

Effect of inclusion of biocholine in the diet of laying hens on performance parameters, egg quality, and serum biochemistry

Romário Duarte Bernardes^{1*} , Heloísa Pagnussatt¹ , Samuel Oliveira Borges¹ , Rayanne Andrade Nunes¹ , Tiago Goulart Petrolli² , Larissa Pereira Castro¹ , Beatriz Garcia do Vale¹ , Arele Arlindo Calderano¹ 

¹ Universidade Federal de Viçosa, Departamento de Zootecnia, Viçosa, MG, Brasil.

² Universidade do Oeste de Santa Catarina, Departamento de Zootecnia, Xanxerê, SC, Brasil.

*Corresponding author:
duarteromario040@gmail.com

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ABSTRACT - The objective of this study was to evaluate the effect of different sources of choline in the diet of laying Lohmann Brown hens (26 to 37 weeks of age) on productive performance, egg quality, and blood parameters. A completely randomized experimental design was adopted, consisting of five treatments, with eight replications and six birds per experimental unit. The treatments consisted of a basal diet without supplementation of any source of choline (NC); NC + supplementation with 939 mg/kg choline chloride 60%; NC + supplementation with 167 mg/kg Biocholine[®]; NC + supplementation with 83 mg/kg Biocholine DS[®]; and NC + supplementation with 300 mg/kg Biocholine DS[®]. The performance parameters evaluated included laying percentage, average feed intake, feed conversion ratio by mass and by dozen eggs, average weight gain, and egg mass. The egg quality parameters comprised the average weights of the egg, yolk, shell, and albumen, as well as the Haugh unit, yolk index, shell weight per surface area, yolk color, and average percentages of the constituent parts. Additionally, blood parameters were analyzed, including glucose, cholesterol, high-density lipoprotein, triglycerides, uric acid, glutamic oxaloacetic transaminase, creatine phosphokinase, and gamma-glutamyltransferase. No statistically significant differences were observed in any of the evaluated parameters, except for glucose levels, which were higher in treatment with 300 mg/kg Biocholine DS[®]. For hens fed diets based on corn and soybean meal, no effect on the performance and egg quality was observed due to supplementation with choline chloride 60% or herbal products that are sources of choline.

Keywords: cholesterol, egg laying, laying hen, triglyceride

1. Introduction

Choline is a B-complex vitamin relevant to poultry nutrition, as it performs three essential functions for the animal. First, it is a component of phospholipids, which have a critical role in the integrity and function of cellular membranes (Gupta et al., 2019). Second, it is crucial to hepatic lipid metabolism, preventing abnormal fat accumulation in the liver (Pour et al., 2014; Lin et al., 2021). Finally, it is a precursor of acetylcholine, a very important neurotransmitter for controlling functions such as heart rate and muscle contractions (Pour et al., 2014; Farina et al., 2017).

The nutritional requirement for choline in birds is achieved by adding choline-rich products to their diets. In general, choline chloride is used; however, there are natural alternatives from selected plants, which have a high content of esterified choline, in addition to high bioavailability. These alternatives can also meet this nutritional requirement (Gangane et al., 2010; Calderano et al., 2015; Petrolli et al., 2020).

Although the choline requirement for birds decreases with age due to the increased capacity of these animals to synthesize it (Saeed et al., 2017; Janist et al., 2019), in laying hens, egg quality and health parameters must be considered, as well as performance parameters. These animals remain in the production system for a long time and commonly experience health problems, such as fatty liver syndrome (Saeed et al., 2017; Navarro-Villa et al., 2019; Zaki et al., 2023). Due to the participation of choline in hepatic lipid metabolism (Saeed et al., 2017; Dong et al., 2019; Olgum et al., 2022), choline supplementation can prevent this situation and maintain good productivity.

Given these considerations, the significance of choline in laying hen nutrition becomes evident. Furthermore, it is essential to investigate the impact of plant-based choline as a substitute for choline chloride. This alternative may provide additional advantages, such as low hygroscopicity, a characteristic that facilitates the handling of choline in feed mills during the diet formulation process (Gupta et al., 2019). Our study may corroborate the findings of other researchers who conducted similar investigations (Chen et al., 2007; Dazuk et al., 2021; Bernardes et al., 2024), thereby contributing to the greater reliability of the data available on the subject.

The hypothesis for the present research is that the inclusion of different sources of choline in the diets of laying Lohmann Brown hens will have a beneficial impact on their productive performance and egg quality. The objective was to evaluate the productive performance, egg quality, and blood parameters of brown laying hens fed diets containing different sources of choline.

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 (case no. 06/2023), and were in accordance 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).

Two hundred and forty Lohmann Brown laying hens, aged 26 to 37 weeks, were used. At 26 weeks, they were weighed and distributed in a completely randomized design consisting of five treatments, eight replications, and six birds per experimental unit. The distribution of birds in their respective experimental units was conducted while considering their individual weights and laying ratios.

The birds were housed in 34 × 47 cm metal cages equipped with trough-type feeders and nipple drinkers. Each experimental unit was composed of three cages housing two birds each.

The experimental treatments were: basal diet without supplementation of any source of choline (NC); NC + supplementation with 939 mg/kg choline chloride 60%; NC + supplementation with 167 mg/kg Biocholine® (Nutriquest, Brazil); NC + supplementation with 83 mg/kg Biocholine DS® (Nutriquest, Brazil); and NC + supplementation with 300 mg/kg Biocholine DS®. The herbal products used were composed of a low-hygroscopicity blend of plant extracts based on *Trachyspermum ammi*, *Citrullus colocynthis*, *Achyranthes aspera*, and *Azadirachta indica*, which are sources of phosphatidylcholine. The products were added to the basal diet in "on-top" form.

The feeding program was divided into two phases: from 26 to 33 weeks (Phase 1) and 34 to 37 weeks (Phase 2). The basal diets (Table 1) were formulated to meet the nutritional recommendations of Rostagno et al. (2017).

During the experimental period, the birds received water and feed *ad libitum*, and a 16-h photoperiod light program was adopted. The experiment lasted 84 days and was subdivided into three data collection periods of 28 days each.

Table 1 - Ingredients and nutritional composition of the experimental diets

Item	Phase 1	Phase 2
Ingredient (%)		
Corn	65.980	67.970
Soybean meal	21.010	19.780
Soy oil	1.230	0.450
Limestone	9.250	9.270
Dicalcium phosphate	1.290	1.290
Common salt	0.420	0.420
DL-Methionine	0.320	0.300
L-Lysine HCL	0.120	0.130
Mineral Premix ¹	0.110	0.110
Vitamin Premix ²	0.100	0.100
L-Valine	0.090	0.090
L-Threonine	0.070	0.070
BHT	0.010	0.010
Calculated values		
Crude protein (%)	15.140	14.740
Metabolizable energy (kcal/kg)	2830	2800
Calcium (%)	3.889	3.893
Available phosphorus (%)	0.318	0.318
Digestible lysine (%)	0.756	0.736
Digestible Met + Cis (%)	0.741	0.721
Digestible arginine (%)	0.878	0.846
Digestible Gly + Ser (%)	1.176	0.141
Digestible threonine (%)	0.582	0.567
Digestible tryptophan (%)	0.159	0.154
Digestible valine (%)	0.703	0.684
Choline (mg/kg)	827.787	805.224

¹ Guarantee levels/kg of product: Mn, 58.36 g; Zn, 54.21 g; Fe, 41.68 g; Cu, 8.31 g; I, 0.843 g; Se, 0.250 g.

² Guarantee levels/kg of product: vitamin A, 9,638,000 IU; vitamin D3, 2,410,000 IU; vitamin E, 36,100 IU; vitamin B1, 2.59 mg; vitamin B2, 6.45 mg; vitamin B6, 3.61 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.

2.1. Performance

To evaluate the performance of the birds, the parameters measured were laying percentage (%), average feed intake (FI, g/bird/day), feed conversion per dozen eggs (kg/dozen), feed conversion per egg mass (kg/kg), average weight gain (kg), and egg mass production (g/bird/day). Bird mortality was quantified and used to correct the performance data.

To determine the laying percentage (LP), daily egg collections were carried out, and at the end of the experiment, the total eggs produced by each experimental unit were divided by the product of the total experimental days and the number of birds, expressed by the formula $LP = (\text{Eggs produced} / (\text{Experimental days} \times \text{Number of birds})) \times 100$. The rations provided to the animals were quantified by weighing them at the beginning and end of the experimental period, and FI was calculated as the difference between these two weights, according to the equation $FI = (\text{Initial weight} - \text{Final weight}) / \text{Number of birds}$. The egg mass (EM) was measured using the equation $EM = (LP \times \text{Average egg weight}) \times 100$, and these data were used to determine the feed conversion to egg mass ($FCEM = FI/EM$). Additionally, throughout the experimental period, eggs were counted to calculate feed conversion per dozen eggs. Finally, the birds were weighed at the beginning and the end of the experiment to calculate the average weight gain ($AWG = \text{Final weight} - \text{Initial weight}$).

The equations used to calculate the variables studied are described by Sakomura and Rostagno (2016).

2.2. Egg quality

In the egg quality analysis, the following parameters were measured: average egg weight (g), average yolk weight (g), average shell weight (g), average albumen weight (g), Haugh unit (HU), yolk index, shell weight per surface area (SWSA), yolk color, average yolk percentage (%), average albumen percentage (%), average shell percentage (%), and shell thickness (mm).

During the last three days of each 28-day cycle, all eggs were collected and subjected to quality analyses. All intact eggs were weighed on a precision scale, broken onto a special glass surface, and subjected to albumen and yolk quality assessments. In addition to the weight of the whole eggs, the individual weights of the shell and yolk were measured. The albumen weight, in turn, was determined through the difference between the weight of the whole egg and the sum of the weights of the shell and the yolk, expressed mathematically as: Albumen weight = Egg weight – (Yolk weight + Shell weight). The shell weight was measured after washing to remove excess albumen and subsequently drying it in air for 24 h.

Haugh unit was calculated following the methodology described by Card and Nesheim (1968), which defines it as $HU = 100 \log (H + 7.57 - 1.7W^{0.37})$, in which H is the albumen height (in mL), and W is the egg weight (in g). The albumen height was measured using a digital micrometer. The yolk index (YI) was obtained from the relationship between the yolk height and the yolk diameter, according to the formula: $YI = \text{Yolk height (mm)} / \text{Yolk diameter (mm)}$. The height of the yolk was measured using a digital micrometer, and the diameter of the yolk was measured using a digital pachymeter.

The SWSA followed the methodology described by Abdallah et al. (1993), who defined it as $SWSA = \{SW / [3.9782 \times (EW^{0.7056})]\} \times 1000$, in which SW = shell weight and EW = egg weight. Yolk color was evaluated using the DSM® color range. The egg yolks were initially placed on a white plate in a well-lit environment. Subsequently, the color fan was positioned over these yolks, and the color that best approximated the fan was selected as the representative color value. The average percentages of yolk, albumen, and shell were calculated relative to the average egg weight ($\%Part = (Part \text{ weight} / \text{Egg weight}) \times 100$). Finally, shell thickness was measured with a digital pachymeter and was measured at three points, and the average was calculated.

2.3. Serum parameters

The serum parameters evaluated were glucose (mg/dL), total cholesterol (mg/dL), high-density lipoprotein (HDL; mg/dL), triglycerides (mg/dL), uric acid (mg/dL), glutamic oxaloacetic transaminase (U/L), creatine phosphokinase (U/L), and gamma-glutamyltransferase (GGT; U/L).

At the end of the experimental period, one bird per experimental unit was selected for blood collection, which was performed on the wing. The blood obtained was placed in a CAT vacuum tube with a coagulation activator and immediately sent for analysis at the ViçosaLab laboratory in Viçosa, Minas Gerais, Brazil.

2.4. Statistical analysis

For each variable, analysis of variance was performed according to the following general model:

$$Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$$

in which Y_{ij} is the measured dependent variable, μ is the overall mean, α_i is the effect of treatments, and ε_{ij} is the random error.

Analysis of variance was performed using the ExpDes.pt package of R statistical software (R software, v.4.0.4). The means were later compared to each other using Tukey's test. The means were considered statistically distinct when the significance value (P) was less than 0.05.

3. Results

No statistically significant difference ($P>0.05$; Table 2) were observed among the treatments evaluated for the performance parameters LP, FI, FCDE, FCEM, AWG and EM.

No statistically significant difference ($P>0.05$; Table 3) were observed among the treatments evaluated for the parameters of egg quality: average egg weight, average yolk weight, average albumen weight, average shell weight, SWSA, average yolk percentage, average percentage of albumen, shell thickness, and average shell percentage.

No statistically significant difference ($P>0.05$; Table 4) were observed among the treatments evaluated for the blood parameters total cholesterol, HDL, triglycerides, uric acid, OGT, creatine phosphokinase (CPK), and GGT. However, there was a statistically significant difference ($P<0.05$) for serum glucose levels.

Table 2 - Performance parameters observed from 26 to 37 weeks

Variable	Treatment					SEM	P-value
	T1	T2	T3	T4	T5		
Laying percentage (%)	98.280	97.383	96.826	97.738	98.636	0.133	0.186
Feed intake (g/bird/day)	109.152	108.073	107.644	109.928	109.127	0.299	0.611
FCDE (kg/dozen)	1.350	1.349	1.383	1.375	1.344	0.015	0.409
FCEM (kg/kg)	1.878	1.865	1.872	1.904	1.884	0.010	0.864
Average weight gain (kg)	0.137	0.117	0.111	0.138	0.124	0.011	0.714
Egg mass (g/bird/day)	58.159	58.042	57.525	58.087	58.163	0.144	0.925

T1 - basal diet without supplementation of any source of choline (NC); T2 - NC + supplementation with 939 mg/kg choline chloride 60%; T3 - NC + supplementation with 167 mg/kg Biocholine® (Nutriquest, Brazil); T4 - NC + supplementation with 83 mg/kg Biocholine DS® (Nutriquest, Brazil); T5 - NC + supplementation with 300 mg/kg Biocholine DS®.

FCDE - feed conversion per dozen eggs; FCEM - feed conversion per egg mass; SEM - standard error of the means (n = 8 per treatment).

Table 3 - Observed egg quality parameters

Variable	Treatment					SEM	P-value
	T1	T2	T3	T4	T5		
Average egg weight (g)	59.181	59.595	59.418	59.155	58.768	0.067	0.807
Average yolk weight (g)	14.720	14.665	14.607	14.873	14.716	0.014	0.658
Average albumen weight (g)	38.636	39.039	38.908	38.416	38.256	0.090	0.658
Average shell weight (g)	5.826	5.891	5.903	5.865	5.796	0.028	0.828
Haugh unit	96.946	96.193	97.101	95.361	97.028	0.487	0.245
Yolk index	0.479	0.479	0.479	0.478	0.472	0.001	0.433
Shell weight per surface area	82.270	82.785	83.125	82.836	82.223	0.201	0.908
Range of colors	5.439	5.486	5.516	5.429	5.450	0.036	0.717
Average yolk percentage (%)	24.870	24.618	24.588	25.156	25.052	0.052	0.343
Average albumen percentage (%)	65.284	65.495	65.476	64.930	65.089	0.079	0.475
Shell thickness (mm)	0.596	0.594	0.607	0.600	0.602	0.001	0.290
Average shell percentage (%)	9.845	9.887	9.936	9.914	9.859	0.028	0.952

T1 - basal diet without supplementation of any source of choline (NC); T2 - NC + supplementation with 939 mg/kg choline chloride 60%; T3 - NC + supplementation with 167 mg/kg Biocholine® (Nutriquest, Brazil); T4 - NC + supplementation with 83 mg/kg Biocholine DS® (Nutriquest, Brazil); T5 - NC + supplementation with 300 mg/kg Biocholine DS®.

SEM - standard error of the means (n = 8 per treatment).

Table 4 - Observed blood parameters

Variable	Treatment					SEM	P-value
	T1	T2	T3	T4	T5		
Glucose (mg/dL)	177.375b	190.375b	196.875ab	199.250ab	215.375a	2.572	<0.001
Total cholesterol (mg/dL)	111.125	122.375	141.375	144.250	109.250	5.702	0.238
HDL (mg/dL)	4.375	5.000	5.500	8.375	4.500	0.447	0.198
Triglycerides (mg/dL)	1364.250	1500.875	1643.125	1606.250	1212.625	123.990	0.422
Uric acid (mg/dL)	3.015	2.296	3.221	3.390	3.851	0.018	0.073
OGT (U/L)	171.000	178.625	193.000	186.875	188.625	1.118	0.199
CPK (U/L)	1966.875	2021.250	2369.500	2046.375	1541.250	131.593	0.125
GGT (U/L)	25.750	26.875	26.625	22.375	22.875	0.894	0.241

T1 - basal diet without supplementation of any source of choline (NC); T2 - NC + supplementation with 939 mg/kg choline chloride 60%; T3 - NC + supplementation with 167 mg/kg Biocholine® (Nutriquest, Brazil); T4 - NC + supplementation with 83 mg/kg Biocholine DS® (Nutriquest, Brazil); T5 - NC + supplementation with 300 mg/kg Biocholine DS®.

HDL - high-density lipoprotein; OGT - oxaloacetic glutamic transaminase; CPK - creatine phosphokinase; GGT - gamma-glutamyltransferase; SEM - standard error of the means (n = 8 per treatment).

a-b - Means followed by different letters in the same row differ from each other by Tukey's test at a 5% significance level (P<0.005).

4. Discussion

Due to the functions of choline in the metabolism of laying hens, it was expected that the treatment without the inclusion of choline sources would present worse performance results when compared with the others. However, this hypothesis was not confirmed. No negative effects were observed on the performance of birds that were not supplemented with choline; consequently, there was no improvement in their performance when supplemented with the different choline sources. The results observed in this study can be explained by the ability of birds to synthesize enough endogenous choline to meet their requirements from eight weeks of age (Janist et al., 2019). Furthermore, choline metabolism interacts with the metabolism of sulfur amino acids, which can partially compensate for dietary choline deficiency (Santana et al., 2014; Farina et al., 2017; Gül et al., 2023). The amino acid methionine is capable of synthesizing choline and, consequently, partially supplying the dietary deficiency of this nutrient. These two factors explain the absence of significant results in the evaluated performance parameters.

Previous research evaluating the effect of dietary choline supplementation on the performance of laying hens has demonstrated divergent results. Some studies have indicated that dietary choline supplementation does not improve bird performance parameters (Janist et al., 2019; Zhai et al., 2013), in agreement with the findings of the present study. In contrast, Moghadam et al. (2021) suggested that choline enhances the performance of laying hens. However, it is important to note that the diet used by these researchers contained flaxseed, aiming to nutritionally enrich the eggs; however, flaxseed is known to reduce the performance of birds due to the presence of antinutritional factors. The findings of Moghadam et al. (2021), together with the results of our research, indicate that choline supplementation may be necessary in diets aimed at specific objectives for birds. For diets based on cereals rich in sulfur amino acids, such as corn and soy, supplementation with dietary choline may become unnecessary.

Another relevant aspect to be highlighted refers to the environmental conditions in which the birds used in the present study were kept. They were raised in an environment characterized by low stress, both thermal and sanitary. Evidence from the literature indicates that laying hens raised under favorable environmental and sanitary conditions present superior performance, associated with greater efficiency in the use of nutrients available in the diet (Al-Tamimi et al., 2019). In this sense, it is important to conduct additional investigations on the supplementation of choline sources in scenarios characterized by more severe sanitary and thermal challenges to validate the practical applicability of the results obtained in the present study.

Sulfur amino acids, especially methionine, are capable of synthesizing endogenous choline because they have some similar metabolic functions (Santana et al., 2014; Farina et al., 2017). However, in severe environmental conditions, these amino acids may be required in greater quantities for other metabolic functions, and are not capable of supplying the choline deficiency.

Considering the role of choline as a precursor in the formation of hepatic lipoproteins (Pour et al., 2014; Lin et al., 2021), it is assumed that choline supplementation in laying hen diets may positively influence egg quality parameters, such as weight and average yolk percentage. This is due to the function of lipoproteins in the transport of lipids to the egg yolk. However, egg quality was not affected by choline supplementation.

The study conducted by Dong et al. (2019) demonstrated that increasing choline levels in diets does not influence egg yolk weight; however, an increase in the concentration of total lipids and phosphatidylcholine in the yolk was observed in response to this increase. Research conducted by Yonke and Cherian (2019) on choline supplementation in diets containing microalgae intended for laying hens yielded results differing from ours. It was observed that choline could improve the albumen height, HU, and feed conversion per dozen eggs. The divergence between these results can be attributed to the concentration of sulfur amino acids methionine + cystine (MET+CYS) in the diets. Our formulations have a higher concentration of these compounds than the diets used by Yonke and Cherian (2019). This finding corroborates the premise that diets based on corn and soybean meal, which are rich in sulfur amino acids, may not require supplementation with additional sources of choline.

As previously mentioned, choline plays a fundamental role in the synthesis of very low-density lipoproteins (VLDL) in the liver, which are responsible for transporting lipids, cholesterol, and triglycerides to peripheral tissues and for egg formation (Dong et al., 2019). Choline deficiency can impair the efficiency of this transport mechanism, leading to an accumulation of cholesterol and triglycerides in the bloodstream. However, our results regarding the analyzed serum parameters indicated that the absence of choline supplementation in the diet did not negatively affect the evaluated animals, supporting the lack of statistically significant differences for most of the parameters studied. The only exception was observed in serum glucose levels, which were higher in the group subjected to treatment with 300 mg/kg Biocholine DS®. As reported in the investigation by Dong et al. (2019), increased dietary choline intake did not promote changes in serum triglyceride and cholesterol levels, corroborating our findings.

5. Conclusions

No effect was observed on the performance and egg quality of Lohmann Brown hens fed diets based on corn and soybean meal with supplementation with choline chloride 60% or herbal products that are alternative sources of choline.

Data availability

The data that support the results of this study are available from the corresponding author upon reasonable request.

Author contributions

Conceptualization: Calderano, A. A. **Data curation:** Bernardes, R. D. **Formal analysis:** Bernardes, R. D. **Funding acquisition:** Calderano, A. A. **Investigation:** Bernardes, R. D. and Pagnussatt, H. **Methodology:** Petrolli, T. G. and Calderano, A. A. **Project administration:** Calderano, A. A. **Resources:** Calderano, A. A. **Software:** Borges, S. O. **Supervision:** Calderano, A. A. **Validation:** Bernardes, R. D. **Visualization:** Bernardes, R. D. **Writing – original draft:** Bernardes, R. D. **Writing – review & editing:** Borges, S. O.; Nunes, R. A.; Castro, L. P. and Vale, B. G.

Conflict of interest

The authors declare no conflict of interest.

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