

Treatment	Protein level (%)	Description
19P	19	Practical formulations with minimal cost, increased protein, and other changes.
21P	21	
19E	19	Experimental formulations – designed to keep similar the main nutrients that could influence nutritional performance (starch, fiber, ether extract), using unusual ingredients.
21E	21	
19E+Aa	19*	

* Amino acids levels similar to treatment 21% CP.

Table 2 - Ingredients and calculated nutritional composition of starter (1-21 days of age) and grower (22-46 days of age) diets

Ingredient (%)	Starter	Grower				
		19P	21P	19E	21E	19E+Aa
Corn	54.67	65.20	58.35	54.08	53.31	53.78
Soybean meal (45.5%)	33.33	25.10	31.37	32.00	34.00	31.95
Soy oil	3.67	3.06	4.11	5.50	5.50	5.50
Meat and bone meal (41%)	6.67	5.15	5.00	-	-	-
Starch	-	-	-	2.74	-	2.75
Corn gluten bran	-	-	-	-	2.20	-
Soybean hull	-	-	-	0.83	0.50	0.83
Limestone	0.23	0.32	0.23	0.93	0.84	0.82
Dicalcium phosphate	-	-	-	1.73	1.68	1.73
Sodium chloride	0.42	0.36	0.36	0.41	0.41	0.41
DL-Methionine (99%)	0.32	0.24	0.18	0.22	0.16	0.22
L-Lisyne HCL (78%)	0.18	0.13	-	0.04	-	0.08
L-Threonine (98.5%)	0.06	0.03	-	-	-	0.08
L-Tryptophan	-	-	-	-	-	0.02
L-Arginine	-	-	-	-	-	0.12
L-Valine	-	-	-	-	-	0.11
L-Isoleucine	-	-	-	-	-	0.09
Vitamin/mineral supplement[1]	0.40	-	-	-	-	-
Vitamin/mineral supplement[2]	-	0.40	0.40	0.40	0.40	0.40
Inert	-	-	-	1.12	1.00	1.10
Total (%)	100.00	100.00	100.00	100.00	100.00	100.00
Calculated analysis						
Crude protein (%)	22.5	19.0	21.0	19.0	21.0	19.0
Metabolizable energy (kcal/kg)	3,050	3,130	3,130	3,130	3,130	3,130
Crude fiber (%)	2.58	2.43	2.61	2.78	2.78	2.78
Starch (%)	-	38.85	35.87	36.01	33.78	35.85
Fats (%)	-	6.58	7.46	8.09	8.13	8.07
Calcium (%)	1.08	0.88	0.85	0.87	0.83	0.84
Available phosphorus (%)	0.49	0.39	0.39	0.41	0.41	0.41
Ash (%)	5.47	4.59	4.77	2.86	2.98	2.85
Digestible lysine (%)	1.20	0.95	0.99	0.95	0.98	0.98
Digestible Met + Cis (%)	0.90	0.74	0.74	0.74	0.74	0.74

Continues...

Table 2 (Continued)

Digestible methionine (%)	0.62	0.49	0.47	0.48	0.46	0.48
Digestible threonine (%)	0.78	0.65	0.71	0.65	0.72	0.72
Digestible tryptophan (%)	0.21	0.19	0.22	0.21	0.23	0.23
Digestible valine (%)	0.91	0.76	0.86	0.79	0.89	0.89
Digestible arginine (%)	1.39	1.12	1.29	1.17	1.27	1.28
Digestible isoleucine (%)	0.82	0.67	0.77	0.74	0.82	0.82
Sodium (%)	0.21	0.18	0.18	0.17	0.17	0.17

[1] Vitamin/mineral supplement (starter). Each 1.0 kg contains: folic acid, 142 mg; pantothenic acid, 2,600 mg; Halquinol, 7,500 mg; *Bacillus subtilus*, 75×10^{e9} cfu; biotin, 13 mg; copper 1,500 mg; choline, 75 g; iron, 26 g; iodine, 250 mg; manganese, 16.25 g; niacin, 8,750 mg; monensin, 30 g; selenium, 60 mg; vitamin A, 2,500,000 IU; vitamin B1, 370 mg; vitamin B12, 3,000 mcg; vitamin B2, 1,280 mg; vitamin B6, 410 mg; vitamin D3, 500,000 IU; vitamin E, 3,750 IU; vitamin K3, 635 mg; zinc, 11.37 g.
[2] Vitamin/mineral supplement (grower phase). Each 1.0 kg contains: folic acid, 877.5 mg; pantothenic acid, 9,900 mg; BHT, 13,750 mg; biotin, 68,750 mg; copper, 10,000 mg; choline, 1,546.3 mg; iron, 30,000 mg; iodine, 1,000 mg; manganese, 90,000 mg; niacin, 38,500 mg; selenium, 212.5 mg; vitamin A, 9,775 IU; vitamin B1, 1,725 mg; vitamin B2, 6,187.5 mg; vitamin B6, 3,900 mg; vitamin B12, 15,225 mg; vitamin D3, 2,255 IU; vitamin E, 25 mg; vitamin K3, 3,150 mg; zinc, 80,000 mg.

2.2. Performance

Broilers and feed were weighed weekly to obtain feed intake (FI), body weight gain (BWG), and feed conversion ratio (FCR). Viability (VIA) was calculated considering the percentage of broilers that died during the period. The productive factor (PF) of the experimental period was calculated using the following formula: (daily weight gain (kg) × viability (%) / feed conversion) × 100.

2.3. Nutrient digestibility

Nutrient digestibility, nitrogen excretion (NE), apparent metabolizable energy (AME), and apparent metabolizable energy corrected for nitrogen balance (AMEn) were obtained using the traditional total excreta collection method (Sakomura and Rostagno, 2016). Thirteen chickens were used per experimental unit, from 28 to 32 days, with seven days of adaptation.

Excreta and diets were analyzed to determine dry matter (DM), CP, crude energy (CE), and EE (AOAC, 2006). Acid hydrolysis was used to determine EE content of excreta. Values for CE were determined using a Parr® brand isoperibol 6200 adiabatic heat pump. Calculations for apparent metabolizable energy (AME) were performed according to Matterson et al. (1965). The apparent DM digestibility coefficient (DMDC), CP digestibility coefficient (CPDC), and EE digestibility coefficient (EEDC) were calculated by: nutrient metabolism (%) = ((ingested nutrient (g) − excreted nutrient (g))/nutrient ingested (g)) × 100.

2.4. Macroscopic muscular evaluation

Twelve *pectoralis major* muscles from each treatment were macroscopically evaluated in a bright environment (white light with minimum intensity of 500 lux) at 38 d and 46 d, by a well-trained assessor for the identification and classification of white striping and wooden breast according to the methodologies of Kuttappan et al. (2012) and Sihvo et al. (2014), respectively.

White striping was classified into grades from 0 to 5, in which: 0 = normal; 1 = discrete white striping; 2 = white striping < 1 mm thick; 3 = white striping > 1 mm thick; 4 = white striping between 2 and 3 mm thick; and 5 = white striping > 3 mm thick (Adapted from Kuttappan et al., 2012).

Wooden breast was classified as PRESENT, when the musculature presented areas with diffuse or focused hardening in large areas, paleness, or surface with clear liquid and presence of petechiae (Adapted from Sihvo et al., 2014) or ABSENT, when normal.

2.5. Histological muscular evaluation

Samples of approximately 4 cm^2 were collected from the *pectoralis major*, *pectoralis minor*, and *latissimus dorsi* muscles of six 38- and 46-day-old broiler chickens per treatment and fixed in 10% buffered formalin for 72 h. Samples were then washed in phosphate-buffered saline (PBS, 0.1 M, pH 7.4), dehydrated in an increasing ethanol series (70-100%), and embedded in paraffin. Subsequently, 4-µm thick sections were made and stained with hematoxylin and eosin (HE) (Bancroft and Stevens, 1996).

For examining the slides, the evaluator (a trained pathologist) used the following criteria: hyaline degeneration, observed by swelling and hypereosinophilia of the fibers; muscle fiber necrosis – observed by fiber fragmentation with or without swelling and interspersed inflammatory cells; fibrosis – assigned an intensity from 1 to 3 when fibrosis was observed; adipocytes – assigned an intensity from 1 to 3 when adipocytes were observed; lymphohistiocytic infiltration – assigned an intensity of 0 to 3 when infiltrate was observed; lesion score – microscopic lesions were classified as normal, mild (less than 4 necrotic fibers in 10 fields), moderate (5-10 necrotic fibers in 10 fields), or marked (more the 11 necrotic fibers in 10 fields).

2.6. Statistical analysis

Nutrient digestibility data were analyzed using the following fixed effect model:

$$Y_{ij} = \mu + T_i + e_{ij},$$

in which Y_{ij} is the response variable, μ is the overall mean, T_i is the fixed effect of treatment (i = 19P, 19E, 21P, 21E, 19E+Aa), and e is the residual error.

Performance and myopathies data were analyzed using the following fixed effect model:

$$Y_{ij} = \mu + T^1 i + T^2 j + Y_{ij} + \varepsilon_{ijk},$$

in which Y_{ij} is the quantitative response variable; μ is the overall mean; $T^1 i$ = 19P, 19E, 21P, 21E, 19E+Aa; $T^2 j$ = 38 or 46 days; Y is the effect of factors "i" and "j", and ε is the random error.

Results were analyzed using Statistical Analysis System (SAS, 2002). Normality of the data was tested by the Shapiro-Wilk test. Normal data were subjected to analysis of variance (F test) to evaluate significant effects among simple factors. Differences between means were considered significant when P≤0.05 and compared by Tukey test at 5% probability. Non-normal data were evaluated by non-parametric statistics, namely analysis by the Wilcoxon test for the dependent variable and Kruskal-Wallis for the independent variable, both at 5% probability. Frequency data were analyzed using the chi-square test.

3. Results

There was no interaction between diet and age for any of the performance variables studied (P>0.05). Broilers that received the 19P diet had higher FI than those that received the 21P and 21E diets (P≤0.05), while FI was intermediate for the other treatments. Regardless of diet, higher FI values were found at 44 days compared with 37 days (P≤0.05) (Table 3).

Diet did not affect broiler chicken BWG (P>0.05). Broilers had higher BWG at 44 days than at 37 days (P≤0.05). Broilers fed the 19P diet had higher FCR compared with those in the other treatments (P≤0.05), but age had no effect on FCR (P>0.05). Neither diet (P>0.05) nor age (P>0.05) had an effect on VIA. Treatment 19E+Aa showed higher PF than treatment 19P (P≤0.05), while the other treatments showed intermediate PF values. Regardless of diet, age had no effect on PF (P>0.05) (Table 3).

Values of DMDC for treatments 19P, 21P, and 19E+Aa were higher than those for treatment 21E, while treatment 19E was intermediate and similar to the others (P≤0.05). Treatment 19E+Aa had better CPDC than the other treatments (P≤0.05). Treatment 19E+Aa had lower NE than treatments 21P and 21E, while treatments 19P and 19E had intermediate values (P≤0.05). Animals that received treatments 19E, 21E, and 19E+Aa had better EEDC compared with animals receiving 19P and 21P (P≤0.05). Treatment 21P had lower AME and AMEn than the other treatments (P≤0.05) (Table 4).

Neither diet (P>0.05) nor slaughter age (P>0.05) had an effect on the white striping score or on the appearance of wooden breast in female broilers (Table 5). For the *pectoralis major* muscle (Figures 1A and 1B), connective tissue intensity was lower for the 19P treatment compared with the other treatments (P≤0.05). The other histological variables were not affected by diet (P>0.05). Age had no effect on any of the evaluated variables in this muscle (P>0.05) (Table 6).

For the *pectoralis minor* muscle (Figure 1C), diet had no effect on any of the evaluated parameters (P>0.05). However, there was an increase in the intensity of necrosis, hyaline degeneration, lymphohistiocytic infiltration, and lesion score at 46 days compared with 38 days (P≤0.05). Age had no effect on fibrosis and adipocyte intensity (P>0.05) (Table 6).

Table 3 - Performance of female broilers according to diets and age (37 and 44 days)

	Performance					
	Initial weight (kg)	FI (kg)	BWG (kg)	FCR	Viability (%)	PF
Diet						
19P	0.937	2.930a	1.689	1.73b	100.00	499.80b
21P	0.928	2.842bc	1.705	1.66a	100.00	524.51ab
19E	0.916	2.901ab	1.720	1.68a	100.00	521.80ab
21E	0.928	2.811c	1.706	1.64a	98.71	524.45ab
19E+Aa	0.926	2.879abc	1.731	1.66a	98.71	526.14a
Age						
37 days	-	2.326b	1.390b	1.67	99.48	516.84
44 days	-	3.419a	2.031a	1.68	99.48	521.84
P-value						
Diet	0.5679	0.0030	0.2577	<0.0001	0.1764	0.0349
Age	-	<0.0001	<0.0001	0.3041	0.5000	0.3992
Diet × age	-	0.9898	0.7996	0.9321	-	0.9156
SEM	-	0.076	0.048	0.040	1.455	22.78

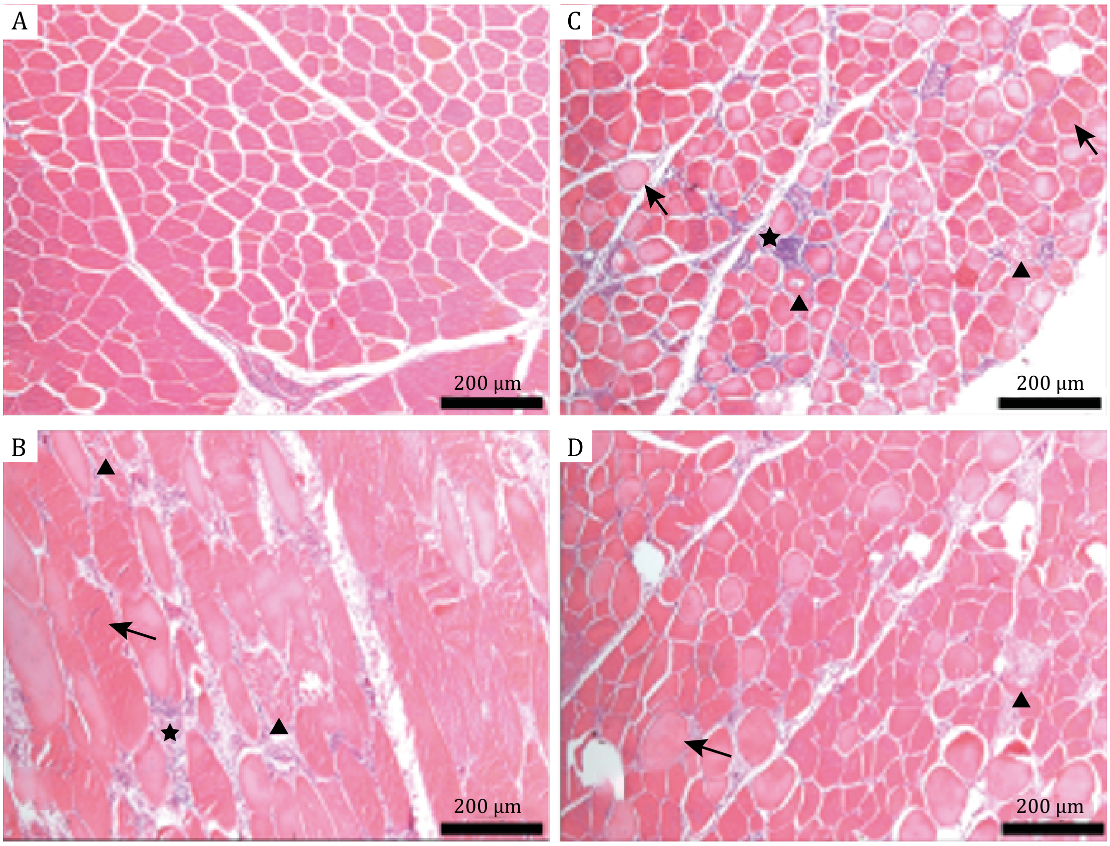
FI - feed intake; BWG - body weight gain; FCR - feed conversion ratio; PF - productive factor; SEM - standard error of the mean.
a,b,c - Means followed by distinct letters in column differ from each other by Tukey test (P<0.05).

Table 4 - Nutrient digestibility and apparent metabolizable energy corrected for nitrogen balance (AMEn) of female broilers fed different diets, from 28 to 32 days of age

Diet	DMDC	CPDC	NE	EEDC	AME	AMEn
19P	74.58a	57.14b	99.45ab	77.25b	3,306a	3,154a
21P	73.16a	56.88b	110.78a	77.82b	3,129b	2,956b
19E	71.49ab	59.28b	95.64ab	80.80a	3,396a	3,227a
21E	69.78b	57.18b	113.55a	80.93a	3,396a	3,219a
19E+Aa	73.66a	63.13a	86.18b	81.09a	3,403a	3,221a
P-value	<0.001	0.049	0.013	0.034	<0.001	<0.001
SEM	0.3245	0.6938	2.5536	0.6208	0.1411	0.1225

DMDC - dry matter digestibility coefficient (%); CPDC - crude protein digestibility coefficient (%); NE - nitrogen excretion (g/kg); EEDC - ether extract digestibility coefficient (%); AME - apparent metabolizable energy (kcal/kg); AMEn - apparent metabolizable energy corrected for nitrogen balance (kcal/kg); SEM - standard error of the mean.
ab - Means followed by distinct letters in column differ from each other by Tukey test (P<0.05).

For the *latissimus dorsi* muscle (Figure 1D), diet had no effect on any of the variables studied (P>0.05). However, higher fibrosis, adipocytes and lymphohistiocytic infiltration were more frequent at 46 days



A - Normal *pectoralis major* muscle; B - *Pectoralis major* muscle with fiber degeneration (→), fiber necrosis (▲), and inflamatory infiltrate (★); C - *Pectoralis minor* muscle with fiber degeneration (→), fiber necrosis (▲), and inflamatory infiltrate (★); D - *Latissimus dorsi* muscle with fiber degeneration (→) and fiber necrosis (▲).
Hematoxylin and eosin.

Figure 1 - Histology of *pectoralis major*, *pectoralis minor*, and *latissimus dorse* muscles from broilers.

Table 5 - Macroscopic evaluation of white striping scores and wooden breast frequency study in *pectoralis major* muscle in female broilers according to diets and slaughter age

	White striping[1]	Wooden breast[2]	
	Score	Absent (%)	Present (%)
Diet			
19P	0.91	62.50	37.50
21P	0.81	54.55	45.45
19E	0.95	58.33	41.67
21E	1.33	37.50	62.50
19E+Aa	0.72	63.64	36.36
Age			
38 days	0.83	53.33	46.64
46 days	1.03	57.14	42.46
P-value			
Diet	0.4383	0.368	
Age	0.4565	0.680	

[1] Means compared by the Kruskal Wallis test (P<0.05) for the independent variable and the Wilcoxon test (P<0.05) for the dependent variable.
[2] Means compared by Chi-square test (P>0.05).

than at 38 days (P≤0.05). Age had no effect on necrosis, hyaline degeneration, and lesion scores in this muscle (P>0.05) (Table 6).

Table 6 - Histological evaluation of muscles according to the diets and age of female broilers

	Variable					
	MFN	HD	FIB	ADP	LHI	LS
Diet			*Pectoralis major*			
19P	5.10	15.15	0.00	0.40	0.70	1.50
21P	6.17	15.69	0.25	0.62	0.87	1.75
19E	5.58	17.14	0.11	0.44	1.11	1.67
21E	5.95	12.69	0.25	0.50	0.62	1.62
19E+Aa	5.64	25.09	0.45	0.54	0.82	1.64
Age						
38 days	6.58	16.68	0.12	0.37	0.75	1.79
46 days	4.66	18.56	0.32	0.64	0.91	1.45
P-value						
Diet	0.879	0.507	0.138	0.897	0.183	0.922
Age	0.057	0.349	0.116	0.079	0.187	0.095
			Pectoralis minor			
Diet						
19P	2.52	13.00	0.25	0.37	0.75	1.00
21P	4.97	21.55	0.33	0.00	1.00	1.55
19E	3.66	15.60	0.20	0.10	0.70	1.22
21E	3.17	16.94	0.12	0.12	0.75	1.12
19E+Aa	4.96	22.44	0.62	0.50	1.12	1.75
Age						
38 days	2.27b	12.28b	0.13	0.13	0.65b	0.87b
46 days	5.71a	24.32a	0.50	0.30	1.10a	1.89a
P-value						
Diet	0.301	0.563	0.607	0.164	0.392	0.423
Age	0.001	0.002	0.076	0.296	0.004	>0.001
			Latissimus dorsi			
Diet						
19P	2.78	14.58	0.45	1.00	0.54	1.00
21P	2.52	15.83	0.40	0.90	0.60	0.90
19E	2.46	20.64	0.33	0.89	0.44	0.89
21E	3.08	16.85	0.20	1.20	0.70	1.10
19E+Aa	2.36	17.69	0.20	0.90	0.40	0.60
Age						
38 days	2.74	15.63	0.13b	0.56b	0.22b	0.83
46 days	2.57	18.16	0.48a	1.33a	0.81a	0.96
P-value						
Diet	0.650	0.494	0.817	0.947	0.972	0.488
Age	0.868	0.262	0.024	0.003	>0.001	0.425

MFN - muscle fiber necrosis; HD - hyaline degeneration; FIB - fibrosis; ADP - adipocytes; LHI - lymphohistiocytic infiltration; LS - lesion score.
a,b - Means followed by distinct letters in the column differ from each other by the Kruskal Wallis test for the independent variable (P<0.05) and the Wilcoxon test for the dependent variable (P<0.05).

4. Discussion

Despite numerous studies aiming to reduce dietary protein in broiler feed (Si et al., 2004; Leeson and Summers, 2005; Attia et al., 2018; Soares et al., 2020), studies considering protein reduction without altering the concentration of lipids, fiber, and starch in the diet cannot be found. These factors may influence nutrient utilization and, consequently, animal performance.

The higher FCR observed for the 19P treatment may be associated with the lower inclusion of oil in the diet since the 19E treatment, which had the lowest FCR, had the same CP content but with higher oil inclusion. Inclusion of lipids in poultry diets improves FCR because lipids reduce gastric movement, slowing the passage of diet in the digestive tract (Baião and Lara, 2005). This, in turn, improves nutrient utilization and increases the feeling of satiety, and thus lipids are known as gastric emptying inhibitors (Khoddami et al., 2018).

In the present study, the experimental feed represented by treatments 19E, 21E, and 19E+Aa, designed to isolate the lipid, fiber, and starch factors, which can affect nutrient utilization, did not affect BWG and FCR. When these factors are isolated, it is possible to perceive the beneficial effects of lipids on broiler performance because for treatments 19P and 21P, in which these factors were not isolated, the former, with lower lipid content, had worse FCR.

In a study using different CP levels (16, 18, 20, or 22%) for male broilers, Soares et al. (2020) observed higher BWG and lowest FCR for the two highest CP levels, which was explained by CP level and inclusion of oil in diets. Similarly, Attia et al. (2018) evaluated a 2% increase in CP contents, in addition to energy increase along with the increase of CP in the initial diets, and growth of broilers under heat stress. The authors found, along with the energy increase, higher BWG and lower FCR for the treatment with higher CP content (22%), whose formulation had about 2% greater inclusion of oil.

According to Cobb (2018), the BWG of female broilers from 21 to 37 days is 1.332 kg and from 21 to 44 days, it is 1.932 kg, while FCR is 1.79 from 21 to 37 days and 1.92 from 21 to 44 days. However, the present study found higher BWG (1.390 kg from 21 to 37 days and 2.031 kg from 21 to 44 days) and lower FCR (1.67 from 21 to 37 days and 1.68 from 21 to 44 days). Thus, FCR did not worsen with increasing age as suggested by Cobb (2018), as the increase in FI was proportional to the increase in BWG observed at 37 and 44 days. This proportional increase may have been achieved by adequate conditions in the experimental environment, which was also reflected in the low mortality rate, leading to similar viability results at 37 and 44 days. The higher PF value of treatment 19E+Aa compared with treatment 19P occurred in response to the BWG and dietary FCR found for these treatments.

The results found for performance in the present study are in agreement with nutrient digestibility and diet energy values. Reduced nutritional levels in a diet improves nutrient digestibility for broiler chickens (Viola et al., 2008); however, the lack of standardization of the nutritional matrix of corn gluten meal (Santos, 2004) and higher inclusion of soybean meal may have worsened DMDC for the 21E treatment, which was similar to that of 19E and lower than the others. The amount and quality of carbohydrates, proteins, and fats contained in different diets can alter the retention time of digestion and thus influence the efficiency of digestion and absorption of nutrients (Soares et al., 2020).

The greater inclusion of synthetic amino acids in the 19E+Aa treatment improved CPDC because these amino acids are absorbed faster and more efficiently than those contained in the intact dietary protein. In addition, the NE values, and also the lowest AME and AMEn values, observed in the 21P treatment confirm that the CP supply may be above the requirements. Excess CP is catabolized and excreted as uric acid, which has a high energy cost to the animal and may be associated with the loss of about 0.7 kcal of energy in the urine for each gram of dietary CP. Thus, the energy of productive processes is converted to nitrogen excretion (Noblet and Perez, 1993).

A reduction in AME and AMEn values was not observed for the 21E treatment, as was observed for 21P. This is due to the higher inclusion of oil in the diet of the 21E treatment compared with the

21P treatment, evidencing the extra-caloric effect of oil in improving energy utilization by broilers. Similarly, higher oil inclusion improved EEDC in treatments 19E, 21E, and 19E+Aa compared with the others. Increased yield of carcass and parts is one of the main objectives of the poultry industry because this factor is responsible for higher profitability of the activity (Nortey et al., 2019). The difference in protein deposition at different ages of broilers is mainly due to changes in metabolism, because the difference between anabolic and catabolic processes decreases with advancing age (Radaelli et al., 2017). When the animal reaches its peak growth, the tendency is for fat deposition to increase and protein deposition to decrease.

Linked to older slaughter age and higher carcass yields, the pursuit of rapid growth and yield of poultry has implications for animal physiology and, consequently, changes in meat quality (Bailey et al., 2015; Petracci et al., 2019). Since pectoral muscle growth is achieved by muscle fiber hypertrophy, which results in increased fiber diameter, there is also reduced space for connective tissue and, as a consequence, increased muscle degeneration. These morphological changes limit blood supply, compromising nutrient supply and the removal of metabolites produced by muscle fibers, thereby altering meat quality (Sandercock et al., 2009) and causing the onset of myopathies.

Mudalal et al. (2015) found a strong association between the occurrence of wooden breast and higher weight and white striping of the *pectoralis major* muscle; however, the myopathies studied here (white striping and wooden breast) were not associated with the diets provided to broilers nor with slaughter age. Furthermore, it was not possible to notice any difference in the intensity of myopathic lesions due to slaughter age.

Although the white striping myopathy has no defined etiology, it seems to be related to high growth rates, and so males have a higher incidence, which can be explained by the greater body weight of these animals (Kuttappan et al., 2012). Wooden breast in the *pectoralis major* of broiler chickens may be associated with white striping myopathy in the most severe cases (Sihvo et al., 2014), but its etiology and the factors that cause its appearance have not been identified, being predominantly described, as well as white striping in fast growing strains.

Histological changes in muscle tissue can be seen in broilers affected by myopathies before macroscopic perception. Changes in muscle tissues can present pathological aspects and usually affect the *pectoralis major, pectoralis minor*, and *latissimus dorsi* (Ecco and Braga, 2015). The pectoral muscles act synergistically for wing movement, with the *pectoralis major* as the main thruster muscle, being composed of fast-twitch myofibers with glycolytic activity, the fibers of which respond rapidly with increased white muscle mass when given high nutritional diets (Roy et al., 2006). However, although tissue lesions were observed, no association was observed between the experimental diets of the present study and histological characterization.

The connective tissue of the pectoral muscle has thousands of cells with tissue connection, support, and filling functions. Despite its regenerative capacity, when damage to skeletal muscle leads to cell destruction, connective tissue proliferation occurs, and when the muscle area is replaced by fibrous tissue, complete regeneration is inhibited. After muscle fiber degeneration, revascularization of the injured area occurs and inflammatory cells are activated to remove necrotic tissue, which requires the formation of healing connective tissue to keep the ends of the myofibrils connected (Cruz et al., 2017; Petracci et al., 2019).

These principles may underlie the lower connective tissue intensity observed for the 19P treatment, which contained lower CP content and oil inclusion, and possibly led to slower muscle development, compared with the other treatments. The changes observed in the *pectoralis minor* and *latissimus dorsi* due to slaughter age were not associated with infectious conditions. However, histological changes were possibly a consequence of ischemia and the rapid growth of these muscles (Velleman et al., 2014).

Whereas white striping and wooden breast myopathies may exhibit similar histological lesions, including myodegeneration and regeneration, fibrosis, and lipidosis (Sihvo et al., 2017; Soglia et al., 2016; Radaelli et al., 2017; Petracci et al., 2019), there is probably some common mechanism that allows both myopathies to involve the same muscle, which can also be observed in the present study. Moreover, in this study, the histological features found were consistent with macroscopic evaluation (Baldi et al., 2018).

5. Conclusions

Diet with 19% crude protein, using only commercially available ingredients, was not adequate because it worsens feed conversion. The diet with 21% crude protein, using only commercially available ingredients, reduces the availability of apparent metabolizable energy and apparent metabolizable energy corrected for nitrogen balance due to high nitrogen excretion, so it should be avoided. The experimental diet 19% crude protein plus synthetic amino acids (19E+Aa) improves the crude protein digestibility coefficient, exhibits low nitrogen excretion, and has feed conversion similar to diets with 21% crude protein (using only commercially available ingredients), 19% crude protein (using experimental ingredients), and 21% crude protein (using experimental ingredients), all with higher oil inclusion. However, the choice of the most appropriate diet should be based on the cost and availability of ingredients. In addition, slaughter of 46-day-old instead of 38-day-old female broiler chickens is recommended. Broilers slaughtered at 46 days have higher weight gain without worsening feed conversion and the intensity of myopathies.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: L.J.C. Lara. Data curation: A.R.C. Abreu, D.P. Vaz and M.M. Saldanha. Formal analysis: D.P. Vaz. Funding acquisition: L.J.C. Lara. Investigation: A.R.C. Abreu, I.C.S. Araújo, M.M. Saldanha, P.A. Leão and R. Ecco. Methodology: A.R.C. Abreu, I.C.S. Araújo, D.P. Vaz, D.O. Fontes and R. Ecco. Project administration: L.J.C. Lara. Resources: L.J.C. Lara. Supervision: L.J.C. Lara. Visualization: D.O. Fontes, R. Ecco and L.J.C. Lara. Writing-original draft: A.R.C. Abreu, I.C.S. Araújo, R. Ecco and L.J.C. Lara. Writing-review & editing: I.C.S. Araújo.

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