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Supplementation of plant phosphatidylcholine sources: effects on performance and carcass traits in broiler chickens

Suplementação de fontes vegetais de fosfatidilcolina: efeito no desempenho e características de carcaça de frangos de corte

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Abstract: Choline is an important nutrient for broiler chickens and plays several important roles in their metabolism. In recent years, there has been an observed increase in the utilization of plant-derived products rich in choline to meet the nutritional requirements of poultry. Given its relevance, the objective of this study was to evaluate the effect of supplementation with two vegetable sources of phosphatidylcholine on the performance and carcass characteristics of broiler chickens. Twelve hundred broiler chicks were distributed among five experimental treatments, as follows: Basal diet (BD)-diet without choline supplementation; BD + Biocholine (218, 197, and 143 mg/kg of diet, respectively); BD + Biocholine DS1 (146, 131, and 96 mg/kg of diets, respectively); BD + Biocholine DS2 (109, 98, and 72 mg/kg of diets, respectively); BD + Biocholine DS3 (87, 79, and 57 mg/kg of diets, respectively). The five experimental treatments were composed of twelve replications and twenty birds each. A significant improvement ($P < 0.05$) in feed conversion was observed in animals supplemented with Biocholine and Biocholine DS during the 1 to 21-day phase. However, no improvement were noted in the other evaluated parameters ($P > 0.05$). In conclusion, supplementation with plant sources of phosphatidylcholine improves feed conversion in broiler chickens from 1 to 21 days of age, however, it does not have any effect on the carcass characteristics of the animals.

Keywords: body composition; feed conversion; weight gain

Resumo: A colina é um nutriente importante para frangos de corte e desempenha vários papéis importantes em seu metabolismo. Nos últimos anos, tem-se observado um aumento na utilização de produtos derivados de plantas ricas em colina para atender as necessidades nutricionais das aves. Dada a sua relevância, o objetivo deste estudo foi avaliar o efeito da suplementação de duas fontes vegetais de fosfatidilcolina sobre o desempenho e as características de carcaça de frangos de corte. Mil e duzentos pintos de corte foram distribuídos em cinco tratamentos experimentais, sendo estes:

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Dieta basal (DB) – dieta sem suplementação com colina; DB + Biocholine (218, 197 e 143 mg/kg de dieta, respectivamente); DB + Biocholine DS1 (146, 131 e 96 mg/kg de dietas, respectivamente); DB + Biocholine DS2 (109, 98 e 72 mg/kg de dietas, respectivamente); DB + Biocholine DS3 (87, 79 e 57 mg/kg de dietas, respectivamente). Os cinco tratamentos experimentais foram compostos por doze repetições e vinte aves cada. Observou-se uma melhora significativa (P < 0,05) na conversão alimentar dos animais suplementados com Biocholine e Biocholine DS na fase de 1 a 21 dias. Entretanto, nenhuma melhora foi observada nos demais parâmetros avaliados (P > 0,05). Em conclusão, a suplementação de fontes vegetais de fosfatidilcolina melhora a conversão alimentar de frangos de corte de 1 a 21 dias de idade, entretanto não apresenta nenhum efeito sobre as características de carcaça dos animais.

Palavras-chave: ganho de peso; composição corporal; conversão alimentar

1. Introduction

Choline is a nutrient that contributes to several metabolic processes, including lipid transport, cell signaling, cell membrane integrity, and biosynthesis of methylated compounds [1,2,3]. This vitamin is essential for the formation of endochondral bone, allowing the adequate proliferation of chondrocytes, bone elongation, and prevention of disorders in the paws, such as perosis [4]. Additionally, choline acts with methionine as a lipotropic factor, improving lipoprotein synthesis and lipid-cholesterol transport, thereby preventing fatty liver syndrome [5,6,7].

In poultry nutrition, the requirement for this nutrient is met through the dietary utilization of products rich in choline. Some of these products are derived from selected plants, which exhibit a high content of esterified choline, along with bioavailability. According to Gupta et al. [8], some advantages of using vegetable choline, as an alternative of use of choline chloride, include high absorption efficiency, low hygroscopicity, low conversion of choline to trimethylamine (TMA), and high phosphatidylinositol and phosphatidylcholine (PC) content.

In research with broilers, it has been estimated that a plant-based product containing 1.6% PC has a choline bioequivalence of 2,520 g/kg [9]. The present study hypothesized that another plant source with 3.2% PC could be utilized and would have at least twice the choline bioequivalence in broiler diets. Therefore, the objective was to evaluate the effects of dietary supplementation of two plant sources of PC on the performance and body composition characteristics of broiler chickens.

2. Material and methods

All procedures adopted in the current study were previously evaluated and approved by the ethics committee on the use of farm animals (Registration protocol: 07/2022), and were in compliance with the ethical principles of animal experimentation established by the Conselho Nacional de Controle de Experimentação Animal (CONCEA). Experiments were carried out in Viçosa, Minas Gerais State, Brazil (20°45’57.19” S, 42°51’35.42” W, and 682 m altitude).

2.1 Experimental design, diets, and animals

One-day-old male broiler chicks (Cobb 500) were used, with an initial weight of 46.1 ± 3.82 g. The birds were distributed in a completely randomized design with five treatments, twelve replications, and twenty birds per experimental unit. The experiment lasted 42 days. Three corn/soybean meal basal diets were formulated to meet the nutritional recommendations given by Rostagno et al. [10] according to the experimental phases (1 to 7, 8 to 21, and 22 to 42 days of age), except for choline levels (Table 1). For the formulation of the basal diets, a choline concentration of 440 mg/kg in corn and 2559 mg/kg in soybean meal was considered [11]. Therefore, the total level of choline in the basal diets was 550, 496, and 361 mg/kg of choline, respectively.

Table 1. Ingredients and nutritional composition of diets (NC)

Ingredientes (%)	1-7 days	8-21 days	22-42 days
Corn	48.112	50.468	59.943
Soybean meal	43.730	41.102	31.685
Soy oil	3.919	4.545	5.042
Dicalcium phosphate	1.898	1.679	1.323
Limestone	0.923	0.840	0.692
Salt	0.533	0.516	0.480
DL-Methionine	0.337	0.321	0.268
L-Lysine HCl	0.134	0.149	0.203
Vitamin Premix[b]	0.150	0.130	0.120
Mineral Premix[a]	0.140	0.120	0.100
Salinomycin (12%)	0.055	0.055	0.055
L-Threonine	0.055	0.055	0.053
L-Valine	0.004	0.009	0.026
Antioxidant (BHT)	0.010	0.010	0.010
Calculated values			
Crude Protein, %	24.00	23.00	19.50
Metabolizable Energy, Kcal/kg	2,975	3,050	3,200
Calcium, %	0.971	0.878	0.705
Available phosphorus, %	0.463	0.419	0.341
Digestible lysine, %	1.307	1.256	1.077
Digestible methionine, %	0.650	0.624	0.533
Digestible Met + Cis, %	0.967	0.929	0.797
Digestible arginine, %	1.524	1.45	1.189
Digestible isoleucine, %	0.942	0.898	0.743
Digestible Gli + Ser, %	1.940	1.854	1.549
Digestible histidine, %	0.574	0.551	0.471
Digestible treonine, %	0.863	0.829	0.711
Digestible tryptophan, %	0.278	0.265	0.217
Digestible valine, %	1.006	0.967	0.829
Digestible leucine, %	1.807	1.746	1.537
Choline, mg/kg	1,331	1,274	1,075

[a]Guarantee levels/kg of product - Mn: 58.36 g; Zn: 54.21 g; Fe: 41.68 g; Cu: 8.31g; I: 0.843g; If: 0.250 g. [b]Guarantee levels/kg of product - Vitamin A: 9,638,000 IU; Vitamin D3: 2,410,000 IU; Vitamin E: 36,100 IU; Vitamin B1: 2,590 mg; Vitamin B2: 6,450 mg; Vitamin B6: 3,610 mg; Vitamin B12: 15.9 mg; Vitamin K3: 1936 mg; Vitamin B5: 12.95 mg; Vitamin B3: 39.2 mg; Vitamin B9: 903.0 mg; Vitamin B7: 89.8 mg.

The plant sources of PC tested were Biocholine and Biocholine DS (minimum 1.6 and 3.2% PC, respectively, Nutriquest, Brazil). The experimental treatments consisted of Basal diets (BD)- diet without choline supplementation; BD + Biocholine (218, 197, and 143 mg/kg of diet, respectively); BD + Biocholine DS1 (146, 131, and 96 mg/kg of diets, respectively); BD + Biocholine DS2 (109, 98, and 72 mg/kg of diets, respectively); BD + Biocholine DS3 (87, 79, and 57 mg/kg of diets, respectively). The experimental treatments are illustrated in table 2. These levels of products supplementation considered the choline bioequivalence of 2,520 mg/kg for Biocholine and 3,780, 5,040, and 6,300 mg/kg of Biocholine DS1, Bicholine DS2 and Biocholine DS3, respectively, and the recommendation of supplementation given by [(10)] of 550, 496, and 361 mg choline/kg of diet, respectively. The addition of the products to the experimental rations was done in an *on-top* way.

Table 2. Description of experimental treatments

Phases	Sources of choline	Experimental treatments				
		BD	BD + Biocholine	BD + Biocholine DS1	BD + Biocholine DS2	BD + Biocholine DS3
1 a 7 days	Biocholine (mg/kg)	0	218	0	0	0
	Biocholine DS (mg/kg)	0	0	146	109	87
8 a 21 days	Biocholine (mg/kg)	0	197	0	0	0
	Biocholine DS (mg/kg)	0	0	131	98	79
22 a 42 days	Biocholine (mg/kg)	0	143	0	0	0
	Biocholine DS (mg/kg)	0	0	96	72	57

Biocholine DS1= level 1 supplementation with Biocholine DS; Biocholine DS2= level 2 supplementation with Biocholine DS; Biocholine DS3= level 3 supplementation with Biocholine DS.

During the research, a 24-hour light program was adopted from 1 to 14 days and then 18 hours of light until the end of the experiment. In addition, feed and water were provided *ad libitum* throughout the experimental period.

2.2 Evaluated parameters

2.2.1 Performance

The birds were weighed at 7, 21, and 42 days of age to evaluate performance parameters. Feed intake (FI), weight gain (WG), and feed conversion ratio (FCR) were measured. Mortalities were recorded daily throughout the study for corrections in performance parameters. The difference between the final weight and the initial weight determined WG. The FI was calculated as the difference between all the feed provided in the evaluated periods and the leftover feed in the feeders at the end of each period. Finally, the FCR was calculated as the ratio between FI and WG.

2.2.2 Carcass yield

At 42 days of age, two birds per experimental unit were slaughtered by electro narcosis to evaluate the carcass yield (CY, %), the yield of parts (%), and the relative weight of some organs. The CY was determined by the ratio of the carcass weight without viscera, head, feet, and neck to the live weight before slaughter; %CY = (Carcass weight x 100) / Live weight. The ratio between the weight of the evaluated part and the weight of the carcass without viscera, feet, head, and neck determined the yield of parts; % RPart = (Part weight x 100) / carcass weight, with breast yield (BY, %) and thigh plus drumstick yield (TDY, %) being evaluated. The relative weight of the organs was determined as the ratio between of the organ weight to the carcass weight, excluding viscera, feet, head, and neck. The formula used was: relative weight = (Organ weight x 100) / carcass weight. The relative liver weight (RLW) and abdominal fat (RWAF) were evaluated.

2.2.3 Body composition

Bird body composition was estimated by Dual Energy X-Ray Absorptiometry (DXA). At 42 days of age, a bird with the closest weight to the average weight of each experimental unit was chosen and slaughtered by cervical dislocation. The estimated values of fat tissue and lean tissue were used to determine the percentage of fat tissue and lean tissue according to the equations proposed by Schallier et el. [(12)]:

1: Total Body Fat Percent = -1.288 + 0.806 x Percent Fat (DXA).

2: Percentage of total lean tissue = 19.95 + 0.805 x Percent lean (DXA).

2.3 Statistical analyses

Data were analyzed via one-way ANOVA using the GLM procedure of SAS (Statistical Analysis System, version 9.4). In addition, a comparison between treatment averages was performed using Tukey’s test. A significance level of 0.05 was applied.

3. Results and discussion

There was no effect of the treatments on the FI and WG of the birds in any of the phases studied ($P > 0.05$; Table 3). However, there was an effect of treatments on FCR from 1 to 21 days of age, and the birds of the basal diet presented the worst FCR in relation to the other treatments ($P < 0.05$).

Table 3. Performance results observed in phases from 1 to 7, 1 to 21, and 1 to 42 days old.

Variables	BD	BD + Biocholine	BD + Biocholine DS1	BD + Biocholine DS2	BD + Biocholine DS3	SEM	P-valor
	One to seven days old						
FI (Kg/bird)	0.161	0.159	0.154	0.157	0.158	0.017	0.226
WG (Kg/bird)	0.146	0.149	0.145	0.148	0.147	0.016	0.554
FCR (Kg/bird)	1.100	1.07	1.06	1.06	1.07	0.04	0.153
One to twenty-one days old							
FI (Kg/bird)	1.305	1.288	1.283	1.292	1.279	0.033	0.499
WG (Kg/bird)	1.036	1.055	1.051	1.059	1.048	0.031	0.416
FCR (Kg/bird)	1.26 b	1.22 a	1.22 a	1.22 a	1.22 a	0.03	0.004
One to forty-two days old							
FI (Kg/bird)	5.173	5.156	5.125	5.191	5.253	0.018	0.247
WG (Kg/bird)	3.410	3.430	3.386	3.440	3.440	0.014	0.722
FCR (Kg/bird)	1.52	1.50	1.51	1.51	1.53	0.06	0.665

FI: Feed intake; WG: Weight gain; FCR: Feed conversion ratio; SEM: Standard Error Mean; Means followed by different letters on the same line differ from each other by Tukey's test at the 5% significance level; Biocholine DS1= level 1 supplementation with Biocholine DS; Biocholine DS2= level 2 supplementation with Biocholine DS; Biocholine DS3= level 3 supplementation with Biocholine DS.

There was no significant effect of treatments on carcass and parts yield and on the relative weights of abdominal fat and liver of birds at 42 days of age. Furthermore, no positive results were observed in body composition parameters with the supplementation of phosphatidylcholine sources (P > 0.05; Table 4).

Table 4. Carcass yield, parts, the relative weight of abdominal fat and liver, and body composition of chickens at 42 days of age.

Variables	BD	BD + Biocholine	BD + Biocholine DS1	BD + Biocholine DS2	BD + Biocholine DS3	SEM	P-valor
	Relative weight of organs at 42 days (%)						
RLW	1.64	1.69	1.68	1.62	1.66	0.02	0.657
RWAF	0.97	0.87	0.88	1.00	0.82	0.03	0.212
Yield of carcass and parts at 42 days (%)							
CY	77.76	78.15	77.04	77.83	77.74	0.18	0.427
BY	30.47	30.80	30.12	30.22	30.28	0.16	0.704
TDY	21.26	21.03	21.12	20.83	21.39	0.12	0.599
Fat and lean tissue at 42 days (%)							
Fat tissue	10.02	9.63	9.96	9.85	9.94	0.18	0.970
Lean tissue	89.16	89.55	89.22	89.33	89.24	0.18	0.971

RLW: Relative liver weight; RWAF: Relative weight of abdominal fat; CY: Carcass yield; BY: Breast yield; TDY: Thigh plus drumstick yield; SEM: Standard Error Mean; Biocholine DS1= level 1 supplementation with Biocholine DS; Biocholine DS2= level 2 supplementation with Biocholine DS; Biocholine DS3= level 3 supplementation with Biocholine DS.

The result found for the FCR in the phase from 1 to 21 days is in line with expectations since choline acts on lipid metabolism, promoting better use of dietary energy [13,14] and consequently leading to better performance results. On the other hand, no improvement was observed in FI and WG in any of the evaluated phases. According to Igwe et al. [1], young birds are more demanding on choline as it is an essential nutrient for metabolic functions necessary for growth.

Unlike the current research, Khose et al. [15] found worsening in all performance parameters when diets were deficient in choline; however, their diets contained smaller proportions of sulfur-containing amino acids, which could explain our result. Furthermore, Khosravinia et al. [13] found positive results for WG with no significant increase in FI. They reasoned that the lipotropic effect of choline may have improved dietary energy utilization and consequently promoted better WG.

Regarding carcass characteristics, it was expected that animals fed with BD would have higher percentages of body fat, since choline deficiency can house fat deposition in organs such as the liver and other parts of the carcass, since that it is an essential nutrient for lipid transport [13]. As with the results for weight gain, the explanation for the lack of changes in carcass composition can be attributed to the amount of sulfur amino acid present in the diet, however, further studies are needed to corroborate our findings.

Research on the performance of broilers supplemented with choline shows controversial results, whether the source is organic or synthetic. These different results can be explained by the link between the metabolism of choline and that of sulfur amino acids [9,5,16,17,18]. Supplementation does not promote any effect when animals are fed diets with a high content of sulfur amino acids since methionine can partially supply the choline deficiency, mainly in diets rich in corn and soybeans. Although the diets in the present study are deficient in choline, since it considers only the choline from corn and soybean meal, they are rich in methionine + cystine. This may explain the lack of positive results on WG and FI.

In research carried out by Lima et al. [11], it was estimated from quadratic regression equations that the choline level that optimizes broiler performance was 1276 and 1010 mg/kg for phases 1 to 7 and 1 to 21 days of age, respectively. As previously mentioned, corn and soybean meal diets are very rich in choline, and although they were formulated according to Rostagno et al. [10], deficient diet choline levels are above those recommended by Lima et al. [11]. These data help to explain the lack of results in the current research and corroborate the hypothesis that the choline requirement is outdated and requires further studies.

4. Conclusion

In conclusion, supplementing with vegetable sources of phosphatidylcholine improves the feed conversion of broiler chickens from 1 to 21 days of age. Based on performance results up to 42 days of age, the Biocholine DS product, with twice the concentration of phosphatidylcholine compared to the Biocholine product, can be used with the bioequivalence matrix of 5.040 mg/kg in diets based on corn and soybean meal for broilers.

Declaration of conflict of interest

The authors declare no conflicts of interest.

Author contributions

Conceptualization: A. A. Calderano and R. D. Bernardes. *Data curation*: R.D. Bernardes, A.A. Calderano, T. G. Petrolli and K.M. Gomes. *Formal analysis:* A. A. Calderano and S. O. Borges. *Investigation*: R. D. Bernardes, H. Pagnussatt, J. V. S. Miranda and L. P. Castro. *Writing (original draft):* R. D. Bernardes. *Writing (proof-reading & editing)*: A. A. Calderano and J.K. Valentin.

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