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Vegetable choline as a replacement for choline chloride in broiler feed

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ABSTRACT. The objective of this study was to determine choline chloride replacement effects by a vegetable choline source, compost by *Trachyspermum amni*, *Citrullus colocynthis*, *Achyranthus aspera*, and *Azadirachta indica* in broiler feed. These compounds are fonts of phosphatidylcholine, a high-disponible molecule for intestinal absorption and choline supply. A total of 640 animals were randomly allocated in a completely randomized design, with four treatments and eight repetitions (n = 20), and zootechnical performance (body weight, weight gain, feed conversion ratio, and productive efficiency index), carcass yield, cuts yield, and organs (heart, liver, proventriculus, gizzard, and small intestine) relative weights were evaluated. Were evaluated two choline chloride levels (600 and 800 mg kg^{-1}) and two vegetable choline levels (100 and 200 mg kg^{-1}), added in a corn-soybean meal basal diet, during 42 days of raising. Results revealed better feed conversion ratio ($p < 0.001$) and production efficiency index ($p < 0.001$) in broilers fed vegetable choline, with no differences on body weight ($p = 0.372$) and weight gain ($p = 0.427$) among broilers. Carcass, cuts yield, and organ relative weights do not alter ($p > 0.05$) due to different group of supplementations. Findings in this trial concludes vegetable choline can adequately replace choline chloride in broiler feed, with improvement on performance and no compromising carcass, cuts or organ development.

Keywords: phosphatidylcholine; poultry nutrition; trimethylamine; vitamin.

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Introduction

Choline is a B-complex vitamin that optimizes energy metabolism, controls fatty liver syndrome and reduces body fat. It is indispensable for efficient nutrient assimilation, as it helps form phospholipids and lecithins, participating in hepatic fat metabolism (Huang, Yang, & Wang, 2007; Zhang, Haitao, Zhao, Guo, & Barri, 2011). It is present in all body tissues, where it constitutes a cell structure element, compounding phospholipids membrane (Zeisel, Mar, Howe, & Holden, 2003). Also, choline is a metil-group (CH_3) donor (Pompeu et al., 2011) for hepatic xenobiotic detoxification metabolism, is fundamental for the transmission of nerve impulses, as it is essential for acetylcholine formation (Rodelas, Magpantay, & Luis, 2011), an important neurotransmitter.

Choline deficiency in birds causes fatty liver syndrome and perosis and disturbances in liver fat mobilization due to the low availability of carrier lipoproteins (Pompeu et al., 2011; Selvan, Saravanakumar, Suresh, Chandrasekeran, & D'Souza, 2018). In fast-growing broiler strains, choline deficiency also results in growth retardation and bone deformation (Igwe, Okonkwo, Uzoukwu, & Onyenegecha, 2015). Affected birds tend to suffer tibial-tarsal rotation, tendon dislocation (gastrocnemius) and more often leg joint thickening; in adult birds, problems such as ascites and cirrhosis can also occur (Selvan et al., 2018).

To avoid the deleterious effects of choline deficiency in diets, high-disponible supplementation fonts is required. Normally, this is done with synthetic sources, usually choline chloride-based molecules, as it is the most widely available form on the market. Nevertheless, approximately 70% of choline chloride is not absorbed in the gut, and instead, intestinal bacteria convert it to trimethylamine (TMA) (Hoyles et al., 2018), a toxic compound (Fallah, Ebrahimnezhad, Maheri-Sis, & Ghasemi-Sadabadi, 2016; Landfald, Valeur, Berstad, & Raa, 2017). TMA is absorbed in small intestine and, through the bloodstream, reaches the body tissues, causing deleterious effects (Zeisel et al., 2003; Craciun & Balskus, 2012). Another occurring problem with choline chloride usage is its highly hygroscopic characteristic, and its presence in vitamin premixes and rations accelerates the destruction of other vitamins present in feed.

There are currently some herbal compounds that contain significant amounts of choline in their composition, being alternatives to choline chloride in broiler feed choline supplementation. They have great high-disponible amounts of phosphatidylcholine, phosphatidylinositol and phosphatidylethanolamine (Calderano, Nunes, Rodrigueiro, & César, 2015; Farina et al., 2017), which due to its high gut-receptors affinity, provides higher choline bioavailability than choline chloride. These vegetable compounds are less hygroscopic, and does not damage the integrity of other molecules present in nutrients. Finally, the molecule is not converted to trimethylamine in gut, and in fact, generates no toxic compounds in digestive tract. The most promissor plants with these proprierties are *Trachyspermum amni*, *Citrullus colocynthis*, *Achyranthus áspera*, and *Azadirachta indica.*

Based on these properties, the objective of this study was to determine the effect of substitution of choline chloride with vegetable choline in broiler feed, on zootechnical performance, carcass, cuts, and organ yields.

Material and methods

This study was conducted in a poultry sector at UNOESC Xanxerê, with protocol submitted and approved by the animal ethics committee (CEUA/UNOESC). All procedures were performed strictly in accordance with the guidelines of recommendations in the Brazilian national council of animal experimentation (Concea), and experimental protocol was approved under number 034/2015.

Animals and experimental design

The present study was carried out at UNOESC Xanxerê poultry facilities, being used 640 male Cobb broilers, 1 to 42 days old, randomly allocated in four treatments (Table 1) with eight repetitions composed of 20 birds each. The animals were housed in 2 m^2 experimental pens, with wood shaving bed. Throughout the trial period, feed and water were provided ad libitum. Rations (corn-soybean meal based) were formulated according to Rostagno et al. (2011) recommendations (Table 2). The phytogenic used (Biocholine, Nutriquest Technofeed, Campinas, São Paulo State, Brazil) was composed by a low-hygroscopicity blend of plant extracts based on *Trachyspermum amni*, *Citrullus colocynthis*, *Achyranthus aspera*, and *Azadirachta indica* (Biocholine™), which are sources of phosphatidylcholine.

Table 1. Treatments used in the trial.

Treatment	Supplement source
T1	600 g ton^{-1} Choline Chloride
T2	800 g ton^{-1} Choline Chloride
T3	100 g ton^{-1} of Choline Herbal Extract
T4	200 g ton^{-1} of Choline Herbal Extract

Performance, carcass and organ evaluation

The birds and feed leftovers were weighed at 7, 21, and 42 days of age to determine zootechnical performance (weight gain, feed intake, feed conversion ratio, and productive efficiency index). Additionally, productive efficiency index (PEI) was calculated, with the following formulae:

$$PEI = \frac{Body\ weight\ (kg)\ x\ viability (\%)}{Feed\ conversion\ ratio\ x\ age\ at\ slaughter\ (d)}\ \mathrm{x}\ 100$$

where: PEI = Production Efficiency Index.

At 42 days of age, to evaluate the carcass and cut yields (breast, wing, thigh, drumstick and back), and organs (heart, liver, proventriculus, gizzard, and small intestine), one bird per unit was slaughtered, following animal welfare and euthanasia norms described by the national research council euthanasia practice guidelines (Brasil, 2013). To calculate the parameters described above, were used the following equations:

$$Carcass\ yield\ (\%)\ \frac{Carcass\ weight}{Body\ weight} x\ 100$$

$$\text{Relative cut weight (\%)} = \frac{Cut\ weight}{Body\ weight}\ x\ 100$$

$$\text{Relative organ weight (\%)} = \frac{\text{Organ weight}}{\text{Body weight}} \, x \, 100$$

Performance data, carcass, cuts, and organ parameters were subjected to analysis of variance, and on presence of significant differences, the means were subjected to the SNK test at 5% significance, using R statistical software.

Table 2. Experimental diets compositions.

Ingredient (kg ton^{-1})	Initial (1 – 21 days)	Final (22 – 41 days)
Corn	544.00	578.66
Soybean meal (46%)	361.65	309.00
Soybean oil	27.79	44.89
Dicalcium phosphate	18.30	18.64
Limestone	8.25	8.41
Salt	3.25	3.32
DL-Methionine (99%)	2.60	3.11
L-Lysine HCl	2.25	1.94
Additives inclusion[1]	1.00	1.00
Supplemental vitamins[2]	15.00	15.0
Supplemental minerals[3]	15.00	15.0
Calculated values		
Metabolic energy (kcal kg^{-1})	2950.00	3100.00
Crude protein (g kg^{-1})	215.00	194.00
Lysine dig. (g kg^{-1})	12.00	10.50
Methionine dig. (g kg^{-1})	5.44	5.05
Met. + Cys. dig. (g kg^{-1})	8.39	7.75
Threonine dig. (g kg^{-1})	7.55	6.84
Tryptophan dig. (g kg^{-1})	2.46	2.13
Arginine dig. (g kg^{-1})	14.14	12.27
Valine dig. (g kg^{-1})	9.25	8.20
Calcium (g kg^{-1})	9.02	8.24
Available phosphate (g kg^{-1})	4.51	4.10
Sodium, g kg^{-1}	1.70	2.05
Potassium, g kg^{-1}	8.49	7.46
Chloride, g kg^{-1}	3.77	3.56

[1]Premix of choline fonts inclusion for each groups tested: 600 mg kg^{-1} CC – 600 g of CC + 400 g of caulin; 800 mg kg^{-1} CC – 800 g of CC + 200 g of caulin; 100 mg kg^{-1} VC – 100 g of V + 900 g of caulin; 200 mg kg^{-1} VC – 200 g of VC + 800 g of caulin; [2]Supplemental vitamins containing, per kg of product: Vit. A – 10,000,000 IU; Vit. D3 – 2,000,000 IU; Vit. E – 30,000 IU; Vit. B1 – 2.0 g; Vit. B2 – 6.0 g; Vit. B6 – 4.0 g; Vit. B12 – 0.015 g; Pantothenic acid - 12.0 g; Biotin - 0.1 g; Vit. K3 - 3.0 g; Folic acid - 1.0 g; Nicotinamide acid - 50.0 g; Selenium - 250.0 mg; and Excipient q.s.p – 1,000 g; [3]Suplemental mineral content per kg of product: Iron - 100.0 g; Cobalt - 2.0 g; Copper – 20.0 g; Manganese - 160.0 g; Zinc - 100.0 g; Iodine – 2.0 g; and Excipient q.s.p – 1,000 g.

Results and discussion

Zootechnical performance

There were no differences in bird body weight ($p = 0.182$), weight gain ($p = 0.182$), feed intake ($p = 0.219$), and feed conversion ratio ($p = 0.549$) in 1-7-day period among broilers from the various groups tested (Table 3). In 1 - 21 day, no alterations were observed in body weight ($p = 0.184$) and weight gain ($p = 0.187$), however, higher feed intake ($p = 0.017$) and feed conversion ratio ($p = 0.045$) were observed in birds fed 800 mg kg^{-1} of choline chloride.

In 1 - 42 days, broilers fed 800 mg kg^{-1} of chorine chloride (CC) had higher feed intake ($p = 0.001$) and feed:gain ratio ($p < 0.001$). Also, birds fed with 100 mg kg^{-1} of vegetable choline (VC) demonstrated lower feed conversion ratio ($p < 0.001$) than broilers fed with 200 mg kg^{-1} of VC and 800 mg kg^{-1} of CC. No differences were observed on body weight ($p = 0.372$) and weight gain ($p = 0.427$) between animals fed all different treatments. Better production efficiency index (PEI) was found in broilers fed with 100 mg kg^{-1} of VC and 600 m kg^{-1} of CC ($p < 0.001$), and 800 mg kg^{-1} of CC has the worst PEI.

Choline is a well-recognized nutrient that prevents fatty liver, perosis and growth retardation in poultry (Selvan et al., 2018), and young chicks have limited ability to perform the first methylation of phosphatidylethanolamine because S-adenosyl-methionine is inefficient as a methyl donor in choline biosynthesis (Jukes, Oleson, & Dornbrush, 1945), not being able to synthetize choline in *de novo* synthesis. So, choline supplementation is extremely important in this situation. Vegetable choline can be adequately used to replace choline chloride in the feed of broilers, ensuring zootechnical performance of the birds. Also, due to its lower inclusion in the diets, it allows the opening of space for the inclusion of other additives in the formulations, without impacting the proportions of the macro ingredients of the feed. The feed conversion ratio of chickens supplemented with CC 800 mg kg^{-1} is worse, due to the high formation of trimethylamine in the intestinal lumen, causing subclinical cell damage in tissues. In the present study, feed conversion and productive efficiency index of the vegetable choline supplemented groups gave results similar to birds supplemented with choline chloride 600 mg kg^{-1}, however, these indices were higher than those of the choline chloride 800 mg kg^{-1} group. The data found agree with those of Calderano et al. (2015) and Farina et al. (2017), who found similar results on performance of broilers when evaluating the substitution of choline chloride with vegetable choline.

Table 3. Performance of broilers supplemented with various sources and choline fonts levels.

		1 – 7 days			
	Body Weight (g)	Weight gain (g)	Feed Intake (g)	Feed Conversion ratio	
600 g ton^{-1} CC	187.34	133.53	150.03	1.14	
800 g ton^{-1} CC	200.45	146.93	168.33	1.15	
100 g ton^{-1} VC	188.63	134.32	151.54	1.12	
200 g ton^{-1} VC	192.15	138.17	155.17	1.12	
P-value	0.182	0.182	0.219	0.549	
CV (%)	5.06	7.05	9.14	9.82	
SE	13.21	12.23	18.63	0.04	
		1 – 21 days			
	Body Weight (g)	Weight gain (g)	Feed Intake (g)	Feed Conversion ratio	
600 g ton^{-1} CC	769.52	714.46	1013.33b	1.42b	
800 g ton^{-1} CC	836.22	781.77	1190.23a	1.52a	
100 g ton^{-1} VC	790.01	736.21	1028.31b	1.40b	
200 g ton^{-1} VC	813.09	759.23	1073.31b	1.41b	
P-value	0.184	0.187	0.017	0.045	
CV (%)	5.97	6.41	7.85	5.08	
SE	15.12	12.43	25.76	0.08	
		1 – 42 days			
	Body Weight (g)	Weight gain (g)	Feed Intake (g)	Feed Conversion ratio	Productive Efficiency Index
600 g ton^{-1} CC	2635.23	2580.21	4009.32b	1.55bc	404.31ab
800 g ton^{-1} CC	2671.64	2616.77	4407.31a	1.68a	377.11c
100 g ton^{-1} VC	2670.15	2615.12	3984.44b	1.52c	417.00a
200 g ton^{-1} VC	2636.44	2581.11	4092,81b	1.58b	395.03b
P-value	0.372	0.427	0.001	<0.001	<0.001
CV (%)	2.79	2.84	3.62	5.08	2.71
SE	33.00	32.12	44.22	0.12	12.21

Means followed by different letters in the column differ statistically by the SNK test (0.05). CC – Choline chloride, VC – Vegetable choline.

An assumption regarding the absence of significant effect on weight gain evaluation in the first week of age may also be based on yolk choline vitelline reserves, due the good reserves it has. Farina et al. (2017) related presence of approximately 6,800 mg kg^{-1} of choline in yolk, being sufficient to meet and supply the necessary choline reserves. Broilers supplemented with 800 mg kg^{-1} CC displayed worse feed conversion and feed intake, nevertheless, feed intake was not different from those of other treatments. This finding suggests supplementation with the vegetable choline improves zootechnical indicators. It can be explained because, during chicken development, there is a greater demand for choline supplementation for production of lean muscles, and were found chickens supplemented with 100 mg kg^{-1} VC obtained the lower feed conversion ratio compared to those of other groups tested.

El-Maaty, Hayam, Rabie, and El-Khateeb (2014) described herbal molecules are known to improve performance of broilers, and the results found in the present research were also consistent with the studies mentioned above, like Khosravinia, Chethen, Umakantha, and Nourmohammadi (2015), who found the performance efficiency index was higher in birds supplemented with vegetable choline than in those fed control feed. Many active compounds act by increasing bile flow, thereby improving feed intake, and consequently, increasing weight gain without change in feed intake in the supplemented group suggests that lipotropic agents (especially vegetable choline and choline chloride) may improve energy use in the feed.

The results of PEI show differences in the performance of the flocks when we change the levels or sources of choline in the diets. This index assesses the general performance of broilers, covering the parameters of weight gain, feed intake, feed conversion ratio, age at slaughter and flock viability in a single index. The lower value found in chickens supplemented with 800 mg kg^{-1} of CC helps to highlight the deleterious effects of trimethylamine on the performance of birds.

Carcass yield, commercial cuts yield and organ relative weight

Carcass and cuts yield data are displayed in Table 4, having no influence of different fonts and levels of choline supplementation on carcass (p = 0.079), breast (p = 0.059), thigh (p = 0.108), drumstick (p = 0.093), wing (p = 0.386), and back (p = 0.217). The results obtained for organ relative weight showed no variation on heart (p = 0.138), liver (p = 0.241), proventriculus (p = 0.388), gizzard (p = 0.271), and small intestine (p = 0.173) relative weights (Table 5).

Table 4. Carcass yield and relative weights of breast, thigh, drumstick, wing and back of broiler chickens supplemented with various sources and levels of choline at 42 days of age.

Treatments	Carcass (%)	Breast (%)	Thigh (%)	Drumstick (%)	Wing (%)	Back (%)
600 g ton^{-1} CC	76.47	24.23	11.01	9.32	8.26	17.84
800 g ton^{-1} CC	76.52	23.94	10.18	9.52	8.14	18.74
100 g ton^{-1} VC	73.09	23.05	11.13	9.26	7.78	19.93
200 g ton^{-1} VC	76.16	23.11	10.67	8.86	8.32	18.74
P-value	0.079	0.059	0.108	0.093	0.386	0.217
CV (%)	2.98	6.69	5.75	12.43	8.09	7.92
SE	0.56	0.34	0.24	0.15	0.14	0.34

Means followed by different letters in the column differ statistically by the SNK test (0.05). CC – Choline chloride. VC – Phosphatidylcholine, CV – Coefficient of variarion, SE – Standard Error.

Farina et al. (2017) also explain one phosphatidylcholine unit (the molecule present in vegetable choline) is equivalent to 2.52 choline chloride units, demonstrating phosphatidylcholine makes diets less expensive than those containing choline chloride. Demattê Filho, Pereira, and Possamai (2015) evaluated alternative sources of choline in the organic production of broiler chickens, and did not observe significant differences in carcass yield, suggesting choline chloride can be replaced by organic sources. Khosravinia et al. (2015) evaluated productive performance of broilers supplemented with various levels of choline chloride and vegetable choline, and they did not found differences in carcass yield percentages in moderate or high energy diets.

Table 5. Relative weights of heart, liver, proventriculus, gizzard and small intestine of broilers supplemented with various sources and choline levels at 42 days.

Treatments	Heart (%)	Liver (%)	Proventriculus (%)	Gizzard (%)	Small Intestine (%)
600 g ton^{-1} CC	0.48	2.18	0.35	1.97	4.95
800 g ton^{-1} CC	0.54	2.20	0.41	2.13	5.01
100 g ton^{-1} VC	0.55	2.34	0.34	2.22	5.04
200 g ton^{-1} VC	0.45	2.17	0.40	2.17	5.29
P-value	0.138	0.241	0.388	0.271	0.173
CV (%)	14.72	11.04	20.87	13.60	8.79
SE	0.01	0.05	0.02	0.06	0.09

Means followed by different letters in the column differ statistically by the SNK test (0.05). CC – Choline chloride, VC – Phosphatidylcholine, CV – Coefficient of variarion, SE – Standard Error.

Choline is a well-known lipotropic factor responsible for the mobilisation of liver fat in the form of lipoproteins toward extra-hepatic tissues where they may be metabolised or deposited (Selvan et al., 2018). As vegetable choline in feed promotes more choline gut absorption efficiency, lower VC levels than CC levels could provide optimal nutrient requirement meeting and improvement on liver function. As a result, the excess fat energy gets diverted towards muscle protein accretion rather than body fat synthesis, resulting in better growth and improved feed conversion ratio.

The absence of alterations observed for heart, proventriculus, gizzard, and small intestine yields were expected, as these organs are not directly influenced by choline levels. Usually, these organs increase or decrease in size according to physiological requirements. The liver is the most affected organ by choline levels, due to responsible for lecithin synthesis, which participates in absorption and transport of liver fat, and our data suggest vegetable choline adequately meets hepatic requirements for choline. Additionally, Kumar, Das, Rao, and Chatterjee (2009) has described no toxic effects of vegetable choline use, confirming biological safety in its use.

Conclusion

The addition of 100 and 200 g ton^{-1} of Vegetable choline, as a source of choline, adequately meets the requirements for this vitamin and can be used as a substitute for choline chloride, improving performance and without impairing carcass yields, carcass cuts or organs in broiler chickens.

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