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Effects of herbal choline as a replacement for choline chloride on myopathy, locomotor system, and hepatic health of broilers

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ABSTRACT - The objective of this study was to evaluate the effects of replacing choline chloride with a plant source of choline on the locomotor system, liver health, and development of breast myopathies in broilers aged 1-42 days of age. We allocated 1,120 one-day-old Cobb broilers to four treatment groups and fed them commercial diets based on corn and soybean meal. The treatments included choline in the form of 1,800.00 mg/kg choline chloride; 1,350.00 mg/kg choline chloride + 450.00 mg/kg herbal choline; 900.00 mg/kg choline chloride + 900.00 mg/kg herbal choline; and 1,000.00 mg/kg herbal choline. Each treatment group had eight replications. Throughout the experiment, gait score, footpad dermatitis, hock burn, and leg deformities (valgus and varus) were evaluated in the birds at 28 and 35 days of age. After slaughter, parameters such as breast myopathies, tibial dyschondroplasia score, and histological slides of the pectoral muscle, liver, and proximal tibial epiphysis were assessed. The results demonstrated good hepatic and locomotor health in the broilers, as no classical signs of choline deficiency were observed. Statistical analyses indicated no significant differences between treatments in terms of liver and locomotor health, suggesting that broilers fed diets supplemented with the plant source did not experience choline deficiency. Additionally, no statistically significant differences were found between treatments regarding breast myopathies. Overall, the tested choline plant source can effectively replace choline chloride in broiler diets.

Keywords: footpad dermatitis, histological analysis, liver, pectoral muscle, tibial dyschondroplasia

1. Introduction

Choline, an essential nutrient for broilers, is not adequately produced endogenously during the early weeks of life (Saeed et al., 2017; de Lima et al., 2018). It plays crucial roles in lipid transport in the liver, methyl group donation, acetylcholine neurotransmitter production, hepatic lipid transport, and bone cartilage maturation (Zhang et al., 2013; Calderano et al., 2015; Igwe et al., 2015). The latter two are crucial as the first indicators of choline deficiency (Farina et al., 2017). Its significance in hepatic lipid transport stems from its involvement in phosphatidylcholine synthesis, which forms membranes of cholesterol-carrying lipoproteins (high-density lipoprotein, low-density lipoprotein [LDL], and very low-density lipoprotein). This process directly affects fat deposition in the liver and body of birds (Khose, 2019).

Choline deficiency in diets leads to a reduction in cancellous and cortical bone tissue, due to the interaction of lecithin, a form of choline, with peroxisome proliferator-activated receptor (PPAR) α and PPAR$^{\gamma}$. These receptors play a protective role in regulating bone metabolism (Zhang et al., 2021). Tibial dyschondroplasia and hepatic steatosis are the initial signs observed when birds are deficient in choline (Ferguson et al., 1978; Lima, 2012).

Choline chloride, a synthetic compound with high hygroscopicity, is commonly used as a choline supplement. However, this property poses challenges in handling and storage and can result in the loss of other diet nutrients, such as vitamins and amino acids, due to its water-absorption potential (Farina et al., 2017). To address these practical issues, several studies have explored alternative sources, particularly plant-based ones rich in phosphatidylcholine (Calderano et al., 2015; Dematté Filho et al., 2015; Khosravinia et al., 2015; Farina et al., 2017; Khose, 2019).

Plant sources of choline offer the advantage of meeting broilers' needs with smaller inclusions (Farina et al., 2017). Commercially used plant sources often recommend using three to five times less raw material compared with choline chloride. Moreover, they have shown additional benefits such as reducing serum LDL levels, along with the inclusion of medicinal plants such as *Ocimum sanctum*, known for their antioxidant properties (Kelm et al., 2000; Dias et al., 2022).

Against this backdrop, this study evaluated the effects of replacing choline chloride with a new plant source of choline. The objective was to evaluate indicators of choline deficiency in the locomotor system and liver, as well as potential effects on fat deposition in broilers' breast muscles.

2. Material and Methods

The experiment was conducted in Goiânia, GO, Brazil (16°35'39.4" S latitude and 49°17'13.7" W longitude), from March to May 2020. All procedures were conducted following the institutional Animal Ethics Committee Registration Protocol (case number 101/19).

The study involved 1,120 one-day-old Cobb 500® male broiler chicks with an average initial body weight of 42 g, obtained from a commercial hatchery (São Salvador Alimentos S/A, Itaberaí, GO, Brazil). A completely randomized design was utilized with four treatments and eight replicates, resulting in 32 experimental units, each containing 35 birds. The treatments included: control = 1,800.00 mg/kg choline in the form of choline chloride (Ch-Chl; 60% content); 1,350.00 mg/kg Ch-Chl + 450.00 mg/kg herbal choline (Ch-H) as the choline source; 900.00 mg/kg Ch-Chl + 900.00 mg/kg Ch-H as the choline source; and 1,000.00 mg/kg Ch-H as the choline source.

All diets were formulated using corn and soybean meal, and the commercial nutrient levels were employed for the respective rearing phases: pre-starter (1 to 7 days), starter (8 to 21 days), grower (22 to 35 days), and finisher (36 to 42 days). The diets were in mash form and were isonutritive (Table 1). Each treatment contained the respective choline sources as follows: control (100) = 1,800.00 mg/kg choline chloride; Ch-Chl + Ch-H (75/25) = 1,350.00 mg/kg + 450.00 mg/kg; Ch-Chl + Ch-H (50/50) = 900.00 mg/kg + 900.00 mg/kg; and herbal choline (100) = 1,000.00 mg/kg, with the inert ingredient (corn starch) being replaced in the diet. The herbal choline comprised *Ocimum sanctum*, *Andrographis paniculata*, *Silybum marianum*, *Glycine max*, and *Azadirachta indica*, while the choline chloride contained 60% choline chloride. The choline sources were added to the diets to replace corn starch and achieve the intended content of 1,800.00 mg/kg choline in the treatments.

To evaluate the incidence of locomotor system problems, myopathies, and hepatic metabolism, the following analyses were performed:

Gait scoring, valgus, and varus

Gait scoring was evaluated at 28 and 35 days, with 10 birds selected from five replicates per treatment, totaling 50 birds per treatment. We followed the method proposed by Stamp Dawkins et al. (2004), which involves observing the birds' gait on a 1-m path and assigning scores from 0 to 2 (0 = normal; 1 = slight difficulty in locomotion; 2 = severe difficulty in walking). Valgus and varus were analyzed

Table 1 - Experimental diet composition for pre-starter (1 to 7 days), starter (8 to 21 days), grower (22 to 35 days), and finisher (36 to 42 days) phases

Ingredient (g/kg)	Pre-starter	Starter	Grower	Finisher
Corn	581.80	627.40	655.00	761.9
Soybean meal	344.80	293.00	269.10	145.8
Meat and bone meal	-	-	-	10.0
Offal meal	34.00	44.67	22.00	30.00
Feather meal	-	-	-	24.67
Bird fat	5.33	6.00	19.33	5.33
Limestone	12.41	10.91	12.33	8.60
Salt	3.19	3.09	3.36	2.80
Sodium bicarbonate	0.81	0.07	-	-
Monocalcium phosphate	4.31	2.13	6.80	-
DL-methionine	3.49	3.01	2.69	1.89
L-lysine	3.90	3.67	3.93	5.19
L-threonine	1.20	1.00	1.01	0.83
Enramycin	0.03	0.05	0.05	0.03
Nicarbazin	-	0.50	-	-
Salinomycin	-	-	0.30	-
Adsorbent	1.80	1.50	1.20	-
Phytase	0.15	0.15	0.11	0.14
Antioxidant	0.04	0.04	0.04	0.04
Vitamin premix[1]	0.50	0.50	0.50	0.50
Mineral premix[2]	0.50	0.50	0.50	0.50
Choline	1.80	1.80	1.80	1.80
	1000.00	1000.00	1000.00	1000.00

[1] Vitamin supplement (PX Vitamínico Frango SSA) provides the following per kilogram of diet: pre-starter/starter: vitamin A, 22,000,000 IU/kg; vitamin D3, 8,000,000 IU/kg; vitamin E, 80,000 IU/kg; vitamin K3, 6,000 mg/kg; vitamin B1, 5,000 mg/kg; vitamin B2, 15 g/kg; vitamin B6, 8,000 mg/kg; vitamin B12, 40,000 mcg/kg; vitamin, B5, 32 g/kg; vitamin B3, 100 g/kg; folic acid, 3,200 mg/kg; biotin, 300 mg/kg; selenium, 1,000 mg/kg. Grower: vitamin A, 10,000,000 IU/kg; vitamin D3, 4,000,000 IU/kg; vitamin E, 50,000 IU/kg; vitamin K3, 6,000 mg/kg; vitamin B1, 4,000 mg/kg; vitamin B2, 12 g/kg; vitamin B6, 8,000 mg/kg; vitamin B12, 40,000 mcg/kg; vitamin B5, 37 g/kg; vitamin B3, 36 g/kg; folic acid, 2,000 mg/kg; biotin, 200 mg/kg; selenium, 800 mg/kg. Finisher: vitamin A, 10,000,000 IU/kg; vitamin D3, 4,000,000 IU/kg; vitamin E, 20,000 IU/kg; vitamin K3, 4,000 mg/kg; vitamin B1, 3,000 mg/kg; vitamin B2, 8,000 mg/kg; vitamin B6, 4,000 mg/kg; vitamin B12, 20.000 mcg/kg; vitamin B5, 19 g/kg; vitamin B3, 40 g/kg; folic acid, 1,400 mg/kg; biotin, 150 mg/kg; selenium, 500 mg/kg.
[2] Mineral supplement (PX Micro mineral Frango SSA) provides the following minerals per kilogram of diet: manganese, 150 g/kg; zinc, 140 g/kg; iron, 100 g/kg; copper, 20 g/kg; iodine, 2,000 mg/kg.

simultaneously with gait scoring (at 28 and 35 days). Birds were held from behind and had their legs stretched to measure the angle of the legs. Negative angles between the tibia and the third finger indicated varus, while positive angles indicated valgus (Almeida Paz et al., 2010).

Footpad dermatitis and hock burn

Locomotor problems were assessed based on gait scoring, hock burn, footpad dermatitis, valgus, and varus in birds at 28 and 35 days of age. The condition of both footpads was observed, and lesions were measured using a millimeter ruler. Lesion severity was scored on a scale from 0 to 3 (0 = healthy foot pad region; 1 = lesions with a diameter of up to 5 mm; 2 = lesions with a diameter greater than 5 mm; 3 = lesions with a diameter greater than 5 mm and presence of an exposed wound). Similar criteria were adopted for hock burn evaluation, and a scale from 0 to 3 was used based on lesion size (0 = no lesion (normal); 1 = small lesion; 2 = large lesion without hemorrhage; 3 = large lesion with hemorrhage) (Almeida Paz et al., 2010).

Liver and muscle histological analysis

For histological analysis, 32 birds were selected and slaughtered at 42 days of age. Liver, breast, drumstick, and thigh samples were collected and placed in labeled containers with 10% buffered formalin solution. For the breast muscle tissue (*Pectoralis major*), sampling was performed following the method proposed by Soglia et al. (2017) on the left side of the breast.

To assess the incidence of tibial dyschondroplasia, two analyses were conducted. Firstly, a visual inspection was performed during the preparation of histological slides after making the tibial cross-section. Scores ranging from 0 to 3 were assigned using the method of Rowland and Edwards (1999). After classification, the samples were collected and stored in labeled containers with formalin to preserve the tissues for slide preparation.

Liver tissue slides were examined for the following characteristics: trabecular pattern (normal or altered), inflammation (graded from 0 to 3), degradation (graded from 0 to 3), necrosis (graded from 0 to 3), congestion (graded from 0 to 3), and sinusoidal vessels (normal or enlarged).

Breast tissue slides were analyzed for the following traits: accumulation of adipose tissue (graded from 0 to 3), inflammation (graded from 0 to 3), degradation (graded from 0 to 3), necrosis (graded from 0 to 3), connective tissue (graded from 0 to 3), and presence of edema (graded from 0 to 3).

For the histological analysis of bone tissue from the tibia, three distinct regions were considered and characterized based on their morphological appearance: the resting zone, proliferating cartilage zone, and hypertrophic cartilage zone. The calcified cartilage zone was used as the lower limit to determine the thickening of the hypertrophic zone in lesion characterization, following the method proposed by Thorp et al. (1995). On the other hand, the method of Oviedo-Rondón et al. (2001) was used to determine the cartilage zones from the histological section of the longitudinal profile of the tibia.

Myopathy analysis

To analyze myopathies in the broilers' breast muscle (*Pectoralis major*), the entire breast section used for visual-tactile analysis was separated from the carcasses of the 32 chickens slaughtered at 42 days of age.

White striping was evaluated following the method described by Kuttappan et al. (2012), which assigns scores from 0 to 3. A score of 0 represents a normal condition, while scores 1, 2, and 3 indicate the presence of smaller than 2 mm, larger than 2 mm, or fatty deposits in the stripping, respectively.

The wooden breast was analyzed according to Tijare et al. (2016) and Petracci et al. (2019). It involved assigning scores on a scale of 0 to 4: 0 for a normal condition, 1 for light hardening (less than 40% hardening in the cranial region of the breast), 2 for moderate hardening (between 40 and 80% hardening in the cranial region), 3 for severe hardening (total hardening), and 4 for extreme hardening (total hardening with the presence of hemorrhagic regions).

Finally, for other myopathies, such as cranial dorsal myopathy, deep pectoral myopathy, and spaghetti meat (unstructured meat), we employed the presence/absence analysis method. These myopathies were evaluated based on their presence or absence without assigning specific scores.

2.1. Statistical analysis

After checking the normality of residuals and assessing the homogeneity of variances of the data, we conducted a non-parametric Kruskal-Walli analysis. To compare means for each variable, we used the Scott-Knott test with a significance level of 5%. These analyses were carried out using the easyanova, ExpDes.pt, and ANOVA packages of the R-code statistical software (version 4.0) (Arnhold, 2013; Ferreira et al., 2014).

3. Results

The analyses revealed a significant statistical difference only for valgus (positive leg angle) at 28 days ($P = 0.025$; Table 2). The highest incidence of valgus was observed in the left leg of birds that were fed treatments containing only herbal choline or a combination of 75% choline chloride and 25% herbal choline. However, there were no significant differences ($P>0.05$) observed for the other variables.

Pectoral myopathies, such as wooden breasts, white striping, and spaghetti meat, were detected. However, the treatments did not have a statistically significant effect ($P>0.05$) on the incidence of these disorders (Table 3). Similarly, in the histological slides of the breast muscle, no statistical differences (P>0.05) were found for any of the analyzed variables (Table 4). No statistical differences were detected in the other histological analyzes (P>0.05), both for the proliferating cartilage zone, hypertrophic cartilage zone, and total area of the proximal epiphysis of the tibia (Table 5), and for the histological evaluation of the liver tissue slides (Table 6).

Table 2 - Median scores and percentage relative to the median count for gait scoring (GS), footpad dermatitis (FPD), hock burn (HB), and valgus and varus in the right (R) and left (L) legs of broilers fed different sources of choline at 28 and 35 days of age

Treatment[1]	GS	FPD	HB	Valgus		Varus	
				R	L[2]	R	L
				28 days			
Ch-Chl	0 (76)	0 (62)	1 (56)	A (82)	A (92) a	A (100)	A (100)
Ch-Chl + Ch-H (75/25)	0 (68)	0 (52)	1 (42)	A (80)	A (68) b	A (100)	A (100)
Ch-Chl + Ch-H (50/50)	0 (72)	0 (62)	1 (52)	A (80)	A (82) a	A (100)	A (100)
Ch-H	0 (66)	0 (62)	1 (56)	A (64)	A (76) b	A (96)	A (100)
P-value	0.728	0.552	0.246	0.123	0.025	0.111	-
				35 days			
Ch-Chl	0 (86)	1 (66)	1 (56)	P (80)	A (82)	A (98)	A (100)
Ch-Chl + Ch-H (75/25)	0 (80)	1 (64)	1 (56)	P (84)	A (78)	A (98)	A (100)
Ch-Chl + Ch-H (50/50)	0 (84)	1 (62)	1 (60)	P (88)	A (92)	A (96)	A (100)
Ch-H	0 (76)	1 (68)	1 (66)	P (88)	A (76)	A (98)	A (98)
P-value	0.559	0.951	0.813	0.638	0.134	0.655	0.394

A - absence; P - presence.
[1] Ch-Chl: 100% choline chloride as the source of choline; Ch-Chl + Ch-H (75/25): 75% choline chloride + 25% herbal choline as the source of choline; Ch-Chl + Ch-H (50/50): 50% choline chloride + 50% herbal choline as the source of choline; Ch-H: 100% herbal choline as the source of choline.
[2] Median scores and percentages followed by different letters in the columns are statistically different at 5% significance using the Scott-Knott test.

Table 3 - Median scores and percentage relative to the median count for muscle myopathy analysis (wooden breast, white striping, and spaghetti meat) in the pectoral muscle of broilers treated with different sources of choline

Treatment[1]	Wooden breast[2]	White striping[2]	Spaghetti meat
Ch-Chl	1 (50.0)	1 (50.0)	A (87.5)
Ch-Chl + Ch-H (75/25)	1 (50.0)	1 (37.5)	A (75.0)
Ch-Chl + Ch-H (50/50)	1 (25.0)	1 (75.0)	A (87.5)
Ch-H	1.5 (50.0)	1 (50.0)	A (100.0)
P-value	0.4373	0.7498	0.5496

A - absence; P - presence.
[1] Ch-Chl: 100% choline chloride as the source of choline; Ch-Chl + Ch-H (75/25): 75% choline chloride + 25% herbal choline as the source of choline; Ch-Chl + Ch-H (50/50): 50% choline chloride + 50% herbal choline as the source of choline; Ch-H: 100% herbal choline as the source of choline.
[2] Scores: wooden breast (0-4); white striping (0-3).

Table 4 - Median scores[1] and percentage relative to the median count for histological analysis of pectoral muscle tissue slides in broilers fed different sources of choline

Treatment[2]	Adipose tissue	Inflammation	Degradation	Necrosis	Connective tissue	Edema
Ch-Chl	1 (50.0)	1 (62.5)	3 (62.5)	3 (62.5)	1 (37.5)	3 (50.0)
Ch-Chl + Ch-H (75/25)	2.5 (100)	2 (50.0)	3 (50.0)	3 (50.0)	2.5 (100)	3 (62.5)
Ch-Chl + Ch-H (50/50)	3 (50.0)	1 (37.5)	3 (50.0)	3 (50.0)	3 (50.0)	3 (50.0)
Ch-H	3 (62.5)	1 (37.5)	3 (62.5)	3 (62.5)	3 (62.5)	3 (62.5)
P-value	0.1451	0.4151	0.8135	0.8135	0.2823	0.9756

[1] 0 = normal; 1 = discrete; 2 = moderate; 3 = pronounced.
[2] Ch-Chl: 100% choline chloride as the source of choline; Ch-Chl + Ch-H (75/25): 75% choline chloride + 25% herbal choline as the source of choline; Ch-Chl + Ch-H (50/50): 50% choline chloride + 50% herbal choline as the source of choline; Ch-H: 100% herbal choline as the source of choline.

Table 5 - Mean values of proliferating cartilage zone (PCZ), hypertrophic cartilage zone (HCZ), and total area (TA) in 42-day-old broilers fed different sources of choline

Treatment[1]	PCZ (mm^2)	HCZ (mm^2)	TA (mm^2)	Score[2]
Ch-Chl	26.74	33.58	102.97	0 (87.5)
Ch-Chl + Ch-H (75/25)	28.14	29.28	106.87	0 (57.1)
Ch-Chl + Ch-H (50/50)	31.96	28.52	117.83	0 (75.0)
Ch-H	28.58	28.69	108.89	0 (87.5)
CV (%)	19.75	20.15	9.78	-
P-value	0.6167	0.4827	0.3348	0.4140

[1] Ch-Chl: 100% choline chloride as the source of choline; Ch-Chl + Ch-H (75/25): 75% choline chloride + 25% herbal choline as the source of choline; Ch-Chl + Ch-H (50/50): 50% choline chloride + 50% herbal choline as the source of choline; Ch-H: 100% herbal choline as the source of choline.
[2] Median tibial dyschondroplasia visual score and its percentage relative to the total analyzed samples.

Table 6 - Median scores[1] and their percentage relative to the median count for histological analysis of liver tissue slides in broilers fed different sources of choline

Treatment[2]	TP	Inflammation	Degradation	Necrosis	Congestion	SV
Ch-Chl	0 (75.0)	1 (87.5)	3 (62.5)	2 (37.5)	0 (75)	1 (37.5)
Ch-Chl + Ch-H (75/25)	0 (75.0)	1 (87.5)	3 (62.5)	2 (37.5)	0 (87.5)	1.5 (100)
Ch-Chl + Ch-H (50/50)	0 (62.5)	1 (75.0)	3 (87.5)	2.5 (87.5)	0 (87.5)	1 (62.5)
Ch-H	0 (62.5)	1 (100)	2 (50.0)	2 (37.5)	0 (62.5)	1 (75)
P-value	0.8414	0.8162	0.3336	0.5158	0.5850	0.5081

TP - trabecular pattern; SV - sinusoidal vessels.
[1] 0 = normal; 1 = discrete; 2 = moderate; 3 = pronounced.
[2] Ch-Chl: 100% choline chloride as the source of choline; Ch-Chl + Ch-H (75/25): 75% choline chloride + 25% herbal choline as the source of choline; Ch-Chl + Ch-H (50/50): 50% choline chloride + 50% herbal choline as the source of choline; Ch-H: 100% herbal choline as the source of choline.

4. Discussion

Gait scoring, footpad dermatitis, hock burn, valgus, and varus analyses (Table 2) were performed to evaluate the effect of choline deficiency, which is known to be associated with tibial dyschondroplasia (de Lima et al., 2018). Our results indicated no choline deficiency since 70% of birds in all treatments did not exhibit walking difficulties, reflected by a gait score of 0 at 28 days. This percentage increased to over 80% at 35 days, indicating good development of their locomotor limbs.

The low incidence of locomotor problems is positive and suggests no serious issues in this regard. Typically, broiler leg problems are associated with an imbalance between rapid growth rate and immature bones and joints, leading to impaired locomotion, pain, poor welfare, increased mortality, reduced slaughter volumes, and significant financial losses (Güz et al., 2021). Notably, importing countries have set a target for animal welfare, accepting grades 1 and 2 in less than 30% of a batch (Almeida Paz et al., 2010).

The incidence of minor footpad dermatitis and hock burn lesions (score 1) can be considered normal for birds and does not pose a significant problem for their locomotion (van der Eijk et al., 2023). Importantly, the bedding material used in the experiment was new and free from high moisture and temperature, which could have increased the incidence of more severe footpad dermatitis and hock burn scores. The overall results of footpad dermatitis and hock burn indicate no major locomotor problems in the birds.

Regarding valgus and varus, a higher incidence of valgus lateral deviation was observed compared with varus, consistent with findings from other authors (Almeida Paz et al., 2010; Almeida Paz et al., 2013; Guo et al., 2019). According to Gonzales and Mendonça Junior (2006), such alterations may be influenced by the bird's genetics. The difference observed at 28 days, with a higher incidence of valgus in the right leg of birds receiving Ch-H alone and 75% Ch-Chl + 25% Ch-H treatments, is more likely attributed to genetic predisposition rather than nutrition-related factors.

The increased incidence of valgus in the right leg at 35 days did not signify a notable leg issue, and it did not significantly affect the birds' gait and locomotion. As stated by Guo et al. (2019), valgus deviation typically occurs unilaterally in more than 80% of cases and does not hinder broiler performance or growth, although it can affect bone quality.

The incidence of wooden breasts and spaghetti meat (Table 3) was already expected. Although the precise factors influencing these muscle myopathies are not fully comprehended, the fast-growing genetics of the birds are deemed responsible, besides affecting muscle fiber development and leading to economic losses.

As for white striping, a myopathy characterized by lipid accumulation in degraded muscle tissue, a lower incidence could be expected (Kuttappan et al., 2016). No significant differences were observed between treatments and, overall, more than 50% of birds in all treatments showed some degree of white striping in the pectoral muscle. These findings support the theory that these myopathies are primarily linked to the bird's genetics rather than nutrition (Lake and Abasht, 2020). At the molecular level, tissues affected by pectoral myopathy in broilers exhibit high levels of multiple long-chain fatty acids, phospholipids, and triglycerides, indicating dysregulation of lipid metabolism genes such as fatty acid translocase (CD36), fatty acid binding protein 4 (FB94), lipoprotein lipase (LPL), and peroxisome proliferator-activated receptor gamma ($PPAR^{\gamma}$) (Tunim et al., 2021).

The histological results of breast tissue align with the macroscopic analysis of myopathy presence (Table 4), showing no significant differences between treatments. Therefore, as observed in the presence of myopathies, adipose tissue accumulation, extensive tissue degradation, and connective tissue accumulation were visually apparent, indicating the presence of traditional myopathies such as white striping and wooden breast. Petracci et al. (2019) associated these myopathies with the rapid growth of birds rather than nutritional effects, as choline directly affects lipid metabolism and did not induce changes in adipose tissue deposition in the breast muscle.

The analysis of the proximal epiphysis of the tibia (Table 5), which quantified growth zones, is consistent with the lesion scoring and locomotor analyses (Table 2), indicating the absence of locomotor problems in response to the treatments. These results lead to the conclusion that there was no choline deficiency in any of the treatments, as the growth plate zones, which are typically affected by choline deficiency, particularly the hypertrophic cartilage zone, showed no abnormalities (Thorp et al., 1995).

No hepatic lesions characteristic of impaired lipid metabolism in the liver, such as hepatic steatosis (vacuolar degeneration), were found in any of the evaluated chickens (Table 6). Animals with choline deficiency typically exhibit a reduction in sinusoidal space and vacuolar degeneration, indicative of hepatic steatosis (Selvam et al., 2018). The histological analysis in this study revealed a normal trabecular pattern and sinusoidal vessels, indicating an overall moderate degree of enlargement.

5. Conclusions

The plant-based choline source effectively met the choline requirements of the broiler chickens, as no signs of nutrient deficiency were observed in any of the treatments. Choline does not play a direct role in muscle tissue formation, and neither the change of its sources nor their combinations in the diet have any histological effect on muscle tissue or the occurrence of myopathies.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

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