Effects of vitamin A on carcass and meat quality of broilers

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ABSTRACT This study evaluated the level and length of time of vitamin A supplementation and its effects on carcass and cuts yield, meat quality, and myopathies in 42-day-old broilers. A total of 1,920 birds were divided into 6 groups, and each group received a different level of vitamin A: 0; 6,000; 16,000; 26,000; 36,000 and 46,000 IU/ kg. From d 1 to 21, the treatments were distributed among 16 replicates with 20 birds. From the 22nd d on, 8 repetitions remained with the initial treatment and the others received diets with no vitamin A supplementation. Twelve birds were slaughtered per treatment to evaluate carcass and cuts yield, shear force, cooking loss, water holding capacity, and the presence of substances reactive to thiobarbituric acid. The remaining birds were slaughtered and evaluated in loco for Wooden Breast (**WB**) and White Striping (**WS**). Wings weight was affected by vitamin A levels. The duration of the vitamin A supplementation process had effects on the weight of breast, legs with a dorsal portion, and meat color in the yellow intensity (b*). Incidence of WB had higher scores in birds supplemented until 42 d of age. WS showed a quadratic response and a lower response with supplementation of 29,700 IU/ kg. Even for WS, a higher occurrence of the normal score was found in birds supplemented until 21 d of age. Minimal quadratic responses were obtained for normal, moderate, and severe scores, in supplementations of 29,301; 29,959, and 29,827 IU/ kg, respectively. WB had lower occurrence rates in birds supplemented until 21 d of age. Consequently, the severe score was more frequent when supplementation was provided until 42 d of age. The level of vitamin A and the length of time during which this supplementation was provided had influence on cuts yield, meat color and the incidence of WB and WS of the 42-day-old birds.

Key words: retinol, white striping, wooden breast, TBARS, meat characteristics

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INTRODUCTION

The current consumer market demands products concerned with quality and health aspects of animal proteins. According to the Brazilian Animal Protein Association, Brazil is the second largest producer of chicken meat in the world (Associação Brasileira de, 2018). However, in order to maintain this huge production and meet the increasing demand for consumption, both genetics and animal nutrition companies need to move together toward the adequate and maximum zootechnical development of poultry.

Advances in genetics, nutrition, management, and health practices in broiler breeding have led mortality and loss rates to increase due to slaughterhouse condemnations caused by metabolic disorders, which are directly related to high production levels (Maschio and Raszl, 2012).

Vitamin and micromineral supplementation of diets are some of the widespread and clearly necessary practices within the productive context. Understanding the role of each vitamin and their interrelationships is essential to determine the correct supplementation to be provided.

According to Sundeen et al. (1980), vitamin A influences hepatic carbohydrate metabolism, where hepatic glycogen deposition is largely reduced in cases of hypovitaminosis A. This vitamin, as well as retinol, has a broad role in macronutrient homeostasis as an essential

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micronutrient and an indispensable lipophilic for the maintenance of the animals' general health, since it seems to act on the metabolism of carbohydrates, lipids, and proteins in the liver, pancreas, skeletal muscle, and adipose tissue, consequently playing a role in the metabolism of glucose and fatty acids (Chen and Chen, 2014).

Due to its functions as an antioxidant, acting on the reduction of oxidation and degradation of membrane lipids (Abd El-Hack et al., 2017), as well as its role in cell differentiation, ensuring the correct keratinization and formation of new tissues (Gerster, 1996), vitamin A supplementation in broilers diets may act on the incidence of myopathies.

When evaluating miodegeneration, quality and chemical composition of chicken breast meat (*Pectoralis major*), Mazzoni et al. (2015) found that most of the fillets produced in the current intensive poultry production system showed histological lesions, with changes in the meat chemical composition and a decrease in water holding capacity.

The main cases of carcass condemnation at slaughterhouses are due to muscle changes, or myopathies, mainly white striping (**WS**) and wooden breast (**WB**). The occurrence of each myopathy is different depending on the region and the genetics used, and in most of them, the causes are unclear.

The increase in muscular mass of birds, associated with sedentary conditions and the prolonged and direct pressure on the muscles, leads to a reduction in capillary blood flow in these regions, which compromises nutrients supply, as well as the excretion of metabolic agents produced by muscle fibers, such as carbon dioxide and lactate. The non-excretion of these metabolic agents leads to ionic disorders, such as regulation of calcium, which is necessary for muscle contraction. This results in myopathies and necrosis (Sosnick, 1993).

The objective of this study was to evaluate the influence of different levels and length of time of vitamin A supplementation on carcass, cuts yield, and meat quality of 42-day-old broilers, as well as the incidence of myopathies in those animals.

MATERIALS AND METHODS

Experimental Program

The study was conducted at the Poultry Research Center of the Western Paraná State University − Marechal Cândido Rondon, Paraná, Brazil. All procedures were in accordance with Normative Act No. 37, from February 15, 2018, by CONCEA, which establishes the Euthanasia Practice Guidelines of the National Council for Animal Experimentation Control, registered under number 22-18.

A total of 1,920 one-day-old male broilers (Cobb 500, Cobb-Vantress Ltd., Cascavel, PR), with an average initial weight of 47.00 ± 2.00 g, were divided, within a completely randomized design, into 6 groups. Each group received a different level of vitamin A supplementation (0; 6,000; 16,000; 26,000; 36,000 and 46,000 IU/ kg).

From 1 to (4 d of age, all birds were subjected to diets with no vitamin A supplementation. Thus, their vitamin A deposits were completely consumed. The birds were divided into 96 pens (experimental unit, **EU**), with 20 birds per EU, and were exposed to diets supplemented with different levels of vitamin A, considering 16 replicates of each treatment.

Two modes of vitamin A supplementation were established, with 8 repetitions of each treatment. In the first supplementation mode, named T-21 d, the birds received diets without any vitamin A supplementation (0 IU/kg) until 21 d of age. Then, they started diets without vitamin A supplementation (0 IU/kg) during the period from 22 to 42 d. In the second supplementation mode, denominated (T-42 d), the birds received feed containing vitamin A throughout the whole experimental period, until 42 d of age.

Experimental Diets

The experimental diets (Table 1) were offered as bran and formulated based on corn and soybean meal,

Table 1. Composition and nutritional characteristics of experimental diets for broilers.

Item (g/kg)	1 to 21 d	22 to 42 d
Corn 8%	49.267	57.110
Soybean meal 45%	42.032	34.563
Soybean oil	4.503	4.542
Dicalcium phosphate	1.605	1.425
Limestone 35%	1.086	0.922
Sodium chloride	0.515	0.459
Lysine sulfate 65%	0.174	0.252
DL-methionine 99%	0.318	0.281
PX Vitamin[1]	0.100	0.065
L-threonin 98.5%	0.044	0.048
Inert	0.138	0.138
Choline chloride 60%	0.093	0.073
Salinomycin 12%	0.055	0.055
Mineral premix[2]	0.050	0.050
Avilamycin 10%	0.050	0.050
Antioxidant	0.010	0.010
Nutritional composition (g /kg)		
Met. energy (kcal/ kg)	3,050	3,150
Crude protein	213.50	205.80
Ether extract	71.80	73.74
Calcium	8.78	7.58
Total phosphorus	6.46	5.91
Available phosphorus	4.20	3.74
Sodium	2.18	1.95
Dig. Lysine	12.56	11.24
Dig. methionine + cystine	9.29	8.32
Dig. tryptophan	2.69	2.31
Dig. threonine	8.29	7.42

[1]Premix Mineral Vitamin for Poultry—Guarantee Levels per Kilogram of Feed - 1 to 21 d old: Vit. D3 (min) 3,055 IU; Vit. E (min) 45.8 IU; Vit. K3 (min) 2.44 mg; Vit. B1 (min) 3.25 mg; Vit. B2 (min) 8.17 mg; Vit. B6 (min) 4.58 mg; Vit. B12 (min) 19.0 mcg; Pantothenic Acid (min) 16.40 mg; Niacin (min) 49.60 mg; Folic Acid (min) 1.145 mg; Biotin (min) 114.5 mcg; Selenium (min) 0.32 mg. 22 to 42 d old: Vit. D3 (min) 1.985 IU; Vit. E (min) 29.8 IU; Vit. K3 (min) 1.59 mg; Vit. B1 (min) 2.11 mg; Vit. B2 (min) 5.31 mg; Vit. B6 (min) 2.98 mg; Vit. B12 (min) 12.93 mcg; Pantothenic Acid (min) 10.66 mg; Niacin (min) 32.24 mg; Folic Acid (min) 0.744 mg; Biotin (min) 74.42 mcg; Selenium (min) 0.21 mg.

[2]Premix Poultry Mineral (1−42 d old)—Levels of Guarantee per Feed Kilogram: Copper (min) 10.00 mg, Iron (min) 50.00 mg, Manganese (min) 80.00 mg, Cobalt (min) 1.00 mg, Iodine (min) 1.00 mg, Zinc (min) 50.00 mg.

according to the requirements proposed by Rostagno et al. (2017), for the starter and grower phase, except for vitamin A requirements. For vitamin A supplementation purposes, only values related to retinyl acetate (1,000,000 IU/ kg) were admitted, and the amounts of vitamin A of the ingredients that made up the experimental diets were not considered. An analysis of the corn and soybean meal used in the experimental diets showed values of 2.37 IU/kg and 1.26 IU/kg for corn and soybean meal, respectively.

The vitamin premix used contained vitamins B, D, E, K, and selenium. The vitamin A supplementation was applied replacing weight by weight the inert material in feed and vitamin A sources (Table 1).

Carcass and Cuts Yield

At 42 d of age, 2 birds were slaughtered per EU (n = 224) to evaluate carcass and cut yield, after fasting for 6 h before slaughter. They were euthanized by a ventral neck cut and electric stunning. Then, the carcasses were scalded at 60°C for 30 s, and the feathers were removed mechanically. After removing the feathers, viscera, head, and feet, the carcasses were weighed and cooled in a static mixture of ice and water for 1 h. They were, then, drained for 10 min and stripped to evaluate cuts yield of butterfly breast fillets, tenderloins, legs with a dorsal portion, and wings.

Meat Quality Assessments

Meat quality analysis (pH, meat color, water holding capacity, cooking loss, and shear force) was performed in the breast muscle (*Pectoralis major*) of one bird slaughtered per EU (n = 112).

pH and Meat Color

At 15 min and 24 h postmortem, the pH was measured in the right pectoralis major muscle using a pH meter (HI 99163, Hanna instruments, Woonsocket, RI). The equipment probe was inserted into the breast fillet at an angle of 45°, with constant washing using deionized water between samples. Each value was expressed as the average of 2 measurements.

At 15 min and 24 h postmortem, color was expressed in the CIELAB color system, lightness (L* - dark to light level), red (a * - red / green intensity) and yellow (b * - yellow/blue intensity) using a colorimeter (CR-400, Konica Minolta Sensing, São Paulo, SP). The colorimeter was calibrated against black and white reference tiles before use. Evaluations were performed at the center of each muscle section, and values were expressed as the average of 3 measurements. Color measurements were determined at room temperature (20−25°C), on the surface of each muscle sample, at 3 randomly selected locations, using diffuse illumination and a 0° angle observer.

Water Holding Capacity, Cooking Loss, and Shear Force

Left breast fillets were used for water holding capacity, following the methodology proposed by Nakamura and Katok (1985). Samples of approximately 1 g of breast muscle were wrapped with filter paper, centrifuged at 2,000 rpm for 4 min, weighed, dried in an oven at 70°C for 12 h, and weighed again to calculate water holding capacity.

To perform cooking loss analysis, breast fillets were weighed, wrapped with laminated paper, and cooked in a commercial electric plate at up to 180°C until reaching the internal temperature of 80°C. The samples were kept at rest until stabilizing at room temperature, and then weighed again to calculate cooking loss (Honikel, 1998).

For shear force analysis (kgf /cm^2), the samples were cut into four rectangles (1.0 × 1.0 × 4.0 cm), with fibers perpendicularly toward a blade (CT3 Texture Analyzer, Brookfield Engineering Laboratories, Inc., Middleboro, MA) coupled with a probe (TA 3/100 and fixture TA − SBA Brookfield Engineering Laboratories), with force calibration of 0.01 kg, deformation of 20 mm and speed of 2.5 mm/s test.

Thiobarbituric Acid Reactive Substances

The concentration of thiobarbituric acid reactive substances (**TBARS**) was measured in the thigh meat of 42-day-old broiler chickens. Evaluations were performed with meat refrigerated at −20°C for 24 h, 20 d, and 40 d. Analyses were performed according to the methodology adapted from Vyncke (1975), and Sorensen and Jorgensen (1995). The aldehydes were extracted by homogenizing a 10 mL solution of trichloroacetic acid (7.5%) and BHT (0.2%), with 2.5 g of meat sample. Then, the supernatant was filtered through qualitative filter paper. Three mL samples were added to 3 mL of a thiobarbituric acid (**TBA**) solution and left to react for 40 min at 80°C in a water bath. After cooling, the samples were read at 538 nm in a spectrophotometer (UV-M51; Bel Photonics, Piracicaba, SP). A standard curve of 1,1,3,3 tetraethoxypropane (**TEP**) was used, and the results were expressed as milligrams of malonaldehyde (**MDA**) per kilogram of sample.

Myopathies

The remaining birds were identified and sent to the poultry processing industry for slaughter and on-site assessment of myopathies in their pectoralis major muscle (WB and WS). The incidence of myopathies was measured by using a severity scale, ranging from 0 to 3; where 0 refers to breast fillets considered normal, 1 indicates mild myopathy, 2) indicates moderate myopathy, and 3 refers to severe myopathy (Kuttappan et al., 2016).

Statistical Analysis

The data were submitted to normality analysis using the Shapiro Wilk test. Subsequently, the analysis of variance (**ANOVA**) was performed. Polynomial regression analysis was used to compare vitamin A supplementation levels using the PROC GLM procedure. The generated equations were obtained through polynomial orthogonal contrasts, and the coefficients were generated through PROC IML. At 42 d of age, in order to assess vitamin A supplementation lengths of time, differences between the means were assessed by the F test at 5% probability.

For the statistical analysis of WS (normal, mild, and moderate scores) and WB (normal, mild, and moderate scores), the data were converted into percentages (%) and adjusted by the Generalized Linear Model (**GLM**), using the gamma distribution in log link function. For the severe scores of WS and WB, the data were transformed into percentages (%) and adjusted by the GLM procedure using the inverse Gaussian distribution in log link function.

The goodness of fit of the model was verified by the Akaike Information Criterion (**AIC**) along with a graphical analysis of the residues. The variables WS and WB were compared by a least square means test (lsmeans), using the chi square statistic.

All statistical evaluations were performed using the statistical software SAS University Edition, Student version (SAS Inst. Inc., Cary, NC).

RESULTS

Carcass and Cuts Yield

Wings relative weight at 42 d of age showed an increasing linear response in birds subjected to vitamin A supplementation until 21 d of life. The birds supplemented until 42 d had a quadratic effect (Table 2), with vitamin A supplementation of 35,179 IU/kg providing the minimum response of wings relative weight.

The F test showed that the relative weights of legs with a dorsal portion ($P = 0.0286$) and breast ($P = 0.0109$) were different from each other when comparing the means of supplementation time lengths (Table 2). A higher weight of legs with a dorsal portion was achieved in the birds supplemented with vitamin A until 42 d of age (T-42). However, for relative breast weight, the highest value was detected in birds supplemented with vitamin A until 21 (T-21) days of life, and with no supplementation during the period between their 22nd and 42nd d of life.

Meat Quality

No differences were found for shear force, cooking loss, water holding capacity, and pH at 24 h postmortem evaluated breast meat at 42 d of age (Table 3).

Vitamin A supplementation had an effect on the pH of the breast meat determined at 15 min postmortem in birds that received vitamin A until 21 d of age (Table 3). The derivation of the quadratic equation indicates vitamin A supplementation of 23,678 IU/ kg for a minimal response.

Breast meat color was influenced by the vitamin A supplementation time length for the yellow/blue intensity variable (b*), both at 15 min ($P = 0.0214$) and at 24 h ($P = 0.0061$) postmortem (Table 4). The averages of b* values in the pectoralis major muscle of broiler chickens at 42 d of age were higher in birds exposed to diets supplemented with vitamin A until 21 d of life, both at 15 min and 24 h postmortem.

Lipid oxidation of thighs meat at 42 d of age did not show statistical difference for any of the variables evaluated (Table 5).

Myopathies

Vitamin A supplementation had influence on WS, with a quadratic response resulting in a minimal response at 29,700 IU/kg of vitamin A supplementation

Table 2. Carcass and cuts yield (%) of broiler chickens fed with different vitamin A supplementations at d 42.

		Vitamin A supplementation (IU/kg)										
	Supplementation[1]	0	6,000	16,000	26,000	36,000	46,000	Average[2]	SEM	*P* (Anova)	*P* (Test F)	*P* (Regression)
Carcass	T − 21	72.918	73.388	73.388	73.906	72.771	72.344	73.1191	1.39589	0.3288	0.3337	0.0829
	T − 42	72.046	72.862	72.795	72.900	73.193	73.050	72.8076	1.23125	0.5803		0.4376
Wing	T − 21	5.23	5.55	5.35	5.67	5.60	5.70	5.51666	0.34208	0.0182	0.4575	0.0088 (L)
	T − 42	5.35	5.64	5.49	5.49	5.54	5.38	5.48166	0.46097	0.0006		0.0392 (Q)
Legs	T − 21	39.654	39.556	40.050	39.455	39.762	39.308	39.6308	1.1452	0.8593	0.0286	0.4954
	T − 42	39.917	40.257	39.605	40.366	40.429	40.279	40.1421	1.07259	0.7092		0.8619
Breast	T − 21	27.265	28.063	28.012	28.238	27.570	27.696	27.8073	0.99573	0.4022	0.0109	0.1108
	T − 42	26.754	27.399	27.561	27.013	27.450	27.061	27.2063	1.14569	0.7570		0.3640
Tenderloins	T − 21	5.23	5.544	5.346	5.665	5.596	5.527	5.48466	0.40144	0.2764	0.6541	0.3049
	T − 42	5.347	5.640	5.489	5.490	5.317	5.381	5.444	0.39883	0.6403		0.5182

L: linear; Q: Quadratic.

Carcass: relative weight (%); Wing: relative weight (%); Legs: relative weight (%) to legs with a portion of the back; Breast: relative weight (%); Tenderloins: relative weight (%).

Wing (T-21 d): 0.00000841 vit A + 9.30975; R^2: 0.13; Wing (T-42d): 0.0000000000660904 vit A^2 - 0.00004650 vit A + 10.10156; R^2: 0.28; Vit. A for minimum response: 35,179 UI /kg; Minimum response: 9.29.

[1]T-21d: birds supplemented with treatments in the period from 4 to 21 days of age; T-42 d: birds supplemented with treatments in the period from 4 to 42 days of age;

[2]Test F ($P \leq 0.05$) for comparing the averages of vitamin A supplementation times.

Table 3. Qualitative assessments of breast meat from broiler chickens fed diets containing different vitamin A supplementation.

		Vitamin A supplementation (IU/kg)										
	Supplementation[1]	0	6,000	16,000	26,000	36,000	46,000	Average[2]	SEM	*P* (Anova)	*P* (Test F)	*P* (Regression)
SF (kgf)	T − 21	3.754	3.468	3.747	3.673	4.828	3.930	3.899	1.33108	0.4105	0.8842	0.9198
	T − 42	3.578	3.856	3.265	3.981	4.295	4.171	3.858	1.53301	0.7750		0.7805
CL (%)	T − 21	34.711	35.290	34.377	34.267	36.066	35.840	35.109	3.51894	0.8718	0.1395	0.5429
	T − 42	32.172	33.671	32.792	35.381	36.326	32.793	33.878	4.51713	0.4008		0.1941
WHC (%)	T − 21	66.381	67.967	68.003	66.567	66.552	66.230	66.952	3.52441	0.8294	0.4228	0.5051
	T − 42	68.569	67.616	66.086	66.113	69.350	67.652	67.564	4.07553	0.5431		0.2753
pH 15 min	T − 21	6.486	6.473	6.398	6.378	6.365	6.516	6.436	0.11868	0.0620	0.5208	0.0041 (Q)
	T − 42	6.494	6.360	3.449	6.456	6.528	6.441	6.454	0.15642	0.4031		0.9780
pH 24 h	T − 21	6.004	5.970	6.018	5.915	5.911	5.925	5.959	0.14360	0.5120	0.9555	0.8332
	T − 42	6.003	5.961	6.010	6.020	5.949	6.064	6.001	0.20573	0.8929		0.6848

Abbreviations: CL, cooking loss; L, linear; pH 15 min, pH breast meat at 15 min postmortem; pH 24 h: pH breast meat 24 h postmortem; Q, Quadratic; SF, shear force; WHC, water holding capacity.

pH 15 min (T-21 d): 0.0000000000660904 vit A^2 - 0.00001180 vit A + 6.50960; R^2: 0.21; Vit. A for maximum response: 23,678; Minimum response: 6.37.

[1]T-21d: birds supplemented with treatments in the period of the 4 to 21 days of age; T-42 d: birds supplemented with treatments in the period of the 4 to 42 days of age;

[2]Test F ($P \leq 0.05$) for comparing the averages of vitamin A supplementation times.

Table 4. Color values of breast meat (15 min and 24 h postmortem) of broiler chickens at 42 d age fed with diets containing different vitamin A supplementation.

		Vitamin A (IU/Kg)										
	Supplementation[1]	0	6,000	16,000	26,000	36,000	46,000	Average[2]	SEM	P (Anova)	P (Test F)	P (Regression)
L* (15 min)	T − 21	53.399	51.720	50.911	50.125	43.970	50.726	50.142	7.1902800	0.1776	0.3337	0.2943
	T − 42	51.216	50.570	50.008	49.375	5.343	48.911	50.071	4.878980	0.9457		0.8760
a* (15 min)	T − 21	4.415	6.606	5.484	5.550	5.636	6.116	5.634	2.449553	0.6134	0.5119	0.8164
	T − 42	7.790	4.995	7.381	4.854	6.349	4.953	6.053	3.687036	0.4201		0.8804
b* (15 min)	T − 21	7.430	8.718	6.709	6.069	6.074	7.503	7.085	2.945354	0.4609	0.0214	0.2253
	T − 42	7.330	5.016	6.753	4.984	6.030	3.243	5.559	3.335864	0.1955		0.5469
L* (24 h)	T − 21	60.020	56.755	56.208	55.726	53.506	58.583	56.799	4.192089	0.0566	0.6830	0.0517
	T − 42	59.189	56.334	55.199	54.804	58.403	54.571	56.416	4.558684	0.2193		0.4115
a* (24 h)	T − 21	4.699	7.100	5.950	6.935	6.096	5.345	6.0208	2.31273	0.2986	0.8959	0.0890
	T − 42	6.644	5.579	6.215	6.128	6.113	5.814	6.082	2.31187	0.9615		0.9704
b* (24 h)	T − 21	10.436	8.850	7.910	8.591	7.291	9.103	8.697	2.692618	0.2888	0.0061	0.0793
	T − 42	9.381	5.579	7.050	6.001	7.031	5.989	7.054	2.93487	0.2338		0.3013

L, linear; Q, Quadratic.

L* = lightness; a* = redness; b* = yellowness.

[1]T-21d: birds supplemented with treatments in the period of the 4 to 21 days of age; T-42 d: birds supplemented with treatments in the period of the 4 to 42 days of age.

[2]Test F ($P \leq 0.05$) for comparing the averages of vitamin A supplementation times.

Table 5. Average values of substances reactive to thiobarbituric acid (TBARS), in MDA mg/kg in the breast meat of broiler chickens refrigerated for 24 h, frozen at −20°C for 20 d and 40 d, fed with diets containing different vitamin A supplementation.

		Vitamin A (IU/Kg)										
	Supplementation[1]	0	6,000	16,000	26,000	36,000	46,000	Average[2]	SEM	*P* (Anova)	*P* (Test F)	*P* (Regression)
24h	T − 21	0.028	0.029	0.023	0.030	0.032	0.030	0.0287	0.01222	0.7579	0.1180	0.6346
	T − 42	0.037	0.034	0.028	0.029	0.033	0.040	0.0335	0.01816	0.7850		0.1359
20d	T − 21	0.026	0.025	0.040	0.030	0.030	0.053	0.0034	0.22564	0.3425	0.62310	0.1625
	T − 42	0.032	0.027	0.059	0.039	0.065	0.060	0.0476	0.03162	0.0795		0.6526
40d	T − 21	0.016	0.015	0.014	0.013	0.014	0.015	0.0144	0.00593	0.9188	0.3046	0.2614
	T − 42	0.013	0.012	0.020	0.014	0.017	0.018	0.0157	0.00672	0.2614		0.6218

L, linear; Q, Quadratic.

[1]T-21 d: birds supplemented with treatments in the period of the 4 to 21 days of age; T-42 d: birds supplemented with treatments in the period of the 4 to 42 days of age.

[2]Test F ($P \leq 0.05$) for comparing the averages of vitamin A supplementation times.

(Table 6). Wooden breast was influenced by the length of time of vitamin A supplementation (F test), and the highest scores were found in broiler chickens supplemented until 42 d of age (T-42; Table 6).

The incidence of WS in the pectoralis major muscle of birds at 42 d of age was influenced by vitamin A levels (Table 7). The occurrence of normal, moderate, and severe scores showed quadratic responses in the birds that were provided with vitamin A until 21 d of age. Minimal responses were obtained with supplementations of 29,301; 29,959 and 29,827 IU for normal, moderate and severe WS scores, respectively.

Table 6. Average scores for Wooden Breast (WB) and White Striping (WS) in the breast meat of broiler chickens fed with diets containing different vitamin A supplementation.

		Vitamin A (IU/Kg)										
	Supplementation[1]	0	6,000	16,000	26,000	36,000	46,000	Average[2]	SEM	*P* (Anova)	*P* (Test F)	*P* (Regression)
WS	T − 21	1.83	0.69	0.60	0.70	0.63	0.67	0.8520	0.0793	<0.0001	0.2154	<0.0001 (Q)
	T − 42	0.97	0.96	1.04	0.96	1.00	0.88	0.9684	0.0523	0.9725		0.6935
WB	T − 21	1.11	1.05	0.85	1.16	0.94	1.09	1.0341	0.0577	0.5996	0.0053	0.5432
	T − 42	1.15	1.39	1.40	1.20	1.29	1.09	1.2537	0.0623	0.3751		0.4096

L, linear; Q, Quadratic.

WS (T-21 d): 0.000000001164109 vit A^2 - 0.00006915 vit A + 1.50037; R^2: 0.43; Vit. A for minimum response: 29,700; Minimum response: 0.4735.

[1]T-21 d: birds supplemented with treatments in the period of the 4 to 21 days of age. T-42 d: birds supplemented with treatments in the period of the 4 to 42 days of age.

[2]Test F ($P \leq 0.05$) for comparing the averages of vitamin A supplementation times.

Table 7. Occurrence of White Striping (WS) (%) in the breast meat of broiler chickens fed with diets containing different vitamin A supplementation.

		Vitamin A (IU/Kg)										
	Supplementation[1]	0	6,000	16,000	26,000	36,000	46,000	Average[2]	SEM	*P* (Anova)	*P* (Test F)	*P* (Regression)
Normal	T − 21	11.25	53.36	56.25	46.02	55.97	50.48	45.56	3.377	0.0017	0.0045	0.0026 (Q)
	T − 42	35.71	35.71	37.09	34.96	33.31	34.48	35.21	2.396	0.9989		0.9368
Light	T − 21	21.25	32.32	30.36	40.45	30.56	35.92	31.64	2.081	0.5411	0.0347	0.5411
	T − 42	39.29	39.39	28.78	42.38	42.38	46.67	39.48	2.133	0.2396		0.2396
Moderate	T − 21	41.25	8.17	10.71	10.85	8.04	10.03	14.84	2.396	0.0002	0.7899	0.0011(Q)
	T − 42	16.96	20.53	26.99	14.50	15.32	17.10	18.57	1.576	0.2100		0.2072
Severe	T − 21	26.25	7.14	2.68	2.68	5.43	3.57	7.96	1.591	0.0001	0.4681	<0.0001(Q)
	T − 42	8.04	5.36	7.14	8.17	8.99	2.75	6.74	1.214	0.0181		0.7152

L, linear; Q, Quadratic.

WS normal (T-21d): - 0.0000000402676 vit A^2 + 0.00236 vit A + 23.,81484; R^2: 0.27; Vit. A for maximum response: 29,301 IU/ kg; Maximum response: 58.39. WS moderate (T-21 d): 0.00000000295402 vit A^2 - 0.00177 vit A + 31.71040; R^2: 0.32; Vit. A for minimum response: 29,959 IU/kg; Minimum response: 5.20. WS severe (T-21d): 0.000000002330068 vit A^2 - 0.00139 vit A + 21.07060; R^2: 0.44; Vit. A for minimum response: 29,827 IU/kg; Minimum response: 0.3405.

[1]T-21 d: birds supplemented with treatments in the period of the 4 to 21 days of age; T-42 d: birds supplemented with treatments in the period of the 4 to 42 days of age.

[2]Test F ($P \leq 0.05$) for comparing the averages of vitamin A supplementation times.

The period during which the birds were supplemented influenced the occurrence of WS myopathy with normal and mild scores (Table 7), with the highest occurrence of this normal-score myopathy in the birds whose diets were vitamin A-free, in the period from 22 to 42 d of age (T-21). In contrast, a higher incidence of mild myopathy was found in the supplemented birds throughout the experimental period (T-42; Table 7).

During the evaluation of WB occurrence in the broiler chickens at 42 d of age, differences in the occurrence of normal and severe scores were found by the F test ($P < 0.05$) between the different lengths of time of vitamin A supplementation (Table 8). Such

Table 8. Occurrence of Wooden Breast (%) in the breast meat of broiler chickens fed with diets containing different vitamin A supplementation.

		Vitamin A (IU/Kg)										
	Supplementation[1]	0	6,000	16,000	26,000	36,000	46,000	Average[2]	SEM	*P* (Anova)	*P* (Test F)	*P* (Regression)
Normal	T − 21	38.75	44.16	50.89	40.45	47.05	41.55	43.81	2.743	0.4500	0.0439	0.8270
	T − 42	42.86	30.36	37.91	35.92	34.20	44.23	37.58	1.972	0.4096		0.3191
Light	T − 21	26.25	20.74	23.21	20.74	21.57	21.63	22.36	1.674	0.9893	0.9859	0.9408
	T − 42	16.96	22.32	10.78	24.45	24.31	20.81	19.94	2.001	0.8637		0.3374
Moderate	T − 21	20.00	20.81	16.07	20.88	21.57	23.35	20.45	1.759	0.7814	0.9073	0.9145
	T − 42	22.32	25.00	24.31	23.56	19.85	17.03	22.01	1.818	0.7889		0.8196
Severe	T − 21	15.00	14.29	9.82	17.93	9.82	13.46	13.39	1.531	0.3728	0.0421	0.6420
	T − 42	17.86	22.32	26.99	16.07	21.63	17.93	20.47	2.23	0.7819		0.7687

L, linear; Q, Quadratic.

[1]T-21 d: birds supplemented with treatments in the period of the 4 to 21 days of age; T-42 d: birds supplemented with treatments in the period of the 4 to 42 days of age.

[2]Test F ($P \leq 0.05$) for comparing the averages of vitamin A supplementation times.

supplementation, until 21 d of age, followed by a diet without any supplementation, increased the occurrence of normal scores of WB, while supplementation with vitamin A until 42 d of age increased the incidence of WB in 42-day-old birds.

DISCUSSION

The influence of vitamin A on cuts yield may be related to its functions in the birds' metabolism. Just a few studies specifically addressing the influence of vitamin A on the deposition of protein (meat) in birds were found. However, vitamin A plays a role in cell development, regulation, proliferation, and differentiation (Blomhoff et al., 1990), factors that directly interfere with the results presented, namely relative weight of wings, breast, and legs with a dorsal portion.

According to Chen and Chen (2014), a few studies have been carried out evaluating the effects of hypervitaminosis A on protein metabolism of animals. The authors state that the induction of toxicity for this purpose should be 400 times the animals' nutritional requirement, which was not the case in this study. In addition to the possible influence of vitamin A on protein metabolism, it may have caused changes in the metabolism of carbohydrates and lipids in birds.

Sundeen et al. (1980), when evaluating diets that are poor in vitamin A, observed that birds with mild hypovitaminosis A had increased glycogen deposition in their pectoralis major muscle, and that, in cases of severe vitamin deficiency, these stocks were reduced. In cases when, after a period of deficiency, birds received diets adequately supplemented with vitamin A, the muscular glycogen content was equal to that of birds adequately supplemented.

The influence of vitamin A on these processes can be related to the differences found in the carcass yield parameters evaluated in this study. Yet, more specific analyses are necessary to understand and really confirm these assumptions.

Normally, after slaughter, the pH of breast muscle is higher than 7, but it reduces to 5.8 to 5.9 after 6 h postmortem (Petracci et al., 2015). Moreover, according to the authors, high amounts of muscle glycogen can reduce the meat pH close to the isoelectric point of myofibrillar proteins, which are responsible for fluid retention in the meat. Nonetheless, although pH was influenced by vitamin A, no other quality variable was affected.

Vitamin A had influence on pectoralis major color, for the blue / yellow intensity (b*), at 15 min and 24 h postmortem. The results indicate that the higher the vitamin A supplementation in the diet, the lower the blue / yellow intensity (b*) in the meat of 42-day-old broilers. Lower blue / yellow intensities (b*) are associated with low stress, since higher stress conditions tend to increase the high rate of postmortem glycolysis, causing a rapid decline in pH due to myoglobin denaturation (Oba et al., 2007). Then, it is possible to infer that Vitamin A supplementation was efficient to reduce oxidative stress at breast meat in broilers, and influenced on carbohydrate metabolism at *Pectoralis major* muscle.

Apparently, dietary restrictions of vitamin A tend to increase the deposition of vitamin E, which results in greater stability, due to a reduction in lipid oxidation and, consequently, increasing meat quality and shelf-life (Ayuso et al., 2015). However, in the evaluations of quality and measurement of substances reactive to thiobarbituric acid, no differences were found that could confirm this statement.

In the evaluation of the incidence of WB and WS myopathies, higher values were found in birds that received vitamin A supplementation during the period of 1 to 42 d of life, which may indicate that higher values of vitamin A stored by the birds interfere with growth, oxidative, and metabolic rates of their pectoralis major muscle.

According to Petracci et al. (2019), the process of muscle tissue degeneration that occurs during the incidence of breast myopathy has a direct influence on the sensory traits of meat, such as color and texture. The occurrence of WB results in greater lightness (L*) and increased yellow intensity (b*) in the affected muscle (Kuttappan et al., 2017). These characteristics can be aggravated to a greater degree of severity (Petracci et al., 2019). Apparently, birds with myopathies showed characteristic gene expression, furthermore, some factors as muscular hypoxia, oxidative stress, higher intracellular calcium levels and diseases related to glycose metabolism at muscle results in a higher number of cases (Abasht et al., 2016).

According to Mudalal et al. (2015), broilers with higher feed efficiency, rapid growth, and high protein deposition may present higher probability to development of myopathies at breast meat. Nevertheless, the results found for supplementation and incidence of myopathies differs from these statements. Greater segregation of the assessments should be performed, in order to measure the real influence of vitamin A on these characteristics linked to the incidence of myopathy.

Vitamin A influenced the occurrence of WS in birds at the age of 42 d. The supplementation in the period of 42 d (T-42) of life reduced the occurrence of normal scores and increased the occurrence of mild scores. It may be an indicative that an excess or prolonged supplementation with vitamin A interferes with cell growth and differentiation, perhaps due to some imbalance in relation to other nutrients important for adequate muscle growth, immune response, and maintenance of homeostasis.

According to Ferreira et al. (2014), after histological examination, the regions affected by the WS of breast fillets face an increase in fat cells and connective tissue, followed by degeneration of muscle fibers. Some authors suggest that fat and connective tissue infiltrate injured muscle fibers. In addition, there are no evidences that the onset of this myopathy is related to an infectious or inflammatory condition, but only an indicative that muscle damage is caused by regenerative myopathy (Ferreira et al., 2014).

Wooden breast is associated with microscopic lesions, with the presence of degeneration, necrosis, areas of regeneration, lipidosis and apparent lesions of pallor and stiffness, and some cases with petechiae and minor hemorrhages. Likewise, white striping does not represent a health risk. However, it does change the appearance and color of meat, in addition to affecting the quality of chilled or marinated meat, which becomes harder, with reduced brine absorption, and greater cooking loss. Apparently, the main factor that interferes with the quality of this meat is the reduction in its ability to retain water (Cruz et al., 2018).

Vitamin A interferes with the regulation of the metabolism of lipids, carbohydrates and proteins. Its effects are attributed mainly by the action of retinoic acid on the genetic expression of numerous metabolic pathways in the body (Chen and Chen, 2014). By evaluating this prerogative and the results found in this study, both the lack and excess of vitamin A influence the incidence and greater severity of the type myopathy evaluated.

CONCLUSIONS

The main conclusion drawn by this experimental research was that, not only vitamin A levels, but also the period during which supplementation takes place, can influence the quality of the meat of broilers slaughtered at 42 d of age. The length of time of vitamin A supplementation influenced the relative weight of wings, legs, and breasts, in yellow/blue intensity (b *), while vitamin A levels and the supplementation period influenced the occurrence of WS myopathy. These findings show the importance of evaluating vitamin A supplementation within quality parameters of chicken meat.

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DISCLOSURES

The authors whose names are listed immediately below certify that they have NO affiliations with or involvement in any organization or entity with any financial interest, or non-financial interest in the subject matter or materials discussed in this manuscript.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2021.101490.

REFERENCES

Abasht, B., M. F. Mutryn, R. D. Michalek, and W. R. Lee. 2016. Oxidative stress and metabolic perturbations in *Wooden breast* disorders in chickens. PLoS One. 11:e0153750.

Abd El-Hack, M. E., K. Mahrose, A. A. Askar, M. A. Alagawany, M. Saeed, F. Abbasi, R. N. Soomro, F. A. Siyal, and M. T. Chaudhry. 2017. Single and combined impacts of vitamin A and Selenium in diet on productive performance, egg quality, and some blood parameters of laying hens during hot season. Biol. Trace Elem. Res. 177:169–179.

Associação Brasileira de Proteína Animal – ABPA. Annual Report. 2018, 176. Accessed Oct. 2021. https://abpa-br.org/wp-content/uploads/2018/10/relatorio-anual-2018.pdf.

Ayuso, M., C. Óvilo, A. Fernández, Y. Nuñez, B. Isabel, A. Daza, C. J. López-Bote, and A. I. Rey. 2015. Effects of dietary vitamin A supplementation or restriction and its timing on retinol and α-tocopherol accumulation and gene expression in heavy pigs. Anim. Feed Sci. Technol. 202:62–74.

Blomhoff, R., M. H. Green, T. Berg, and K. R. Norum. 1990. Transport and storage of vitamin A. Science. 250:399–404.

Chen, W., and G. Chen. 2014. The roles of vitamin A in the regulation of carbohydrate, lipid, and protein metabolism. J. Clin. Med. 3:453–479.

Cruz, C. E. B., E. R. Freitas, N. de M. Braz, R. P. R. Salles, and I. N. G. da Silva. 2018. Blood parameters and enzymatic and oxidative activity in the liver of chickens fed with calcium anacardate. Rev. Cienc. Agron. 49:343–352.

Ferreira, T. Z., R. A. Casagrande, S. L. Vieira, D. Driemeier, and L. Kindlein. 2014. An investigation of a reported case of white striping in broilers. Appl. Poult. Res. 23:16.

Gerster, H. 1996. Vitamin A – functions, dietary requirements and safety in humans. Int. J. Vit. Nutr. Res. 67:71–90.

Honikel, K. O. 1998. Reference methods for the assessment of physical characteristics of meat. Meat Sci. 49:447–457.

Kuttappan, V. A., B. M. Hargis, and C. M. Owens. 2016. White striping and woody breast myopathies in the modern poultry industry: a review. Poult. Sci. 95:2724–2733.

Kuttappan, V. A., C. M. Owens, C. Coon, B. M. Hargis, and M. Vazquez-A Non. 2017. Research note: incidence of broiler breast myopathies at 2 different ages and its impact on selected raw meat quality parameters. Poult. Sci. 96:3005–3009.

Maschio, M. M., and S. M. Raszl. 2012. Economic impact from convictions post-mortem total and partials in a poultry slaughterhouse. Revista E-Tech: Tecnologias Para Competitividade Industrial. 2012:26–38.

Mazzoni, M., M. Petracci, A. Meluzzi, C. Cavani, P. Clavenzani, and F. Sirri. 2015. Relationship between pectoralis major muscle histology and quality traits of chicken meat. Poult. Sci. 94:123–130.

Mudalal, S., M. Lorenzi, F. Soglia, C. Cavani, and M. Petracci. 2015. Implications of White striping and wooden breast abnormalities on quality traits raw and marinated chicken meat. Animal. 9:728–734.

Nakamura, M., and K. Katok. 1985. Influence of thawing method on several properties of rabbit meat. Bull. Ishikawa Prefec. Coll. Agric. 11:45–49.

Oba, A., P. A. de Souza, H. B. A. de Souza, F. R. Leonel, E. R. L. Pelicano, N. M. B. Zeoula, and I. C. Bolelli. 2007. Qualidade da carne de frangos de corte submetidos a dietas suplementadas com crômio, criados em diferentes temperaturas ambientais. Acta Sci. Anim. Sci. 29:143–149.

Petracci, M., S. Mudalal, F. Soglia, and C. Cavani. 2015. Meat quality in fast-growing broiler chickens. Poult. Sci. J. 71:363–374.

Petracci, M., F. Soglia, M. Madruga, L. Carvalho, E. Ida, and M. Estévez. 2019. Wooden-breast, white striping, and spaghetti meat: causes, consequences and consumer perception of emerging broiler meat abnormalities. Compr. Rev. Food Sci. Food Saf. 18:565–583.

Rostagno, S. H., L. F. T. Albino, M. I. Hannas, J. L. Donzele, N. K. Sakomura, F. G. Perazzo, A. Saraiva, M. L. T. Abreu, P. B. Rodrigues, R. F. Oliveira, S. L. T. Barreto, and C. O. Brito. 2017. Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais. Viçosa: UFV 4:488.

Sorensen, G., and S. S. Jorgensen. 1995. A critical examination of some experimental variables in the 2-thiobarbituric acid (TBA) test for lipid oxidation in meat products. Zeitschrift Lebensmittel Untersuchung Forschung A. 202:205–210.

Sosnicki, A. A. 1993. Focal myonecrosis effects in turkey muscle tissue. Recipr. Meat Conf. Proc. 46:97–102.

Sundeen, G., J. F. Richards, and D. B. Bragg. 1980. The effect of vitamin A deficiency on some postmortem parameters of avian muscle. Poult. Sci. 59:2225–2236.

Vyncke, W. 1975. Evaluation of the direct thiobarbituric acid extraction method for determining oxidative rancidity in Mackerel. Fette Seifen Anstrichmittel. 77:239–240.