Interactions between lipid source and vitamin A on broiler performance, blood parameters, fat and protein deposition rate, and bone development

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ABSTRACT A total of 2622 male broilers were distributed in a 2×5 factorial design, using 2 lipid sources (soybean oil and palm fat), 5 levels of vitamin A supplementation (0, 3,000, 6,000, 12,000, and 24,000 IU kg^{-1}), with 10 replicates, and also 1 control diet (**CD**) for each lipid source used (7 replicates), each experimental unit being composed of 23 birds. During the first 21 d (how were the birds fed) and from 22 to 42 d of age, a redistribution of the treatments was carried out in a $2 \times 2 \times 5$ factorial design: half of the repetitions of each treatment received the diet of the initial treatment, and the others received the CD with its type of lipid source. In the phase from 1 to 21 d of age, the effect of lipid source on feed intake (**FI**) and feed conversion ratio (**FCR**), and the effect of vitamin supplementation on FI and weight gain (**WG**) were observed, with a quadratic response for both variables. At 42 d of age, the lipid source and vitamin A level influenced the FI, whereas the WG and FCR showed interactions between period and the level of vitamin A supplementation. Neither lipid source resulted in blood parameters out of the typical pattern for birds, and the same was observed in relation to dietary vitamin A supplementation. From 1 to 21 d of age, a vitamin A supplementation of 15,585 IU kg^{-1} was estimated, and at 42 d, 15,527 IU kg^{-1} and 15,148 IU kg^{-1} were estimated for the periods 1 to 21 d and 1 to 42 d, respectively.

Key words: lipid, palm, soy, vitamin A

INTRODUCTION

The use of oils and fats in the formulation of broiler diets is advantageous, especially in high energy diets. However, it is essential to understand and study their possible interactions with other feed nutrients (Abawi et al., 1985). Comparing the digestion of fat with that of carbohydrate and protein is a complex field, because fats need emulsification and micelle formation, besides the different variations and interactions involved between the nutrients (Ravindran et al., 2016).

Degummed soybean oil (**SO**) is a vegetable oil widely used in feed formulation. However, referring to the food industry, SO and palm fat (**PF**) are the main vegetable lipids used (Abdulla et al., 2016). Regardless of the source, oils and fats are important energy sources; both have the same general structure but have different chemical properties (McDonald et al., 2010). Lipids may be saturated with only single bonds between the carbons (weak chemical reactivity) or may be unsaturated with one or more double bonds in their carbon structure (more reactive and susceptible to oxidation; Dossier Oleos, 2014).

The chemical characteristics of a lipid interfere with the amount of metabolizable energy, its absorption, and use by the animal organism. According to Sanz et al. (2010), diets containing saturated fat are less metabolically useful compared with diets containing unsaturated fats. Tancharoenrat et al. (2013), evaluating the digestibility of 5 different lipid sources in broiler diets, did not observe differences in the digestibility of SO, PF, poultry fat and a mixture of beef tallow, poultry oil, and soybean.

Fats may influence the absorption of other nutrients such as fat-soluble vitamins. Dietary vitamin A is mostly

in the retinyl ester form, which is hydrolyzed in the intestinal lumen; however, retinyl esters are hydrophobic and depend on micellar solubilization to be absorbed, this being the reason why vitamin A can be poorly utilized in low-fat diets (Combs, 2008).

Although vitamins have known functions and influences on performance and metabolism, few studies have been conducted to evaluate what would be the new vitamin requirements for broilers, as well as the effect of dietary excess on broiler development. Unlike water-soluble vitamins, dietary excesses of fat-soluble vitamins are stored in the body, making these vitamins potentially toxic, and their toxicity may be associated with high levels of fat and water-soluble vitamins (Abawi and Sullivan, 1989).

Vitamin A is well known for its influence on visual processes, modulation of the immune system (Ramalho, 2010), antioxidant action (Abd El-Hack et al., 2017), intestinal integrity (Guerra, 2016), cell differentiation (Gerster, 1996), and bone development (Wang et al., 2002; Ramalho, 2010; Souza et al., 2011). So, it becomes important to evaluate the supplementation of a fat-soluble vitamin and its interaction with the type of dietary fat, demonstrating the importance of its real requirements and effects on broiler development. Thus, the objective of this study was to evaluate the influence of 2 lipid sources and different levels of vitamin A on performance, blood parameters, fat and protein deposition, and bone growth of broilers at 21 and 42 d of age, considering different periods of diet supplementation.

MATERIALS AND METHODS

Birds and Study Design

The University Animal Ethics Committee approved the test under number 01/19. The slaughter of the birds was performed according to the guidelines described by CONCEA (National Council for Control of Animal Experimentation) in Normative Resolution No. 37 of February 2018.

One-day-old, Cobb 500 broiler chicks were housed in an experimental masonry shed divided into pens (1.96 m^2) with a concrete floor lined with pine shavings litter. Each pen was equipped with tubular feeders, nipple drinkers, electrical elements for heating, hoods, evaporative plates to assist cooling, and cooling air changes.

A total of two thousand six hundred sixty-two 1-day male broiler chicks, with an average initial weight of 43.35 ± 0.59 g, were distributed into a 2×5 factorial design, consisting of 10 treatments: 2 sources of lipids (SO and PF), and 5 levels of vitamin A supplementation (0; 3,000; 6,000; 12,000; 24,000 IU kg^{-1}), with 10 replicates, plus a control diet (**CD**) for each fat source used (7 replications), with 23 birds per experimental unit (**EU**). These birds were raised under these treatments until completed 21 d.

For evaluation at 42 d, with 22 d of age, the birds were redistribute into a $2 \times 2 \times 5$ factorial design,

using 2 lipid sources (SO and PF), 2 supplementation periods and 5 vitamin A levels (0; 3,000; 6,000; 12,000; 24,000 IU kg^{-1}), with 5 repetitions, keeping the CD for each fat source used at the period 1 to 21 d old. From 22 to 42 d old, 2 vitamin A supplementation periods were considered. First period (1–21 d) was formed by birds that received the initial treatments until 21 d old and after this period (22–42 d old) were fed with the respective CD of its lipidic supplementation. Second period (1–42 d) was formed by birds that received the same vitamin supplementation during the whole period.

The CD was supplemented with 8,000 and 6,700 IU kg^{-1} of vitamin A from 1 to 21 and 22 to 42 d of age, respectively. The nutritional levels were chosen based on higher and lower level than those proposed by the Brazilian tables (Rostagno et al., 2011), so that we could have a regression and/or symptom of hypovitaminosis and hypervitaminosis.

Dietary Treatments

From 1 to 3 d of age, all birds received diets with no vitamin A supplementation, except for the birds in the CD group. Thus, the birds were submitted to treatments from 4 to 21 d of age. From 22 to 42 d of age, treatments were redistributed in each treatment: 5 replicates still receiving the initial treatment diet and the other 5 replicates received the CD for their lipid source. For redistribution of the treatments, the characteristics of the EU were evaluated to guarantee homogeneity between the treatment replications. Experimental units in the CD treatment continued to receive the same treatment; no redistribution or change of diet was carried out for these birds.

The experimental diets for each phase were formulated based on corn and soybean meal, according to the requirements proposed by Rostagno et al. (2011), except for vitamin A requirements. Vitamin A was added on top, and in the preparation of the experimental diets, 2 vitamin premixes were used: 1 mineral vitamin premix (Vitmin Px) containing vitamins D, E, and K, B vitamins and selenium, and a second vitamin premix (VitA Px) containing only vitamin A (retinyl acetate) (Table 1).

For diet formulation, the apparent metabolizable energy values for SO and PF were 9,315 kcal kg^{-1} and 7,510 kcal kg^{-1}, respectively. Vitamin A supplementation was performed with the inclusion of VitA Px replacing the inert material, thus not changing the nutritional composition of the diets (Table 2).

Broiler Performance

To determine weight gain (**WG**), feed intake (**FI**), and feed conversion ratio (**FCR**), all birds of each EU were weighed, together with the leftover feed, at 21 and 42 d of age. Feed intake and FCR were determined and corrected using the weight of dead birds, according to Sakomura and Rostagno (2016).

Table 1. Nutritional composition (kg premix) of mineral vitamin premix (Px Vitmin) and vitamin A premix with vitamin A (Px Vit A) used in the composition of experimental diets.

Nutrient	Px Vitmin[1]	Px Vit. A[2]
Vitamin A (KUI kg^{-1})	-	33,333
Vitamin D$_3$ (KUI kg^{-1})	2,100	-
Vitamin E (UI kg^{-1})	35,000	-
Vitamin K$_3$ (mg kg^{-1})	1,880	-
Vitamin B$_1$ (mg kg^{-1})	2,500	-
Vitamin B$_2$ (mg kg^{-1})	6,250	-
Niacin (mg kg^{-1})	37,500	-
Acid. Pantothenic (mg kg^{-1})	12,500	-
Vitamin B$_6$ (mg kg^{-1})	3,500	-
Vitamin B$_{12}$ (mg kg^{-1})	15.00	-
Folic acid (mg kg^{-1})	875	-
Biotin (mg kg^{-1})	88	-
Selenium (mg kg^{-1})	330	-

[1]Dosage used: 1 kg ton^{-1} of feed in the phase of 1 to 21 d of age; and 0.720 kg ton^{-1} of feed at the age of 22 to 42 d.

[2]Replacement with inert according to vitamin A supplementation in the feed, where: 0 IU kg^{-1} - 0 kg ton^{-1} of feed; 3,000 IU kg^{-1} - 0.090 kg ton^{-1} of feed; 6,000 IU kg^{-1} - 0.180 kg ton^{-1} of feed; 12,000 IU kg^{-1} - 0.360 kg ton^{-1} of feed; 24,000 IU kg^{-1} - 0.720 kg ton^{-1} of feed.

Blood Analysis

Blood was collected by puncture of the ulnar vein at 41 d of age, from 3 birds per EU after 6 h fasting. Samples were identified and centrifuged (Centrifuge Kasvi K14–4000, Kasvi, São Paulo, BR) for 10 min at 2,500 rpm (1,050 g). The obtained serum was stored at $-20°C$ for later analysis (Nunes et al., 2018). Blood serum analyses were performed for total cholesterol (**COL**), triglycerides (**TAG**), aspartate aminotransferase (**AST**), alanine aminotransferase (**ALT**), cholesterol (high-density lipoprotein: **HDL** and low-density lipoprotein: **LDL**) using commercial kits (Elitech Clinical Systems, ELITech Group, Paris, France) in an automatic calibration spectrophotometer with high-performance reading (Flexor EL200 Biochemical Analyzer).

Fat and Protein Deposition Rate

For evaluation of fat deposition rate (**FDR**) and protein deposition rate (**PDR**) on the first day of the experiment (in the housing), a total of twenty 1-day-old birds were chosen randomly and sacrificed. These birds were used to calculate FDR and PDR, which were measured by comparing the birds slaughtered at the beginning the experiment with those sacrificed at 21 d (n = 114) and at 42 d (n = 42). At 21 and 42 d, 1 bird per EU for each age was selected, duly identified by treatment, and frozen at $-20°C$ for analysis of dry matter, ether extract, and crude protein, according to AOAC (1996). To determine the FDR and PDR (g day^{-1}), the adapted methodology of Fraga et al. (2008) was performed.

Bone Analysis

To evaluate the incidence of tibial dyschondroplasia and the interference of vitamin A supplementation

with bone growth, the tibia was removed from the left leg of 1 bird per EU (n = 114) at 42 d of age and decalcified with 50% formic acid and 20% sodium citrate (Fernandes et al., 2007). After decalcification, the bone was embedded in paraffin (Beçak and Paulete, 1976). The 5-μm sections were made by microtome and stained with hematoxylin-eosin to observe and measure the epiphyseal disk area to characterize the incidence of tibial dyschondroplasia. For analysis of the tibial epiphyseal cartilage slides, 2 distinct regions characterized by morphological appearance were considered: the proliferative cartilage zone (growth plate [**GP**]) and the hypertrophic cartilage zone. The images were measured with the aid of a computer image analyzer (PROPLUS IMAGE 4.1).

Statistical Analysis

For statistical evaluation, the data were subjected to normality analysis using the Shapiro-Wilk test. Afterward, the analysis of variance was performed, considering the isolated effects and the interaction between the studied factors: fat source and vitamin A levels at 21 d of age and at 42 d of age, fat source and vitamin A levels at different diet supplementation periods (1–21 d or 1–42 d). For the assessment of isolated effects, the F test at 5% probability was used to compare supplementation periods and lipid sources, and regression analysis for comparisons between vitamin A levels by the PROC GLM procedure and the generated equations were obtained through polynomial orthogonal contrasts and the coefficients generated by the PROC IML. The means were compared with CD treatment using the Dunnett's test at 5% probability. The statistical software used for the evaluations was SAS University Edition, student version.

RESULTS

Broiler Performance

At 21 d of age, there was no interaction ($P > 0.05$) between lipid source and vitamin A supplementation (Table 3). Soybean oil and PF influenced FI ($P < 0.001$) and FCR ($P < 0.001$). However, WG was not affected ($P = 0.988$). Vitamin A supplementation provided a quadratic response for WG and FI; however, it did not affect the bird's FCR ($P = 0.105$; Table 3). The equation obtained for FI estimated that the value of 12,721 IU kg^{-1} of vitamin A determined the best FI at 1,210 g; for WG, it was seen that supplementation of 15,585 IU kg^{-1} of vitamin A resulted in a WG of 891 g.

Feed intake and WG values were significantly different ($P < 0.001$) compared with the CD, according to Dunnett's test. Broilers that received 0 or 3,000 IU kg^{-1} of vitamin A exhibited a reduction in FI and showed the lowest WG compared with the CD

Table 2. Composition and nutritional characteristics of basal diets with soybean oil (SO) and palm fat (PF) (as fed) for broilers.

Item (g kg^{-1})	1–21 d		22–42 d	
	SO	PF	SO	PF
Maize 8%	56.400	54.500	59.900	57.800
Soybean meal 45%	36.100	36.500	32.170	32.500
Palm fat	-	4.810	-	5.820
Soybean oil	3.290	-	4.035	-
Dicalcium phosphate	1.551	1.557	1.340	1.340
Limestone 35%	1.065	1.050	1.000	0.994
Salt	0.497	0.496	0.427	0.429
Lysine sulfate 65%	0.352	0.343	0.370	0.360
DL-methionine 99%	0.311	0.312	0.296	0.298
Px Vitmin[1]	0.100	0.100	0.072	0.072
L-threonin 98,5%	0.077	0.077	0.072	0.072
Sodium bicarbonate	-	-	0.068	0.066
Inert	0.072	0.072	0.072	0.072
Choline chloride 60%	0.061	0.061	0.056	0.056
Salinomycin 12%	0.055	0.055	0.055	0.055
Premix mineral[2]	0.050	0.050	0.050	0.050
Avilamycin 10%	0.010	0.010	0.010	0.010
Antioxidant	0.010	0.010	0.010	0.010
Nutritional composition (g kg^{-1})				
Met. Energy (kcal kg^{-1})	3,050	3,050	3,150	3,150
Crude protein	213.50	213.50	198.00	198.00
Ethereal extract	61.30	75.80	69.30	87.00
Calcium	8.40	8.40	7.60	7.60
Total phosphorus	6.20	6.20	5.70	5.70
Available phosphorus	4.00	4.00	3.50	3.50
Sodium	2.10	2.10	2.00	2.00
Dig Lysine	12.20	12.20	11.30	11.30
Dig Methionine + Cystine	8.80	8.80	8.30	8.30
Dig Tryptophan	2.40	2.40	2.20	2.20
Dig Threonine	7.90	7.90	7.30	7.30

[1]Premix Mineral Vitamin for Poultry—Guarantee Levels per Kilogram of Feed - 1 to 21 d old: Vit. D3 (min) 2,100 IU; Vit. E (min) 35 IU; Vit. K3 (min) 1.88 mg; Vit. B1 (min) 2.50 mg; Vit. B2 (min) 6.25 mg; Vit. B6 (min) 3.50 mg; Vit. B12 (min) 15.0 mcg; Pantothenic Acid (min) 12.50 mg; Niacin (min) 37.50 mg; Folic Acid (min) 0.87 mg; Biotin (min) 88 mcg; Selenium (min) 0.33 mg. 22 to 42 d old: Vit. D3 (min) 1.512 IU; Vit. E (min) 25.2 IU; Vit. K3 (min) 1.35 mg; Vit. B1 (min) 1.80 mg; Vit. B2 (min) 4.50 mg; Vit. B6 (min) 2.52 mg; Vit. B12 (min) 10.8 mcg; Pantothenic Acid (min) 9 mg; Niacin (min) 27.0 mg; Folic Acid (min) 0.63 mg; Biotin (min) 63.3 mcg; Selenium (min) 0.24 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.

treatment. Broiler performance supplemented with 6,000 IU kg^{-1} of vitamin A was similar to those fed with the CD.

At 42 d of age, there were no effects ($P > 0.05$) between the period, lipid source, and vitamin A supplementation on broiler performance (FI, WG, and FCR; Table 4)

A significant difference ($P < 0.05$) was observed in broiler FI with lipid source and vitamin A supplementation level. The FI was higher ($P = 0.0367$) in broilers fed diets with PF compared with those fed with SO. Broiler FI showed a quadratic response ($P < 0.0001$) with the level of vitamin A supplementation, with the maximum response being calculated at 16,082 IU kg^{-1}.

Weight gain and FCR showed interactions between vitamin A supplementation and period ($P < 0.0001$). Feed intake, WG, and FCR were significantly different by Dunnett's test ($P < 0.05$) when broilers were fed no vitamin A compared with the CD treatment. The WG of broilers at 42 d of age differed between periods when birds received no vitamin A, with the best WG when

supplementation was administered until 21 d of age (Table 5). The WG of birds fed diets containing vitamin A supplementation from 1 to 21 d and from 22 to 42 d showed a quadratic effect ($P < 0.05$), and the levels that provided the maximum responses were 15,527 IU kg^{-1} and 15,148 IU kg^{-1}, respectively.

Broilers receiving treatments with no vitamin A from 1 to 42 d of age showed the worst FCR compared with the birds that received supplementation from 1 to 21 d of age. Regardless of the period evaluated, broilers receiving no vitamin A showed the worst WG and FCR (<0.0001) compared with the CD treatment, according to Dunnett's test.

Blood Analysis

All blood parameters evaluated at 42 d of age were influenced by period, lipid, and vitamin A supplementation (Table 6).

Serum ALT levels showed an interaction with period, lipid source, and vitamin A level in the diet (Table 7).

Table 3. Performance of 21-day-old broilers fed diets containing 2 lipid sources with different vitamin A supplements.

Item	FI (g)	WG (g)	FCR (g g^{-1})
Palm	1.172[b]	829	1.417[b]
Soy	1.146[a]	828	1.386[a]
Vitamin A (UI kg^{-1})			
CD	1.202	870	1.383
0	1.010[1]	716[1]	1.413
3.000	1.164[1]	839[1]	1.389
6.000	1.187	845	1.407
12.000	1.202	860	1.400
24.000	1.199	851	1.411
Mean	1,159	827	1.401
CV	6.87	7.55	2.95
SEM	7.46	5.86	0.004
P-value			
Source x Vitamin A	0.892	0.199	0.088
Lipid Source	<0.001	0.988	<0.001
Vitamin A	<0.001	<0.001	0.105
Regression	<0.001(Q)	<0.001(Q)	-
Dunnett	<0.0001	<0.001	0.184

Abbreviations: FI, feed intake; WG, weight gain; FCR, feed conversion ratio; Q, quadratic; CD, control diet (8,000 UI vitamin A kg^{-1})

FI: $-0.000001vit.A^2 + 0.025442vit.A + 1048.3489$; R^2: 0.63; Vit A 1° Derivation: 12,721; Estimated Response: 1,210.

WG: $-0.0000006vit.A^2 + 0.0193637vit.A + 746.21053$; R^2: 0.54; Vit A 1° Derivation: 15,585; Estimated Response: 891.

[a,b]Different letters in the line differ statistically by the t test (*P* < 0.05).
[1]Differ from Control by Dunnett test (*P* < 0.05).

There was a difference between the sources of lipids used within the period of 1 to 21 d for the levels of 3,000 and 24,000 IU kg^{-1} of vitamin A, and for both lipid sources, the birds that consumed SO had the highest serum ALT values.

In the evaluation of the period from 1 to 42 d of age, there was a difference between the lipid sources for no vitamin A supplementation and the broilers fed with SO presenting higher serum ALT concentrations. According to regression analysis, an increasing linear response was observed for treatments containing PF. All birds receiving vitamin supplements from 1 to 21 d of age that received the SO diets differed by Dunnett's test from those fed the CD. However, in the evaluation of the supplementation period from 1 to 42 d of age, birds receiving vitamin A at supplementation levels of no or 24,000 IU kg^{-1} differed from those fed the CD regardless of lipid source.

Serum AST concentrations of broilers at 42 d of age were influenced by the lipid source and vitamin A supplementation within 1 to 21 d when supplemented with no or 24,000 IU kg^{-1} of vitamin A; the highest AST values were obtained from SO diets. Similarly, in the period from 1 to 42 d, the highest serum AST concentrations were observed in birds fed SO diets; however, this difference was only found for those supplemented with 12,000 IU kg^{-1} of vitamin A.

According to regression analysis, from 1 to 21 d of age, the serum AST of birds fed diets containing PF had a quadratic effect (*P* < 0.05), and the level that provided the maximum response was 15,706 IU kg^{-1} of vitamin A.

For diets containing SO and vitamin A supplementation in the period from 1 to 21 d of age, AST showed a linear response. When the supplementation period

Table 4. Performance of 42-day-old broilers fed diets containing 2 lipid sources with different vitamin A supplements.

Item	FI (g)	WG (g)	FCR (g g^{-1})
Supplementation			
Up to 21 d[1]	5.304	2.955[a]	1.797[b]
Up to 42 d[2]	5.294	2.832[b]	1.893[a]
Lipid Source			
Palm	5.330[a]	2.908	1.844
Soy	5.280[b]	2.911	1.828
Vitamin A (UI kg^{-1})			
CD	5.348	3.020	1.783
0	5.166[3]	2.517[3]	2.170[3]
3.000	5.315	2.962	1.797
6.000	5.362	3.303	1.786
12.000	5.306	3.000	1.770
24.000	5.346	2.987	1.791
Mean	5.305	2.909	1.849
CV (%)	2.49	8.02	9.12
SEM	12.398	21.854	0.016
P-value			
Period × Lipid × Vitamin	0.262	0.138	0.054
Period × Lipid	0.431	0.615	0.803
Period × Vitamin	0.573	<0.0001	<0.0001
Lipid × Vitamin	0.122	0.922	0.411
Period	0.636	<0.0001	<0.0001
Lipid source	0.037	0.467	0.154
Vitamin A	<0.0001	<0.0001	<0.0001
Dunnett	<0.0001	<0.0001	<0.0001
Regression	0.004(Q)	-	-

Abbreviations: FI, feed intake; WG, weight gain; FCR, feed conversion ratio; Q, quadratic; CD, control diet (8,000 IU of vitamin A kg^{-1} at 1 to 21 d of age, and 6,700 IU of vitamin A kg^{-1} at 22 to 42 d of age)

FI: $-6.14037 \times 10^{-7}vit.A^2 + 0.01975vit.A + 5,215.42325$; R^2: 0.15; Vit A 1° Derivation: 16,082; Estimated Response: 5,347.

[a,b]Different letters in the line differ statistically by the t test (*P* < 0.05).
[1]Up to 21 d: broilers supplemented with treatments from 4 to 21 d of age, and diet-free from vitamin A from 22 to 42 d of age.
[2]Up to 42 d: broilers supplemented with treatments from 4 to 42 d of age.
[3]Differ from Control by Dunnett test (*P* < 0.05).

considered was from 1 to 42 d of age, a quadratic response in AST levels was observed for birds fed SO, and the level that provided the maximum response was 13,096 IU kg^{-1}. On the other hand, for broilers fed with PF, AST showed a quadratic response in the period from 1 to 21 d of age but a linear response in the period from 1 to 42 d of age.

By Dunnett's test, in the supplementation period from 1 to 21 d of age, differences in AST levels were found in treatments containing no or 3,000 IU kg^{-1} vitamin A when the diet contained PF, compared with the CD. The diets containing SO differed from the CD when supplemented with 24,000 IU kg^{-1} of vitamin A.

For the period from 1 to 42 d of age when the base diet was PF, treatments with no or 12,000 IU kg^{-1} of vitamin A differed for the AST variable from the CD by Dunnett's test, whereas for SO diets over this period, a difference was observed between CD and the treatments supplemented with vitamin A at 3,000 and 12,000 IU kg^{-1}.

For the interaction between period, lipid source, and vitamin A level in the diet of birds at 42 d of age, a difference was found in the serum COL concentration between lipid sources in the period of 1 to 42 d of age when the diet contained 12,000 IU kg^{-1} of vitamin A, with higher serum concentrations obtained in birds fed SO diets.

Table 5. Deployment of the interaction between vitamin A supplementation period and vitamin A supplementation level for weight gain (WG) and feed conversion ratio (FCR) for 42-day-old birds supplemented up to 21 and up to 42 d.

Vit. A (UI kg^{-1})	WG (g)			FCR (g g^{-1})		
	1–21 d	1–42 d	P-value	1–21 d	1–42 d	P-value
CD	3.020	3.020	-	1.772	1.772	-
0	2.788[a,1]	2.246[b,1]	<0.0001	1.847[b,1]	2.315[a,1]	<0.0001
3.000	2.992	2.931	0.208	1.781	1.813	0.308
6.000	3.010	2.996	0.715	1.786	1.786	0.997
12.000	2.983	3.016	0.372	1.792	1.748	0.087
24.000	3.002	2.970	0.429	1.778	1.804	0.369
P Regression	0.0009(Q)	0.0001(Q)		0.127	0.128	
P Dunnett	<0.0001	<0.0001		0.005	<0.0001	

Q: quadratic, CD: Control diet (8,000 IU of vitamin A kg^{-1} at 1–21 d of age, and 6,700 IU of vitamin A kg^{-1} at 22–42 d of age).

WG (1–21 d): -0.000000840477vit.A^2 + 0.02610vit.A + $2,849.18746$; R^2: 0.28; Vit A 1° Derivation: 15.527; Estimated Response: 3.052.

WG (1–42 d): -0.00000341vit.A^2 + 0.10331vit.A + $2,423.99964$; R^2: 0.68; Vit A 1° Derivation: 15,148; Estimated Response: 3,206.

[a,b]Different letters in the line differ statistically by the t test ($P < 0.05$).
[1]Differ from Control by Dunnett test ($P < 0.05$).

As for cholesterol proportions, differences in serum HDL and LDL concentrations were obtained in birds in the period of 1 to 42 d of supplementation when they received vitamin supplementation at 12,000 IU kg^{-1}, with higher serum concentrations of these variables when the diet contained SO.

However, for diets with vitamin A supplementation, from 1 to 21 d of age, significant variations in serum LDL concentrations were found, when birds were fed with 3,000 or 6,000 IU kg^{-1} of vitamin A. For supplementation with 3,000 IU kg^{-1} of vitamin A, PF diets resulted in higher serum LDL concentrations, whereas supplementation with 6,000 IU kg^{-1} of vitamin A was SO.

The HDL levels after treatment with 24,000 IU kg^{-1} of vitamin A differed by Dunnett's test from the CD diet in the period of 1 to 42 d of age. Triglyceride levels were higher in birds fed PF diets from 1 to 42 d of age when vitamin A supplementation was at 24,000 IU kg^{-1}. In the same period, an increasing linear response in TAG was obtained for both dietary lipid sources.

Table 6. Blood parameters of broilers of 42-day-old broilers fed diets containing 2 lipid sources with different vitamin A supplements.

Item	ALT (UI l^{-1})	AST (UI l^{-1})	COL (mg dl^{-1})	HDL (mg dl^{-1})	LDL (mg dl^{-1})	TAG (mg dl^{-1})
Supplementation						
Up to 21 d[1]	15.11	308.03	123.76	78.50	20.80	40.17
Up to 42 d[2]	14.66	318.15	127.60	79.76	20.87	37.77
Lipid source						
Palm	12.39	300.84	127.45	81.09	20.65	39.37
Soy	16.37	329.61	124.23	77.85	20.90	38.55
Vitamin A (UI kg^{-1})						
CD	10.34	330.54	127.18	82.24	20.31	39.92
0	12.97	232.02[3]	134.63	83.02	20.71	36.27
3.000	12.97	330.72	122.74	9.02	22.43	34.17
6.000	16.07[3]	337.70	126.31	78.75	20.60	40.85
12.000	15.71	333.72	121.41	76.28	20.88	40.00
24.000	16.71[3]	331.27	123.32	78.59	19.56	43.55
Mean	14.364	315.09	125.85	79.49	20.777	38.96
CV (%)	44.46	24.76	14.17	10.56	17.18	24.73
EPM	0.601	7.339	1.677	0.790	0.336	0.907
P-value						
Period × Lipid × Vitamin	<0.0001	0.006	0.009	0.031	0.004	0.003
Period × Lipid	<0.0001	0.211	0.658	0.048	0.195	0.060
Period × Vitamin	0.036	<0.0001	0.349	0.697	0.012	0.002
Lipid × Vitamin	0.019	0.328	0.288	0.130	0.079	0.838
Period	0.642	0.339	0.265	0.425	0.911	0.165
Lipid source	<0.0001	0.0001	0.471	0.135	0.951	0.545
Vitamin A	0.035	<0.0001	0.114	0.115	0.091	0.007
Dunnett	0.030	<0.0001	0.207	0.137	0.229	0.051

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; COL, cholesterol; LDL, low-density lipoprotein; HD, high-density lipoprotein; TAG, triglycerides; CD, Control diet (8,000 IU of vitamin A kg^{-1} at 1 to 21 d of age, and 6,700 IU of vitamin A kg^{-1} at 22 to 42 d of age)

[1]Up to 21 d: broilers supplemented with treatments from 4 to 21 d of age, and diet free from vitamin A from 22 to 42 d of age.
[2]Up to 42 d: broilers supplemented with treatments from 4 to 42 d of age.
[3]Differ from Control by Dunnett test ($P < 0.05$).

Table 7. Deployment of the interaction between vitamin A supplementation period, the lipid source, and the vitamin A supplementation of the blood variables of 42-day-old broilers.

Item	Supplementation	Lipid source	Vitamina A (UI kg^{-1})						P regression	P Dunnett
			CD	0	3.000	6.000	12.000	24.000		
ALT	Up to 21 d[1]	Palm	11.71	12.24	5.02[b]	15.93	15.71	5.53[b]	0.057	0.053
		Soy	8.46	17.65[3]	19.22[a,3]	19.09[3]	14.48[3]	23.23[a,3]	0.223	<0.0001
		P-value	-	0.228	<0.0001	0.409	0.581	<0.0001		
	Up to 42 d[2]	Palm	11.7	5.1[b,3]	13.9	16.4	13.9	20.9[3]	<0.0001 (L)	<0.0001
		Soy	8.5	16.9[a,3]	13.7	12.8	15.7	17.1[3]	0.718	0.007
		P-value	-	0.023	0.909	0.295	0.588	0.106		
AST	Up to 21 d[1]	Palm	363	237[b,3]	272[3]	347	308	305[b]	0.006(Q)	0.002
		Soy	288	314[a]	253	329	334	381[a,3]	0.003(L)	0.003
		P-value	-	0.007	0.554	0.662	0.336	0.009		
	Up to 42 d[2]	Palm	363	183[3]	367	306	275[b,3]	322	0.026(L)	<0.0001
		Soy	288	195	431[3]	369	418[a,3]	317	<0.0002(Q)	<0.0001
		P-value	-	0.711	0.102	0.227	0.005	0.884		
COL	Up to 21 d[1]	Palm	131	142	120	119	124	124	0.161	0.122
		Soy	126	128	108	134	113	126	0.181	0.255
		P-value	-	0.412	0.057	0.329	0.107	0.799		
	Up to 42 d[2]	Palm	131	130	136	131	110[b]	135	0.108	0.088
		Soy	126	138	128	122	139[a]	109	0.115	0.127
		P-value	-	0.502	0.444	0.458	0.039	0.054		
HDL	Up to 21 d[1]	Palm	86.67	88.76	80.09	77.88	78.69	80.91	0.194	0.074
		Soy	76.77	78.10	72.95	79.49	70.93	77.23	0.414	0.492
		P-value	-	0.169	0.059	0.776	0.062	0.401		
	Up to 42 d[2]	Palm	86.7	80.7	86.2	78.7	70.1[a,3]	81.1	0.099	0.024
		Soy	76.8	84.5	76.8	78.9	85.4[b]	75.1	0.118	0.116
		P-value	-	0.479	0.069	0.953	0.019	0.314		
LDL	Up to 21 d[1]	Palm	19.49	20.68	23.78[a]	18.98[b]	20.79	21.76	0.244	0.212
		Soy	21.26	19.54	18.97[b]	24.70[a]	18.39	20.42	0.524	0.053
		P-value	-	0.467	0.042	0.004	0.115	0.573		
	Up to 42 d[2]	Palm	19.49	22.52	23.52	19.91	19.22[b]	17.01	0.071	0.075
		Soy	21.3	20.08	23.45	18.83	25.12[a]	19.06	0.051	0.032
		P-value	-	0.252	0.980	0.616	0.026	0.386		
TAG	Up to 21 d[1]	Palm	38.50	45.90	35.20	34.90	43.00	36.30	0.129	0.100
		Soy	38.00	42.00	36.20	48.30	36.00	43.90	0.269	0.260
		P-value	-	0.579	0.811	0.097	0.239	0.113		
	Up to 42 d[2]	Palm	38.50	28.80	35.80[a]	44.60	36.50	53.90[a,3]	<0.0001(L)	<0.0001
		Soy	38.00	28.40	29.50[b]	35.60	44.50	40.10[b]	0.016(L)	0.051
		P-value	-	0.932	0.008	0.241	0.248	0.009		

Abbreviations: ALT, alanine aminotransferase (UI l^{-1}); AST, aspartate aminotransferase (UI l^{-1}); COL, cholesterol (mg dL^{-1}); HD, high-density lipoprotein (mg dl^{-1}); LDL, low-density lipoprotein (mg dl^{-1}); TAG, triglycerides (mg dl^{-1}); Q, quadratic; L, linear; CD, Control diet (8,000 IU of vitamin A kg^{-1} at 1 to 21 d of age and 6,700 IU of vitamin A kg^{-1} at 22 to 42 d of age)

ALT (Palm 1–42 d): 0.00049248vit.A + 9.65505; R^2: 0.46.
AST (Palm 1–21 d): -0.0000004 vit.A^2 + 0.0125651vit.A + 246.2107692; R^2: 0.32; Vit A 1° Derivation: 15,706; Estimated Response: 345.
AST (Soy 1–21 d): 0.0038492 vit. A+ 287.77750; R^2: 0.30.
AST (Palm 1–42 d): 0.00247vit.A + 268.28250; R^2: 0.08.
AST (Soy 1–42 d): -0.00000114vit.A^2 + 0.02986vit.A + 251.96615; R^2: 0.39; Vit A 1° Derivation: 13.096; Estimated Response: 447.
TAG (Palm 1–42 d): 0.00086167vit.A + 32.165; R^2: 0.45.
TAG (Soy 1–42 d): 0.00054333 vit. A + 30.73; R^2: 0.21.
[a,b]Different letters in the line differ statistically by the t test ($P < 0.05$).
[1]Up to 21 d: broilers supplemented with treatments from 4 to 21 d of age, and diet free from vitamin A from 22 to 42 d of age.
[2]Up to 42d: broilers supplemented with treatments from 4 to 42 d of age.
[3]Differ from Control by Dunnett Test ($P < 0.05$).

Fat and Protein Deposition Rate

A significant interaction at 21 d of age ($P > 0.05$) was found between lipid source, and vitamin A supplementation of broiler diets with FDR and PDR (Table 8).

The lipid source did not influence ($P > 0.05$) FDR or PDR. Vitamin A supplementation had a quadratic effect for FDR: at the level of 13,264 IU kg^{-1} of vitamin A, a daily FDR of 3,498 g d^{-1} was obtained. Fat deposition rate at 21 d of age differed statistically by Dunnett's test ($P < 0.05$) when the birds received diets with no vitamin A supplementation. For PDR, a significant difference at 5% probability was found by Dunnett's test for vitamin A supplementation at 3,000 and 6,000 IU

kg^{-1}, and these levels resulted in higher PDR levels when compared with the CD (8,000 IU kg^{-1}).

An interaction between the source of fat used and vitamin A supplementation levels and PDR at 21 d were observed ($P < 0.0001$) (Table 9). The use of PF did not show a significant response in PDR when supplemented with vitamin A. For diets containing SO, regression analysis showed a quadratic effect ($P = 0.0054$), and in the derivation of the equation, supplementation with 13,574 IU kg^{-1} of vitamin A resulted in a PDR of 7.934 g day^{-1}. In supplementation with 0 and 3,000 IU kg^{-1} of vitamin A, the PF diets showed better PDR results than those containing SO. However, in the supplementation with 6,000 IU kg^{-1} of vitamin A, the best effect on PDR was observed

Table 8. Fat deposition rate (FDR) and protein deposition rate (PDR) of 21-day-old broilers fed diets containing 2 lipid sources with different vitamin A supplements.

Item	FDR (g d^{-1})	PDR (g d^{-1})
Lipid source	3.223	7.558
Palm		
Soy	3.281	7.501
Vitamin A (UI kg^{-1})		
CD	3.248	7.134
0	2.611[1]	6.681
3.000	3.331	8.344[1]
6.000	3.302	7.779[1]
12.000	3.552	7.557
24.000	3.307	7.348
Mean	3.252	7.529
CV	16.114	10.173
SEM	0.050	0.073
P-value		
Source x Vitamin A	0.396	<0.001
Source lipid	0.416	0.466
Vitamin A	<0.001	<0.001
Regression	<0.001(Q)	0.006(Q)
Dunnett	<0.0001	<0.0001

Q: quadratic, CD: Control diet (8.000 UI vitamin A kg^{-1}).
FDR: -0.0000000401768vit.A^2 + 0.00011744vit.A + 1048.342.78711; R^2: 0.23; Vit A 1° Derivation: 14,615; Estimated Response: 3.645.
[1]Differ from Control by Dunnett test ($P < 0.05$).

when the diet contained SO, no effects on the other vitamin A supplements at 21 d of age.

At 42 days of age, there was no difference in the influence of lipid source and vitamin A supplementation levels on PDR and FDR in broilers carcass (Table 10), and also PDR at treatment 0 IU kg^{-1} did not differ from CD by Dunnet test ($P > 0.05$).

Bone Analysis

There was an interaction between period, lipid source, and vitamin A supplementation level for GP size ($P = 0.0164$). However, there was no effect on bird tibia hypertrophic cartilage at 42 d of age (Table 11).

In the GP evaluation, statistical differences were found within the evaluated periods and

Table 9. Protein deposition rate (PDR) of 21-day-old broilers fed diets containing 2 lipid sources with different vitamin A supplements.

Vitamin A (UI kg^{-1})	PDR (g day^{-1})		
	Soy	Palm	P-value
CP[1]	7.034	7.235	0.542
0	6.413[b]	6.988[a]	0.019
3.000	7.915[1,b]	8.772[1,a]	<0.0001
6.000	8.277[1,a]	7.280[b]	0.0004
12.000	7.639	7.473	0.554
24.000	7.366	7.328	0.878
Mean	7.501	7.558	
P (Regression)	0.008 (Q)	0.132	
P (Dunnett)	<0.0001	<0.0001	

Q: quadratic, CD: Control diet (8,000 UI vitamin A kg^{-1}).
FDR: -0.000000007vit.A^2 + 0.00018516vit.A + 6.9829946; R^2: 0.27; Vit A 1° Derivation: 13,225; Estimated Response: 8.207.
[a,b]Different letters in the line differ statistically by the t test ($P < 0.05$).
[1]Differ from Control by Dunnett test ($P < 0.05$).

Table 10. Fat deposition rate (FDR) and protein deposition rate (PDR) of 42-day-old broilers fed diets containing 2 lipid sources with different vitamin A supplements.

Item	FDR (g day^{-1})	PDR (g day^{-1})
Supplementation	8.82	10.49
Up to 21 d[1]		
Up to 42 d[2]	8.98	10.60
Lipid Source	8.87	10.46
Palm		
Soy	8.78	10.39
Vitamin A (UI kg^{-1})	8.44	9.77
CD		
0	8.64	10.24
3.000	9.06	10.57[3]
6.000	8.87	10.61[3]
12.000	9.09	10.76[3]
24.000	8.79	10.54[3]
Mean	8.82	10.43
CV (%)	0.097	0.098
EPM	11.75	10.02
P-value		
Period × Lipid × Vitamin	0.053	0.186
Period × Lipid	0.202	0.731
Period × Vitamin	0.982	0.900
Lipid × Vitamin	0.540	0.941
Period	0.386	0.541
Lipid source	0.566	0.521
Vitamin A	0.582	0.539
Dunnett	0.279	0.019
Regression	-	-

CD: Control diet (8,000 IU of vitamin A kg^{-1} at 1–21 d of age, and 6,700 IU of vitamin A kg^{-1} at 22–42 d of age)
[1]Up to 21 d: broilers supplemented with treatments from 4 to 21 d of age, and diet free from vitamin A from 22 to 42 d of age.
[2]Up to 42 d: broilers supplemented with treatments from 4 to 42 d of age.
[3]Differ from Control by Dunnett test ($P < 0.05$).

supplementations (Table 12). In the period from 1 to 21 d of age, when the birds received 12,000 IU kg^{-1} supplementation of vitamin A, birds fed PF diets had greater GP sizes compared with those fed SO diets. Considering the period from 1 to 42 d, there was a difference at the level of 6,000 IU kg^{-1} of vitamin A, and in the same way, similar to the previous period, the birds that received PF showed larger tibial GP compared with those that received SO. Supplements of 6,000 and 12,000 IU kg^{-1} of vitamin A differed from the CD (Dunnett's test) from 1 to 21 d of age when PF was used in the diet, whereas from 1 to 42 d of supplementation with 6,000 IU kg^{-1} of vitamin A differed from the CD.

DISCUSSION

The results obtained with supplements above 6,000 IU kg^{-1} of vitamin A were similar to those found for the CD at 21 d of age. This differs from NRC (1994) recommendations, which indicate supplementation at 1,500 IU kg^{-1} of vitamin A for broiler chickens of 1 to 45 d old. However, it is necessary to realize that, in some cases, dietary supplementation above the minimum required values will impact the nutritional value of poultry products, poultry health, and welfare (Leeson, 2007).

According to D'Ambrosio et al. (2011), for optimal absorption of retinoids, they should be consumed together

Table 11. Growth plates (GP) and hypertrophic cartilage zone (HCZ) of broilers of 42-day-old broilers fed diets containing 2 lipid sources with different vitamin A supplements.

Item	GP (mm^2)	HCZ (mm^2)
Supplementation	20.384	42.311
Up to 21 d[1]		
Up to 42 d[2]	17.959	39.966
Lipid source	19.975	40.988
Palm		
Soy	17.734	39.966
Vitamin A (UI kg^{-1})	16.772	38.942
CD[1]		
0	17.748	37.932
3.000	48.507	42.959
6.000	20.585	36.334
12.000	19.212	42.646
24.000	19.643	42.823
Mean	18.880	40.483
CV (%)	28.844	23.559
EPM	0.581	1.011
P-value		
Period × Lipid × Vitamin	0.016	0.264
Period × Lipid	0.169	0.845
Period × Vitamin	0.892	0.376
Lipid × Vitamin	0.466	0.719
Period	0.023	0.233
Lipid source	0.017	0.643
Vitamin A	0.609	0.130
Dunnett	0.451	0.091

CD: Control diet (8,000 IU of vitamin A kg^{-1} at 1 to 21 d of age, and 6,700 IU of vitamin A kg^{-1} at 22 to 42 d of age)

[1]Up to 21 d: broilers supplemented with treatments from 4 to 21 d of age, and diet free from vitamin A from 22 to 42 d of age.

[2]Up to 42 d: broilers supplemented with treatments from 4 to 42 d of age.

with some fat, because fat is necessary to facilitate entry of the retinoids into the enterocytes in the intestinal lumen. Also, a fat "charge" is required for chylomicron formation, because retinoids, similar to other dietary lipids, enter the body as a component of triglyceride-rich chylomicrons.

The effect of oil and fat source on performance results at 21 or 42 d may be due to several factors, such as the presence and number of double bonds, their position in the carbon chain, the amount of triglycerides, free fatty acid (**FA**) composition, the bird's age and sex, and even the intestinal microbiota (Abdulla et al., 2016). These factors will interfere with the digestibility and absorption capacity of the bird to metabolize energy in the consumed diet.

Abawi and Sullivan (1989) conducted studies evaluating fat-soluble vitamins and their interactions in 28-day-old broiler chickens. The authors found that FCR was affected by vitamin A concentration in the diets. Low levels of vitamin A (1,000 IU kg^{-1}) showed the best results, differing from the results found in this study for broilers at 21 d of age. Khoramabadi et al. (2014), evaluating wheat-based diets with different vitamin A supplementation with or without xylanase inclusion, found no effect of treatment on the FI of birds at 21 d of age. However, vitamin A supplementation (at 9,000 or 15,000 IU kg^{-1}) improved WG and FCR ($P < 0.005$) when compared with their levels in birds fed diets without vitamin A supplementation, because diets without supplementation may result in vitamin A deficiency.

Vitamin A supplementation values to obtain optimal response, as found by the equations for FI and WG at 21 and 42 d of age, are higher than those indicated by the NRC (1994), which suggests a requirement of 1,500 IU kg^{-1} of vitamin A regardless of the broiler rearing phase, and those of Rostagno et al. (2017), which indicated vitamin A supplementation levels of 13,538 IU kg^{-1} (1–7 d); 12,216 IU kg^{-1} (8–21 d), 9,637 IU kg^{-1} (22–33 d), and 7,873 IU kg^{-1} (34–42 d).

Khoramabadi et al. (2014) found better WG and FCR levels in broilers fed with xylanase enzyme and higher doses of vitamin A. According to these researchers, there is an influence of vitamin A on increased nutrient digestibility and a role in maintaining intestinal health or integrity.

The lipid source did not influence ($P > 0.05$) FDR or PDR, a similar energy balance of the formulated diets being demonstrated regardless of lipid source.

Fat deposition in the body depends on the net balance between fat absorbed from the diet, fat synthesis, and fat catabolism in the body for energy (Mohammed et al., 2012). Thus, the higher FDR observed in birds that received PF as a lipid source may be related to the fact that PF is mostly composed of saturated FAs. Factors such as degree of saturation of the dietary FA may influence fat deposition by birds, because of different signs of backward regulation of endogenous synthesis, differences in the absorption of each tissue, or to some alteration in the metabolism of FA (Sanz et al., 2010).

Table 12. Deployment of the interaction between vitamin A supplementation period, the lipid source, and the vitamin A supplementation of the growth plate (GP) (mm^2) of 42-day-old broilers.

Item	Supplementation	Lipid	Vitamin A (UI kg^{-1})					
			0	3.000	6.000	12.000	24.000	*P*-value
GP	Up to 21d[1]	Palm	21.41	20.77	24.24	25.86[a]	21.57	0.4239
		Soy	18.39	17.03	21.05	15.05[b]	20.2	0.3682
		P-value	0.4330	0.2845	0.3730	0.0119	0.6324	
	Up to 42 d[2]	Palm	17.37	18.55	23.76[a]	14.30	18.59	0.1077
		Soy	14.72	17.35	13.28[b]	22.19	18.88	0.4311
		P-value	0.4903	0.3565	0.0121	0.3188	0.9520	

[a,b]Different letters in the line differ statistically by the t test ($P < 0.05$).

[1]Up to 21 d: broilers supplemented with treatments from 4 to 21 d of age and diet free from vitamin A from 22 to 42 d of age.

[2]Up to 42 d: broilers supplemented with treatments from 4 to 42 d of age.

Even though they were influenced by vitamin A supplementation at 21 d of age, the FDR values obtained in this study were similar to those found by Nunes et al. (2015) for broilers of the same age fed diets with different levels of ME and digestible lysine levels, which averaged 3.511 g d^{-1}. However, they were lower than those obtained by Dias (2015) of 6.45 g d^{-1} for broilers at 21 d of age fed diets containing different levels of lysine. For the mean values of PDR found in this study (7.529 g d^{-1}), the results are between those obtained by Nunes et al. (2015) of 4.707 g d^{-1} and Dias (2015) of 13.8 g d^{-1}.

According to Cottrell (1991), PF differs from others because of its high concentration of palmitic acid in position 2 of the carbon chain, this being a beneficial feature for the proper digestion and utilization of triglycerides. In addition, the crude palm is rich in carotenoids (provitamin A), and even after being refined and turned into PF, very low proportions of these compounds remain in the material. These characteristics may have contributed to the better PDR in the PF diets supplemented with lower vitamin A levels compared with SO diets. The animal has a daily energy requirement that can be subdivided into the amount required for protein deposition and fat deposition (McDonald et al., 2010). However, for this to occur, numerous biochemical pathways and different substances are necessary for correct and proper deposition. The results of this study demonstrate that PF was more efficient for PDR in low vitamin A supplements than was SO at 21 d of age. However, no differences between fat sources or of vitamin A supplementation levels were found at 42 d of age.

According to Aburto and Britton (1998), high levels of vitamin A may interact with vitamin D3 and affect bone growth and metabolism. The evaluations showed that at 42 d of age, the tibial GP of birds that had received vitamin supplementation treatments up to 21 d were influenced by the fat sources, with no effect observed for vitamin A supplementation. Larger GP were found in birds fed PF diets. According to Henning et al. (2015), hypervitaminosis A increases osteoclast formation and consequently increases bone fragility, as it decreases cortical bone mass. Also, there are some indications that increased serum retinoid levels may result in reduced bone mineral density and increased bone mineral density fracture rate. In this study, the results indicated that growth of the GP is influenced by the 3 factors evaluated (period * lipid * vitamin), and regardless of the period evaluated, PF diets resulted in the highest values of this variable, whereas the vitamin level was influenced by the time of supplementation. Changes in vitamin D3 metabolism caused by high doses of vitamin A can be avoided when vitamin D3 supplementation is adequate (Aburto and Britton, 1998). The results indicate that vitamin A supplementation levels did not interfere with vitamin D3 functions in bone tissue.

According to Velasco et al. (2010), fat intake by poultry influences blood concentrations of triglycerides, lipoproteins, and FA, in addition to altering fat and meat composition. Blood analysis can be a way to find information concerning bird health or even to make diagnoses when necessary. The enzymes alanine aminotransferase (**ALT**) and aspartate aminotransferase (**AST**) are commonly used for determination of liver and cardiac injury, as they are released into the bloodstream after cell damage; after the lesion, the first to be detected in the liver serum is AST, and soon after ALT can be identified (Nelson and Cox, 2014).

Alterations in the enzymes ALT and AST were found in birds at 42 d of age, indicating the influence of all factors evaluated in this study. According to Harrison and Lightfoot (2006), AST is sensitive to but not definitive of liver damage as it can be released by hepatocytes and damaged muscle tissue, but its elevation can be considered to indicate liver damage. The same authors emphasize that the high rate of lipid peroxidation in diets rich in polyunsaturated fats may result in a formation of high levels of FA hydroperoxides and consequent tissue damage; in these cases, the use of AST is an alternative for verification. This is consistent with our results, because broilers fed SO had higher blood AST values than those fed PF, resulting from a possible liver intoxication by SO, and this effect occurred independent of period. According to regression analysis, it was possible to find an increasing linear response to vitamin A supplementation from 1 to 21 d of age; that is, excessive increases in supplementation may result in a bird's intoxication.

Similarly, as for AST, changes in ALT concentrations were found in birds at 42 d of age; however, when the evaluation was performed within the period, PF influenced the ALT values as a function of vitamin A level in both periods evaluated. In this case, we can consider that both fats used and vitamin A level interfere with the ALT values because both can cause liver damage, due either to lipid oxidation or possible intoxication as a result of excess of vitamin A in the diet.

Cholesterol is a steroid, more specifically an unsaturated alcohol, and has functions that ensure the proper functioning of animal cells as it is a constituent of their membranes and is also a precursor of substances such as hormones and bile acids. Triglycerides are glyceryl esters of FA and represent the major lipid components of fat and fat deposits in animal diets (Cox and Palmiere, 1990). According to Aguilar et al. (2011), high-fat diets may affect the blood serum lipid profile, an important factor in the modulation of lipid metabolism. According to these authors, saturated FA intake tends to increase LDL serum concentrations and reduce HDL. The LDL and HDL results obtained in this study for birds at 42 d of age are not in accordance with the previous statement; the statement was valid only for LDL in birds supplemented for 1 to 21 d of treatment with 3,000 IU kg^{-1} of vitamin A.

The COL values only showed an interaction between lipid source and vitamin A supplementation in treatments where birds received the same vitamin A supplementation during the period from 1 to 42 d of age and with 12,000 IU kg^{-1} of vitamin A. Although there was no statistical difference ($P < 0.05$), variations in values

presented within each fat type and different supplements occurred.

The TAG values for the same treatments showed an effect of vitamin A supplementation and an interaction between supplementation levels and dietary fat type. It can be stated that higher vitamin A levels tend to increase serum TAG levels in poultry at 42 d of age in birds receiving supplementation from 1 to 42 d of age (linear effect). The higher concentration of TAG caused by increased vitamin A supplementation may be correlated with the need for joint intestinal absorption of vitamin A and FA; however, higher evaluations are necessary to confirm and understand this response in birds. Generally, high levels of saturated FA increase the levels of triglycerides and LDL in the blood (Velasco et al., 2010); however, the results found in this study do not match this statement.

Although vitamin A supplement levels used in this study are above those indicated by the NRC (1994) and the Brazilian tables (Rostagno et al., 2011, 2017), no results were found indicating that birds had hypervitaminosis A. However, it is possible to indicate that the absence of vitamin A supplementation in the diet caused this deficiency, because this treatment influenced most of the analyzed variables, both at 21 d of age and at 42 d old. However, it can be observed that even having been submitted to vitamin A deficiency in the period of 1 to 21 d, when supplementation was administered in the period of 22 to 42 d (CD with 6,700 IU kg^{-1} of vitamin A), the birds showed an improved WG; however, this improvement was still below that shown by birds that received adequate vitamin A supplementation. These results indicate that after a deficiency, even if birds are once again supplemented with vitamin A, adequate zootechnical performance will not be improved.

In the comparative evaluations of periods, supplementation with 3,000 to 24,000 IU kg^{-1} of vitamin A from 1 to 42 d, and the same supplements given up to 21 d of age with subsequent substitution in a period of 22 to 42 d with a diet containing 6,700 IU kg^{-1} of vitamin A did not negatively affect the parameters evaluated. The lipid source used in the diet affected bird's performance at 21 d of age, but at 42 d of age, there was an effect on FI. None of the evaluated lipids indicated results outside the blood standards for poultry; the same effect was observed for vitamin A supplementation in the diet. For broilers from 1 to 21 d, the best supplementation level was 15,585 IU kg^{-1} of vitamin A, whereas for birds at 42 d, it would be 15,527 IU kg^{-1} (supplemented until 21 d) and 15,148 IU kg^{-1} (supplemented up to 42 d). There was no evidence of the interference of vitamin A supplements on hypertrophic cartilage, but the influence of vitamin level, fat source, and supplementation time in the growth zone. Hypovitaminosis may be reduced with vitamin A supplementation for birds, but the results are not similar to those for birds that have been adequately supplemented.

DISCLOSURES

The authors declare no conflicts of interest.

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