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PRODUÇÃO ANIMAL

REPLACEMENT OF CHOLINE CHLORIDE BY A VEGETAL SOURCE OF CHOLINE IN DIETS FOR BROILERS

SUBSTITUIÇÃO DO CLORETO DE COLINA POR UMA FONTE DE COLINA VEGETAL EM DIETAS PARA FRANGOS DE CORTE

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Abstract:
The objective of this study was to evaluate the use of a vegetal source of choline as a replacement of choline chloride in the diet for broilers from one to 21 and 22 to 42 days of age. In the first experiment, we used 1500 Cobb male broilers at one day of age, distributed in a completely randomized design with ten treatments, five repetitions and 30 birds per experimental unit. In the second experiment, we used 1000 Cobb male broiler at 22 days of age, distributed in a completely randomized design with ten treatments, five repetitions and 20 birds per experimental unit. The treatments consisted of five levels of choline chloride 60% (400, 500, 600, 700 and 800 mg kg^{-1}) and five levels of vegetal source of choline (100, 150, 200, 250 and 300 mg kg^{-1}). There was no difference between the performance parameters of broilers in the periods of one to 21 and 22 to 42 days of age. The use of up to 100 mg kg^{-1} of a vegetal source of choline can replace the use of choline chloride, in the studied levels, in corn-soybean meal diets for broilers from one to 21 and 22 to 42 days of age.
Keywords: nutritional requirement; performance; poultry; vitamin

Resumo:
O objetivo deste estudo foi avaliar o uso de uma fonte de colina vegetal em substituição ao cloreto de colina em dietas para frangos de corte de um a 21 e 22 a 42 dias de idade. No primeiro experimento foram usados 1500 frangos de corte machos Cobb com um dia de idade, distribuídos em um delineamento inteiramente ao acaso com dez tratamentos, cinco repetições e 30 aves por unidade experimental. No segundo experimento foram usados 1000 frangos de corte

machos Cobb com 22 dias de idade distribuídos em um delineamento inteiramente ao acaso com dez tratamentos, cinco repetições e 20 aves por unidade experimental. Os tratamentos consistiram em cinco níveis de cloreto de colina 60% (400, 500, 600, 700 e 800 mg/kg) e cinco da fonte de colina vegetal (100, 150, 200, 250 e 300 mg/kg). Não houve diferença entre as médias dos parâmetros produtivos nas duas fases experimentais. O uso de até 100 mg/kg de uma fonte vegetal de colina pode substituir o uso de cloreto de colina, nos níveis estudados, em dietas a base de milho e farelo de soja para frangos de um a 21 e 22 a 42 dias de idade.
Palavras-chave: Aves; desempenho; exigência nutricional; vitamina

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Introduction

Choline is an essential nutrient for poultry. In the metabolism, it participates in the acetylcholine formation, an important neurotransmitter. Choline is also part of structure of the phosphatidylcholine, being the predominant form present in the body. Phosphatidylcholine is an essential phospholipid and is made in all nucleated cells via the choline pathway. It is involved in the absorption and transport of fats to the liver and subsequent mobilization and transport of liver fat. In addition, it is a part of the composition of cellular membranes and organelles, such as mitochondria and microsomes. Choline also acts as a methyl group donor, after oxidized to betaine. Betaine can be used to convert homocysteine to methionine in the transmethylation pathway in the liver.

Methylation of phosphatidylethanolamine, the first step in endogenous choline biosynthesis, allows S-adenosyl-methionine to donate a methyl group and therefore spare choline in mammalian species(1). In contrast, avian species have limited capacity to carry out this initial biosynthetic step(2). Thus, especially young chicks have a high dietary requirement of choline(3). Emmert and Baker(4), using a choline-deficient basal diet, showed an almost linear response to incremental addition of choline chloride up to 1115 $mg.kg^{-1}$ of feed in chicks from 10 to 22 days of age.

Choline is usually added to animal diets in the choline chloride form. However, this source has some disadvantages such as high hygroscopicity, the acceleration of oxidative loss of vitamins in the diet, and the formation of trimethylamine in the gastrointestinal tract of the birds(5). Trimethylamine is a short-chain aliphatic amine that is formed from dietary choline in a reaction catalyzed by enzymes within gut bacteria(6). This metabolite is found in high concentrations in fish, and is responsible for the characteristic odor of seafood(7).

However, choline is also present in plants in the phosphatidylcholine form, free choline and sphingomyelin. Currently there are natural products, produced from selected plants, with high content of choline in esterified form and with high bioavailability, which may be an important alternative to the use of synthetic choline chloride. Many researches have shown that these products can replace choline chloride in diets for poultry(8-11).

The objective of this study was to evaluate the use of a vegetal source of choline to replace choline chloride in diet for broilers from one to 21 and from 22 to 42 days of age.

Material and Methods

The experiments were carried from November to December 2008 at Fazenda Experimental Antônio Carlos dos Santos Pessoa, located on Linha Guará in Marechal Candido Rondon - PR, belonging to the Universidade Federal do Oeste do Paraná (UNIOESTE).

In the first experiment, we used 1500 Cobb male broilers at one day of age distributed in a completely randomized design with ten treatments, five replications and 30 birds per experimental unit. In the second experiment, we used 1000 Cobb male broilers at 22 days of age distributed in a completely randomized design with ten treatments, five replications and 20 birds per experimental unit. For the formation of the experimental units, birds were weighed individually and grouped by weight range so that all the plots presented similar average weight.

The broilers were housed in a shed consisting of 50 experimental boxes with 1.30 x 1.35 m, totaling an area of 1,755 m^2. The management of drinkers, troughs, curtains and birds followed the recommendations of the strain manual, with water and diet supplied *ad libitum* throughout the experimental periods. The birds used in the second experiment were fed starter feed until 21 days of age according to the recommendations by Rostagno et al.[12].

The continuous lighting program (24 hours of natural light + artificial light) was adopted during all the period. The birds were warmed individually on each experimental unit, using infrared lamp (250 watts).

The broilers received the experimental diets (Table 1) in the period of 1 to 21 and 22 to 42 days of age. The diets were isonutrient and isocaloric in accordance with the requirements proposed by Rostagno et al. [12].

The inclusion of choline chloride 60% and the vegetal source of choline (Biocholine®) were performed during the whole experimental periods (1 to 21 and 22 to 42 days of age) replacing the inert material (Table 2).

The parameters evaluated at 21 and 42 days of age were final weight, weight gain, feed intake, feed conversion and viability. For statistical analyses, we adopted the SNK test at 5% probability for mean comparison. For the study we used the program SAEG (System for Statistical and Genetic Analyses).

Table 1: Experimental diets

	Experiment 1	Experiment 2
Ingredients	**1 - 21**	**22 - 42**
Corn (8.57% CP)	60.164	63.325
Soybean meal (44.0% CP)	27.312	23.550
Corn gluten (60.0% CP)	7.890	6.030
Soybean oil (%)	0.500	3.197
Limestone (%)	0.951	0.874
Dicalcium phosphate (%)	1.864	1.654
Salt (%)	0.503	0.471
L-Lysine HCl (78%)	0.327	0.361
DL-Methionine (99%)	0.185	0.199
L-Threonine (99%)	0.004	0.039
Antioxidant[1]	0.010	0.010
Anticoccidial[2]	0.050	0.050
Minerals[3]	0.050	0.050
Vitamins[4]	0.100	0.100
Inert[5]	0.090	0.090
Total	100.00	100.00
Metabolizable Energy (kcal/kg)	3,007.00	3,200.00
Crude Protein (%)	22.500	19.999
Calcium (%)	0.908	0.818
Available phosphorus (%)	0.454	0.408
Digestible Lysine (%)	1.150	1.071
Digestible Met + Cis (%)	0.863	0.803
DigestibleThreonine (%)	0.748	0.696
Digestible Tryptophan (%)	0.211	0.187
Digestible Valine (%)	0.949	0.838
Digestible Isoleucine (%)	0.870	0.762
Digestible Leucine (%)	2.270	1.989
Digestible Arginine (%)	1.240	1.096
Sodium (%)	0.218	0.205
Potassium (%)	0.679	0.615
Choline (mg/kg)	1,150.00	1,060.15

[1]BHT; [2]Coxistac - salinomycin; [3]Contents/kg – Fe, 100g; Cu, 16g; Mn, 150g; Zn, 100g; I, 1,5 g; [4]Contents/kg – Vit. A, 8,000,000 UI; Vit. D_3, 2,000,000 UI; Vit. E, 15,000 mg; Vit. B_1, 1.8 g; Vit. B_2, 6.0 g; Vit. B_6, 2.8 g; Vit. B_{12}, 12,000 mcg; Pantothenic acid, 15 g; Vit. K, 1.8 g; folic acid, 1.0 g; nicotinic acid, 40.0 g; Se, 0.3 g; [5]Washed sand.
CP: Crude Protein

Table 2: Experimental treatments

Treatments	
Treat. 1	400 mg of choline chloride 60% kg^{-1} of feed
Treat. 2	500 mg of choline chloride 60% kg^{-1} of feed
Treat. 3	600 mg of choline chloride 60% kg^{-1} of feed
Treat. 4	700 mg of choline chloride 60% kg^{-1} of feed
Treat. 5	800 mg of choline chloride 60% kg^{-1} of feed
Treat. 6	100 mg of vegetal source of choline kg^{-1} of feed
Treat. 7	150 mg of vegetal source of choline kg^{-1} of feed
Treat. 8	200 mg of vegetal source of choline kg^{-1} of feed
Treat. 9	250 mg of vegetal source of choline kg^{-1} of feed
Treat 10	300 mg of vegetal source of choline kg^{-1} of feed

Results and Discussion

At one to 21 days of age, there was no significant difference ($p>0.05$) of the performance parameters among the different levels of choline chloride and vegetal source of choline in diets (Table 3). Therefore, either the supplementation level of 400 mg of choline chloride or of 100 mg of vegetal source of choline were enough to keep broiler's performance. Waldroup & Fritts[13] studied the supplementation of corn-soybean meal based diets with 1000 $mg.kg^{-1}$ of choline from choline chloride and also did not observe influence on weight gain and feed conversion of broilers up to 35 days of age. However, literature data are controversial regarding the effect of choline chloride on birds' performance in general. Pompeu et al.[14] evaluated the supplementation levels of 100, 200, 300 and 400 mg of $choline.kg^{-1}$ of feed for broilers at 1 to 21 days of age and observed that feed conversion was influenced linearly, concluding that 400 mg of choline was the best level for this parameter.

In this experiment, as expected, broilers fed both vegetal source of choline and choline chloride presented similar performance. This result was similar to those findings by Chatterjee & Misra[8] and Muthukumarasamy el al.[9] that weight gain, feed intake, feed conversion and viability of broiler chickens were similar when replacing the choline chloride by a vegetal source of choline in the diets.

In the second experiment, there was no significant difference ($p>0.05$) of the performance parameters among the different levels of choline chloride and vegetal source of choline in diets (Table 4). Therefore, either the supplementation level of 400 mg of choline chloride or of 100 mg of vegetal source of choline were enough to keep broiler's performance. Contrary to this result for choline chloride levels, Emmert and Baker[4], using a choline-deficient basal diet, showed an almost linear response to incremental addition of choline chloride up to 1,115 $mg.kg^{-1}$ of feed for broilers from 10 to 22 days of age. Increasing choline chloride to 2000 $mg.kg^{-1}$ of feed resulted in further weight gain improvements, but to a lesser extent. This study is particularly interesting because the diet was treated to ensure inhibition of *de novo* choline synthesis.

Table 3: Performance of birds from 1 to 21 days of age

Treat.	FW^{ns}	WG^{ns}	FI^{ns}	FC^{ns}	Viability^{ns}
Choline chloride 60% (mg kg^{-1})					
400	780.2	737.9	1200.1	1.627	98.67
500	784.7	742.5	1235.2	1.665	99.33
600	776.0	733.8	1210.8	1.651	97.33
700	767.4	725.1	1198.7	1.654	96.00
800	737.1	694.8	1161.1	1.672	99.33
Vegetal source of choline (mg kg^{-1})					
100	793.0	750.7	1200.9	1.599	98.00
150	782.0	739.7	1201.2	1.624	97.33
200	786.3	743.7	1205.4	1.620	98.00
250	780.9	738.6	1205.4	1.619	99.33
300	782.3	740.0	1216.5	1.644	97.33
CV %	4.17	4.41	4.37	2.48	2.90

ns - not significant at 5% probability; FW = final weight; WG = weight gain; FI = feed intake; FC = feed conversion.

Table 4: Performance of the birds from 22 to 42 days of age

Treat.	FW^{ns}	WG^{ns}	FI^{ns}	FC^{ns}	Viability^{ns}
Choline chloride 60% (mg kg^{-1})					
400	2051.5	1361.5	2462.6	1.815	95.00
500	2042.2	1346.2	2484.8	1.851	94.00
600	2261.1	1566.1	2730.2	1.744	95.00
700	2072.9	1388.4	2475.3	1.787	93.00
800	2274.8	1585.4	2695.1	1.697	93.75
Vegetal source of choline (mg kg^{-1})					
100	2265.6	1574.3	2745.1	1.744	98.75
150	2338.6	1661.7	2862.7	1.726	95.00
200	2253.4	1551.9	2741.5	1.768	98.00
250	2296.5	1601.5	2797.6	1.753	95.00
300	2307.3	1613.3	2812.0	1.742	98.00
CV %	5.30	7.92	6.50	3.79	5.56

ns - not significant at 5% probability; FW = final weight; WG = weight gain; FI = feed intake; FC = feed conversion.

In the second experiment, as expected, broilers fed both vegetal source of choline and choline chloride presented similar performance. This result was similar to those verified by Kumar[15] that the performance of broiler chickens was similar when replacing the choline chloride by a vegetal source of choline in the diets. In another study, Yu[16] observed that the vegetal source of choline can replace the choline chloride in the diets besides producing an improvement in weight gain and feed conversion of birds. Devegowda et al.[17] also observed that broilers supplemented with vegetal source of choline in the feed showed a reduction of fat in the abdomen and liver.

An explanation for the variable effects of the choline levels in the literature can be the methionine level in the experimental diets. Castro et al.[18] observed significant interaction for egg production and egg mass of Japanese quail with linear effect in response to choline levels when the methionine level was 0.65%. However, when the methionine level was 0.75%, there was no response to choline levels. Similarly, Reis et al.[19] concluded that there is no need for adding choline to the diet of Japanese quails when these are fed diets containing methionine and cystine at more than 0,91%. This effect can be explained by the endogenous synthesis of choline in the liver by donating methyl groups by methionine.

The present study showed that, regardless of the level of choline chloride and vegetal source of choline used in the diets, the response in broiler performance was the same. This confirms previous observations that the vegetal source of choline, produced from selected plants with high content of choline in esterified form and high bioavailability, may be an important alternative for synthetic choline chloride in corn-soybean meal diets for broilers from one to 21 and 22 to 42 days of age.

Conclusions

The use of up to 100 $mg.kg^{-1}$ of a vegetal source of choline can replace the use of choline chloride in corn-soybean meal diets for broilers from one to 21 and 22 to 42 days of age.

References

1. Kroening GH, Pond WG. Methionine, choline and threonine interrelationships for growth and lipotropic action in the baby pig and rat. Journal Animal Science. 1967;26:352–357. http://dx.doi.org/10.2134/jas1967.262352x

2. Baker DH, Sugahara M. Nutritional investigation of the metabolism of glycine and its precursors by chicks fed a crystalline amino acid diet. Poultry Science. 1970;49:756–60. http://dx.doi.org/10.3382/ps.0490756

3. Molitoris BA, Baker DH. Choline utilization in the chick as influenced by levels of dietary protein and methionine. Journal Nutrition. 1976;106:412–418. Disponível em: http://jn.nutrition.org/content/106/3/412.full.pdf. Acesso em 15 janeiro 2015.

4. Emmert JL, Baker DH. A Chick bioassay approach for determining the bioavailable choline concentration in normal and overheated soybean meal, canola meal and peanut meal. Journal Nutrition. 1997;127:745–752. Disponível em: http://jn.nutrition.org/content/127/5/745.full. Acesso em 15 janeiro 2015.

5. Zeisel SH, Dacosta KA, Youssef M, Hensey S. Conversion of dietary choline to trimethylamine and dimethylamine in rats: dose-response relationship. Journal Nutrition. 1989;119:800-804. Disponível em: http://jn.nutrition.org/content/119/5/800.full.pdf. Acesso em: 15 janeiro 2015.

6. Zeisel SH, Wishnok JS, Blusztajn JK. Formation of methylamines from ingested choline and lecithin. Journal of Pharmacology and Experimental Therapeutics. 1983;225:320-324.Disponível em: http://jpet.aspetjournals.org/content/225/2/320.long. ACesso em 25 janeiro 2015.

7. Zeisel SH, Dacosta KA. Increase in human exposure to methylamine precursors of N-nitrosamines after eating fish. Cancer Research. 1986;46:6136-6138. Disponível em: http://cancerres.aacrjournals.org/content/46/12_Part_1/6136.full.pdf. Acesso em 15 janeiro 2015.

8. Chatterjee S, Misra SK. Efficacy of herbal biocholine in controlling fatty liver syndrome in commercial broilers on high metabolic energy diet. Fytomedica. 2004;5:37-39. Disponível em: http://eurekamag.com/research/004/140/004140101.php. Acesso em 25 janeiro 2015.

9. Muthukumarasamy B, Sahu BK, Swain RK, Samantaray DP. Studies on the effect of biocholine supplementation in commercial broilers. Indian Journal of Poultry Science. 2004;39(3):246-251. Disponível em: http://www.indianjournals.com/ijor.aspx?target=ijor:ijps&volume=39&issue=3&article=008. Acesso em 15 janeiro 2015.

10. Chen YJ, Young KB, Chang SH, Tsai TP, Chen CC. Effect of biocholine as a replacement of synthetic choline supplement on the egg laying performance in laying hen. Fhytomedica. 2007;8:75-81.

11. Gangane GR, Gaikwad NZ, Ravikanth K, Maini S. The Comparative effects of synthetic choline and herbal choline on hepatic lipid metabolism in broilers. Veterinary World. 2010;3(7):318-320.

12. Rostagno HS, Albino LFT, Donzele JL, Gomes PC, Oliveira RF, Lopes DC, et al. Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais. Viçosa, MG: Universidade Federal de Viçosa, 2005. Português.

13. Waldroup PW, Fritts CA. Evaluation of separate and combined effects of choline and betaine in diets for male broilerus. International Journal of Poultry Science. 2005;4(7):442-448.

14. Pompeu MA, Lara LJC, Baião, NC, Ecco R, Cançado SV, Rocha JSR, et al.. Suplementação de colina em dietas para frangos de corte machos na fase inicial de criação. Arquivo Brasileiro de Medicina Veterinária e Zootecnia. 2011;63(6):1446-1452. Português.

15. Kumar A. Comparative efficacy of herbal biocholine and synthetic choline chloride (60%) in commercial broilers. Poultry Technology. 2009;3:38-40.

16. Yu C. Effect of biocholine as a replacement of synthetic choline chloride on the growth and performance of commercial broilers. Livestock International. 2009; 1:12-13.

17. Devegowda G, Chethan PS, Umakantha P, Shashidara RJ. The biological evaluation of BioCholine® as a substitute to choline chloride on performance of commercial broilers. Livestock International. 2011;1:12-14.

18. Castro SF, Fortes BDA, Carvalho JCC, Bertechini AG, Queiroz LFB, Garcia Jr AAP . Relação metionina e colina dietética sobre o desempenho de codornas japonesas (*Coturnix coturnix japonica*) em postura. Ciência Animal Brasileira. 2011;12(4):635-641. Português. http://dx.doi.org/10.5216/cab.v12i4.9976

19. Reis SR, Barreto SLB, Paula E, Muniz JCL, Viana GSV, Mencalha R, et al. Níveis de suplememtação de colina na dieta de codornas japonesas em postura. Revista Brasileira de Agropecuária Sustentável. 2012;2(1):118-123. Português.