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Effect of Encapsulated Choline Chloride on Performance, Carcass Characteristics and Some Blood Parameters in Broiler Chicks

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Abstract
This study examined the effects of various levels of dietary encapsulated choline chloride (ECC) on growth performance, carcass characteristics, serum and liver lipids content in Ross 308 broilers. A total of 480 day-old broilers were assigned to eight dietary treatments with six replicates of 10 birds each from 1 to 21 days of age. Eight experimental diets were provided from a basal non-added choline chloride diet as follow: six diets were made by the addition of ECC at the rate of 300, 600, 900, 1200, 1500 and 1800 mg/kg and a negative and a positive control diet with zero and 1500 mg/kg commercial choline chloride (CCC), respectively. The body weight gain, feed intake, choline chloride (CC) intake, live body weight, as well as carcass, breast muscle, thigh muscle, and liver weights were linearly ($P < 0.05$) related to the dietary levels of ECC. Dietary supplementation of ECC at the levels of 1200 and 1500 mg/kg decreased ($P = 0.014$) liver total lipid percentage, compared to those fed the negative control diet. The birds fed diets containing 900 and 1200 mg ECC/kg had lower liver triglyceride concentration than those fed negative control diet ($P = 0.001$). Furthermore, supplemented ECC at the levels of 1200 and 1800 mg/kg, led to an increase ($P = 0.006$) in the concentration of phospholipid in the liver. The ECC requirements were estimated at 1335.1, 1371.6 and 1353.3 mg/kg of diet for maximizing body weight gain, carcass weight, and breast weight, respectively ($P < 0.001$). It is concluded that the ECC may be used as an alternative to CC in the diet at the rate of 1500 mg/kg with no adverse effect on productive performance, breast and thigh muscle weight, and liver fat content in broilers.

Introduction

Choline is a vital dietary nutrient for the building and maintenance of cell membranes. It helps for normal maturation of the cartilage matrix of bone as well as the prevention of perosis in poultry (Jukes, 1940). Choline prevents abnormal lipid accumulation in the liver of broilers (Hassan *et al.*, 2005).

The typical form of choline or commercial choline chloride (CCC) is widely used in poultry diet formulations. Among the disadvantages of the CCC, the formation of trimethylamine in the gastrointestinal tract of broilers is more concerning (Zeisel *et al.*, 1989). Also, CCC may decrease vitamin B and C dispersion in the mixing proses by the increase of free water amount of feed ingredients (Pesti *et al.*, 1980).

There are several main reasons to use encapsulation technology in animal feed such as: improving the survivability and stability of materials against environment conditions (light, oxygen, and humidity), avoiding caking properties and easier to mix with other materials (Vilstrup, 2001). There are various methods employed in choline encapsulation among them encapsulation of CC with proteins and fats provides protection in simulated gastrointestinal conditions, and therefore, it is a good way of delivering viable CC to the intestine (Messman *et al.*, 1996).

Choline requirements in broilers are dependent on protein, sulfur amino acid contents of the diets and the age of the chickens (Pesti *et al.*, 1980). The NRC (1994) recommendation established choline

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recommended level at 1300 and 1000 mg/kg of the diet of broilers for 0-21 and 22-42 days of age. However, Aviagen Ross 308 recommendation (2014) determined 1700, 1600 and 1500 mg choline/kg of diet for broilers during 0-10, 11-24 and 25-42 days of age.

So far, there is no document on the effect of encapsulated choline chloride (ECC) in poultry. Therefore, the purpose of this study was to investigate the effects of ECC as an alternative to commercial choline chloride (CCC) on performance, organ characteristics, liver lipids content, blood parameters and choline requirement in broiler chickens during 1 to 21 days of age.

Materials and Methods

Birds and management

All procedures of this study were approved by the Ferdowsi University of Mashhad Animal Care and Use Committee (Protocol No. 29818). Four hundred eighty 1-day-old broilers chicks (Ross × Ross 308, 1:1 mixed-sex, male: female), were obtained from a commercial hatchery (Fariman hatchery Inc., Mashhad, Iran), weighed and randomly assigned to eight dietary treatments with six replicates of 10 birds each. Each floor pen of 0.8 × 0.8 × 1.0 m (L × W × H) was covered with litter and equipped with a hanging feeder and two nipple drinkers. Feed and water were provided on *ad-libitum* basis, and experimental diets were prepared in mash form. The temperature was initially set at 32°C on day 1 and reduced by 3.5°C/week to reach a constant temperature of about 21°C on day 21. The lighting program consisted of 24 L:0 D from 1 to 7 days, and 23 L:1 D until 21 days.

The basal negative control diet was formulated according to the Aviagen Ross 308 recommendation (Aviagen, 2014) based on corn and isolated soybean protein (Table1) except for methionine that was 10% lower than Aviagen recommendation. Isolated soybean protein was used to formulate a basal diet with choline deficiency, which it's analyzed choline content was 44.8 mg/kg. Eight experimental diets were prepared with the addition of 6 graded concentrations of powder ECC (300, 600, 900, 1200, 1500 and 1800 mg/kg) and negative (N-Con) and positive (P-Con) control. The negative control diet did not have CC supplementation and positive control diet supplemented by 1500 mg CC/kg as a powder format (75% CCC, Nutrex Nv, Lille, Belgium)

Table 1. Ingredients and calculated composition of basal diet.[1]

Item	Amount
Ingredient (%, as-fed basis)	
Corn grain (7.8% crude protein)	64.0
Isolated soybean protein (79.8% crude protein)	19.3
Corn starch	10.0
Soybean oil	1.75
Limestone	1.50
Dicalcium phosphate	2.00
Sodium chloride	0.40
Vitamin premix[2]	0.25
Mineral premix[3]	0.25
DL-Methionine	0.10
L-Lysine HCL	0.15
Sand	0.30
Total	100
Calculated composition	
Metabolizable energy, kcal/kg	3238
Crude protein, %	23.0
Calcium, %	0.92
Available phosphorus, %	0.49
Digestible lysine, %	1.30
Digestible methionine, %	0.42
Digestible methionine + cystine, %	0.73
Digestible threonine, %	0.87

[1]Eight hundred kg of basal diet was provided and divided into eight equal parts; one parts kept as a negative control, 1500 mg commercial choline chloride (Nutrex Nv, Lille, Belgium)/kg was mixed with another part as a positive control diet and six other parts were mixed with encapsulated choline chloride at the rate of 300, 600, 900, 1200, 1500 and 1800 mg/kg, respectively.

[2] Provides per kg of diet: vitamin A (retinol), 8,800 IU; vitamin D_3 (cholecalciferol), 3,300 IU; vitamin E (DL-α-tocopheryl acetate), 18.5 IU; vitamin K_3 (menadione), 2.2 mg; vitamin B_1 (thiamin), 2.2 mg; vitamin B_2 (riboflavin), 5.5 mg; vitamin B_3 (niacin), 28.0 mg; vitamin B_5 (pantothenic acid), 6.6 mg; vitamin B_6 (pyridoxine), 3.5 mg; vitamin B_9 (folic acid), 0.7 mg; vitamin B_{12} (cyanocobalamin), 0.02 mg; vitamin H_2 (biotin), 0.05 mg; antioxidant 1.0 mg.

[3] Provides (mg/kg of diet): Mn (manganese sulfate) 88.0, Fe (iron sulfate) 55.0, Zn (zinc sulfate) 88.0, Cu (copper sulfate) 5.5, Se (sodium selenite) 0.3.

The CCC was encapsulated (powder form) in the Research Institute of Food Science and Technology (Mashhad, Iran) and was approved by the Intellectual Property Center of Iran (Patent No. 90158). The calculated and analyzed CC in the eight diets are shown in Table 2. Choline contents of the experimental diets were measured by high-performance liquid chromatography (HPLC; Camag Co., Muttenz, Switzerland) according to the method of Tao *et al*. (1984).

Table 2. Amount of choline chloride experimental diets.

Diets [1]	Choline chloride supplemental (mg/kg)	Choline chloride concentration (mg/kg)
N-Con	0	24
ECC_{300}	300	323
ECC_{600}	600	634
ECC_{900}	900	930
ECC_{1200}	1200	1233
ECC_{1500}	1500	1520
ECC_{1800}	1800	1810
P-Con	1500	1290

[1] N-Con, negative control diet; ECC, encapsulated choline chloride; P-Con, positive control diet with 1500 mg commercial choline chloride (75% choline chloride, Nutrex Nv, Lille, Belgium)/kg.

Measurements

Birds and feed were weighed on days 1 and 21 for determination of the body weight gain (BWG), feed intake (FI), choline chloride intake (CCI), choline chloride intake per unit of body weight gain (CCI: BWG). Mortality was recorded daily and feed conversion ratio (FCR) corrected by mortality. The FCR was expressed as FI divided by BWG.

At the end of the experiment (d 21), 2 male chickens from each replicate of treatments (total 12/treatment) were randomly selected, and their blood samples were taken from the brachial vein. The blood samples were centrifuged at 2,000 × *g* for 5 min at 4°C, and sera were collected and frozen at -20°C until use. Serum triglyceride and phospholipid concentrations were assessed using an automatic biochemical analyzer (A15; BioSystems, Barcelona, Spain) with commercial assay kits according to the manufacturer's instructions (Pars Azmoon Co. kits, Tehran, Iran), and were reported as milligram per deciliter (mg/dL).

Following blood sample collection, birds were sacrificed by cervical dislocation and then carcass and internal organs including breast muscle, thigh muscles, liver, and heart were removed and weighed. Organ weights were expressed as a percentage of live body weight. The liver samples were frozen at -20°C for further analysis. Total lipid and triglyceride contents of liver were measured according to Folch *et al*. (1957) method. The determination of total liver phospholipid contents by a molybdenum blue colorimetry method was conducted according to Rizki *et al*. (2006) method. The concentrations of total liver triglyceride and phospholipids were displayed as milligrams per gram of wet weight of liver and the total lipid of the liver was expressed as a percentage of liver weight.

Statistical analysis

First, the data were tested for normality by UNIVARIATE plot normal procedure and analyzed by the PROC GLM of SAS (Version 9.4; SAS Institute Inc., Cary, NC, USA). Duncan's test was performed for comparison of the means (Duncan, 1955). The differences between means were considered significant when $P < 0.05$. Linear and quadratic effects were determined for the relationship between increasing concentrations of CC in the diets and response parameters through PROC REG of SAS (SAS Institute, 2003). Preplanned orthogonal contrast analysis was conducted to compare the positive control diet (1500 mg/kg CC) with each of the dose-response diet (1500 mg/kg ECC). The linear broken-line method was used to estimate the ECC requirement by using the NLIN procedure of SAS when a significant break-point was observed (Robbins *et al*., 2006).

Results and Discussion

Growth performance

It was thought that animals can synthesize enough choline from other nutrients to meet their requirement for this important substance (Zeisel and Blusztajn, 1994). It is reported that CC supplementation in the feeds is crucial for the improvement of body functions and the growth of broilers (Emmert and Baker, 1997).

The effects of dietary treatments on the performance of broilers during 1-21 days of age are presented in Table 3. The BWG, FI, CCI, and CCI per BWG were affected ($P < 0.05$) by different dietary levels of CC and progressive additions of ECC linearly altered ($P < 0.05$) these parameters. The linear responses of FI, CCI and CCI per BWG to ECC supplementation revealed that the highest responses achieved with 1800 mg ECC/kg was supplied ($P < 0.05$). Dietary supplementation of ECC at the levels of 1500 and 1800 mg/kg and also, 1500 mg CC/kg increased BWG, compared to birds fed the un-supplemented CC diet ($P < 0.05$). The results were in line with the reports of Emmert and Baker (1997) and Dilger *et al*. (2007), who found a linear response ($P < 0.05$) graded increase supplementation of CC in the chicks fed the choline-deficient basal diet, indicating the positive role of supplemental choline on BWG. Igwe *et al*. (2015) conducted a study on mix-sex broilers over 8 weeks and their results demonstrated that the highest BWG and FI were observed in birds receiving 2000 mg CC/kg diet ($P < 0.05$). However, Calderano *et al*. (2015) reported that there were not any significant differences between the sources or levels of CC supplementation on the performance of broilers. This variation in the effects of supplemental choline on performance is due to the different levels of supplemental choline as well as the content of choline in the basal diet.

Broilers fed a diet containing 1500 mg/kg ECC had higher ($P < 0.001$) CCI and CCI per BWG when compared with those fed the positive control diet (1500 mg CC/kg) from 1 to 21 days of age. The reason might be due to an improvement in FI capacity and BWG in the new generation of broilers, thereby total CCI and CCI per unit of BWG may have increased in this study. Menten *et al*. (1997) reported that adding 1000 mg/kg of CC to the basal diet containing 1140 mg of choline caused a significant increase in FI.

The treatment which had no choline supplementation (negative control) in our study had numerically the highest FCR which shows poor utilization of the feed but it was not significant; this is also in agreement with other reports of researchers who did not find any effect of different supplementation levels or sources of choline on FCR and mortality (Hassan *et al*. 2005; Waldroup and Fritts, 2005). Similar to our results, Farina *et al*. (2017) reported that supplementation of choline at the level of 600 mg/kg in the corn and soybean isolated protein diet containing 304 mg choline/kg for broiler chickens could improve BWG but did not affect the FCR during 1-21 days of age. Similar results were obtained by Molitoris and Baker (1976). In another study, Swain and Johri (2000) used a diet containing 1300 mg CC/kg and did not find any response in FCR at 0-42-d of age. The variations in response to an addition of CC in the broiler diets may be due to differences in the amount of sulfur amino acid such as methionine in the diet (Farina *et al*., 2017).

Carcass characteristics

The effects of dietary treatments on carcass part yields of broilers at 21 days of age are presented in Table 4. The weight of heart as well as yields of the carcass, breast muscle, thigh muscle, liver, and heart were not affected ($P > 0.05$) by different dietary levels of CC levels. Similarly, Khosravinia *et al*. (2015) and Selvam *et al*. (2018) reported an improvement in BWG and FCR of broilers by dietary supplementation of CC and lecithin extract in a period of 42 days. The live body weight, as well as weights of the carcass, breast muscle, thigh muscle, and liver, were affected ($P < 0.05$) by different dietary CC levels and progressive additions of ECC linearly altered ($P < 0.05$) live body weight and breast muscle weight. Furthermore, an increase in supplemental ECC quadratically altered ($P < 0.05$) carcass weight of broilers at 21 days of age. Dietary supplementation of ECC at the level of 1500 mg/kg decreased ($P = 0.043$) liver weight, compared to birds fed un-supplemented CC diet and increased ($P = 0.048$) live body weight compared to those fed ECC at the level of 300 mg/kg. The birds fed 1500 mg ECC or CC had the greater carcass, breast and thigh weights in comparison to the birds fed 300 mg ECC ($P < 0.05$); indicating that the ECC or CC might have positively altered the muscle development. Similarly, Waldroup and Fritts (2005) observed improvement in the breast weight at 42-day broilers with 1000 mg/kg of CC supplementation on a diet containing 1193 mg/kg of CC, without changing the weight gain and carcass yield.

Choline deficiency causes fatty liver syndrome in poultry (Selvam *et al*., 2018). In this study, birds fed a diet with choline-deficient in a negative control diet had a larger liver, due to increased accumulation of triglycerides in the liver. The chickens used in this study showed a markedly lower liver weight by supplemental ECC, particularly at the level of 1500 mg/kg of diet; thus, the use of ECC in this work was therapeutic. Harms and Russell (2002) reported that CC supplementation in diets of broilers decreased liver weight.

Liver and serum lipids

Broilers fed diets containing graded concentrations of ECC had higher ($P < 0.05$) total lipid and triglyceride concentrations in the liver as well as, decreased ($P < 0.05$) phospholipid concentration at 21 days of age (Table 5). Dietary supplementation of ECC at the levels of 1200 and 1500 mg/kg decreased ($P = 0.014$) liver total lipid concentration, compared to birds fed un-supplemented CC. The birds fed 900 and 1200 mg ECC had lower triglyceride concentration in the liver than the birds fed un-supplemental CC diet ($P = 0.001$). However, supplemented ECC at the levels of 1200 and 1800 mg/kg, led to an increase ($P = 0.006$) in liver phospholipid concentration of broilers at 21 days of age.

Table 3. Growth performance and choline chloride intake (CCI) of broilers fed graded additions of encapsulated choline chloride from 1 to 21 days of age.[1]

Diets[2]	Body weight gain (g/day)	Feed intake (g/bird/day)	Choline chloride intake (mg/bird/day)	Choline chloride intake: body weight gain (mg/g)	Feed conversion ratio (g/g)	Mortality (%)
N-Con	31.09^{c}	52.22^{b}	11.70^{h}	0.376^{h}	1.692	1.67
ECC_{300}	31.16^{c}	52.37^{b}	19.53^{g}	0.627^{g}	1.680	1.67
ECC_{600}	32.85^{bc}	54.88^{ab}	38.64^{f}	1.176^{f}	1.671	0.0
ECC_{900}	32.27^{ab}	57.12^{ab}	57.12^{e}	1.337^{e}	1.667	3.33
ECC_{1200}	35.07^{ab}	57.96^{ab}	67.41^{d}	1.926^{d}	1.656	5.00
ECC_{1500}	35.62^{a}	57.56^{ab}	84.61^{b}	2.376^{b}	1.616	1.67
ECC_{1800}	35.58^{a}	58.57^{a}	97.23^{a}	2.733^{a}	1.647	1.67
P-Con	35.41^{a}	57.83^{ab}	74.61^{c}	2.107^{c}	1.643	3.33
SEM[3]	0.548	1.271	1.392	0.038	0.034	1.768
			P-Value			
Model	<0.001	0.002	<0.001	<0.001	0.855	0.635
Linear response	<0.001	0.046	<0.001	<0.001	0.344	0.225
Quadratic response	0.068	0.528	-	0.098	0.464	0.228
P-Con *vs.* ECC_{1500}	0.781	0.878	<0.001	<0.001	0.718	0.509

[a-h] Values within the same column with uncommon superscript letter significantly differ ($P < 0.05$).
[1] Each value represents the mean of 6 replicate pens with 10 broilers each.
[2] N-Con, negative control diet with no added choline chloride; ECC, encapsulated choline chloride which subscript number shows the added mg ECC/kg diet; P-Con, positive control diet with 1500 mg commercial choline chloride (Nutrex Nv, Lille, Belgium)/kg.
[3] *SEM*, standard error of the means.

Table 4. Processing yield of broilers fed graded additions of encapsulated choline chloride from 1 to 21 days of age.[1]

Diets[2]	Live body weight (g)	Carcass		Breast muscle		Thigh muscle		Liver		Heart	
		Weight (g)	Yield (%)	Weight (g)	Yield (%)	Weight (g)	Yield (%)	Weight (g)	Yield (%)	Weight (g)	Yield (%)
N-Con	765.00^{ab}	475.13^{ab}	62.15	152.53^{ab}	19.92	139.37^{ab}	18.21	22.63^{a}	2.95	5.22	0.68
ECC_{300}	723.33^{b}	433.17^{b}	59.86	144.53^{b}	19.99	119.58^{b}	16.53	21.05^{b}	2.92	4.67	0.64
ECC_{600}	808.33^{ab}	495.08^{ab}	61.28	165.43^{ab}	20.47	135.77^{ab}	16.84	23.25^{ab}	2.87	5.63	0.69
ECC_{900}	806.67^{ab}	504.12^{ab}	62.40	172.07^{ab}	21.24	140.72^{ab}	17.42	23.75^{ab}	2.95	5.02	0.62
ECC_{1200}	814.17^{ab}	500.87^{ab}	61.45	166.22^{ab}	20.34	143.53^{ab}	17.66	21.47^{ab}	2.63	5.20	0.64
ECC_{1500}	876.67^{a}	528.45^{a}	60.25	180.32^{a}	20.47	149.55^{a}	17.09	21.33^{b}	3.12	5.50	0.63
ECC_{1800}	785.00^{ab}	458.70^{ab}	59.00	161.62^{ab}	20.59	135.70^{ab}	17.36	21.93^{ab}	2.79	5.13	0.66
P-Con	854.17^{ab}	536.50^{a}	62.81	191.33^{a}	22.39	147.13^{a}	17.20	22.72^{ab}	2.66	4.95	0.58
SEM[3]	32.056	20.031	1.209	8.979	0.555	5.856	0.393	1.371	0.120	0.292	0.068
					P-Value						
Model	0.048	0.013	0.312	0.021	0.066	0.032	0.137	0.043	0.106	0.384	0.157
Linear response	0.032	0.017	0.498	0.020	0.141	0.121	0.394	0.441	0.394	0.586	0.222
Quadratic response	0.095	0.042	0.404	0.070	0.263	0.252	0.419	0.598	0.470	0.654	0.340
P-Con *vs.* ECC_{1500}	0.622	0.778	0.143	0.391	0.119	0.772	0.832	0.062	0.060	0.190	0.219

[a-b] Values within the same column with uncommon superscript letter significantly differ ($P < 0.05$).
[1] Each carcass characteristics data is the mean of eighteen values.
[2] N-Con, negative control diet with no added choline chloride; ECC, encapsulated choline chloride which subscript number shows the added mg ECC/kg diet; P-Con, positive control diet with 1500 mg commercial choline chloride (Nutrex Nv, Lille, Belgium)/kg.
[3] *SEM*, standard error of the means.

Table 5. Liver and blood serum parameters of broilers fed graded additions of encapsulated choline chloride at 21 days of age.[1]

Diets[2]	Liver			Serum	
	Total lipid (% of liver)	Triglyceride (mg/g)	Phospholipid (mg/g)	Triglyceride (mg/dL)	Phospholipid (mg/dL)
N-Con	31.68[a]	7.28[a]	19.27[c]	30.36	96.53
ECC_{300}	28.89[ab]	6.07[ab]	21.03[bc]	32.17	92.44
ECC_{600}	25.92[bc]	5.58[b]	23.49[ab]	36.50	88.54
ECC_{900}	25.15[bc]	5.19[c]	24.28[abc]	40.10	96.32
ECC_{1200}	21.72[c]	5.13[c]	26.15[a]	35.90	84.14
ECC_{1500}	24.44[c]	5.54[abc]	25.24[ab]	33.40	85.68
ECC_{1800}	23.63[bc]	5.43[ab]	27.92[a]	41.12	95.50
P-Con	25.68[bc]	6.05[ab]	25.24[ab]	39.70	91.89
SEM	1.710	0.613	0.605	6.785	9.894
P-Value	0.014	0.001	0.006	0.293	0.215

[a-e] Values within the same column with uncommon superscript letter differ ($P < 0.05$).
[1] Each value represents the mean of duplicated analysis of 18 samples per each treatment.
[2] N-Con, negative control diet with no added choline chloride; ECC, encapsulated choline chloride which subscript number shows the added mg ECC/kg diet; P-Con, positive control diet with 1500 mg commercial choline chloride (Nutrex Nv, Lille, Belgium)/kg.
[3] *SEM*, standard error of the means.

The reduction of lipid and triglyceride contents in the liver of broilers in the present study might be attributed to the lipotropic effect of ECC (Workel, 2005). The results are supported by Devegowda *et al.* (2011) who reported that fat content was decreased in the abdomen and liver of the broilers when they fed with choline fortified diets. Similarly, when 760 mg/kg choline was added to the broiler diet, their liver fat content was diminished (Rao *et al.* 2001). Choline can promote fat metabolism in two ways; preventing the abnormal accumulation of fat by improving it's transporting as lecithin or with boosting the usage of fatty acids in the liver (Workel, 2005). The ECC did not affect phospholipid content in the liver compared to CC which is in agreement with findings of Wen *et al.* (2014) showed that increasing diet choline reduced, total lipid and triglyceride concentration in liver, but liver phospholipid was linearly increased ($P < 0.05$) in Pekin ducks from hatch to 21 days of age.

In their experiment, CC concentrations in the diets of broilers did not affect the blood serum indices such as triglyceride and phospholipid ($P > 0.05$) which is in agreement with findings of Khose *et al.* (2017) and Fouladi *et al.* (2008).

Choline chloride requirements

Methionine and betaine that are dietary nutrients involved in methyl groups, can affect the dietary requirement of choline (Jukes *et al.*, 1945). When dietary choline is not sufficient, the high sensitivity to the marginal levels of methionine and cystine may happen (Baker and Sugahara, 1970). In this study methionine content of the basal diet decreased to 90% of Ross 308 recommendation (Aviagen International, 2014). Although, other methyl donor content such as folic acid and betaine (3 methyl glycine, used as methyl donor) levels in experimental diets provided as recommended.

Pesti *et al.* (1980) demonstrated that regression equations have the best way to estimate choline requirements for broilers. In our study, required CC to maximize BWG, carcass weight, and breast weight in 21-day broiler chickens was estimated by linear broken-line regression model at 1335.1, 1371.6 and 1353.3 mg/kg, respectively ($P < 0.001$; Table 6).

Table 6. Choline chloride requirement of Ross × Ross 308 broilers from 1 to 21 days of age based on linear broken-line model analyses.

Response criteria	Estimated ECCI requirement[1]	95% confidence interval[2]	*SEM*[3]	*P*-Value
Body weight gain, g	1335.1	1103.5 to 1566.7	1.476	<0.001
Carcass weight, g	1371.6	1104.2 to 1584.7	1.495	<0.001
Breast weight, g	1353.3	1099.8 to 1567.4	1.486	<0.001

[1] The linear broken-line model is $y = L + U \times (R - x)$, where L is the ordinate, R is the abscissa of the break-point, the value of R is zero at values of $x > R$, and U is the slope of the line at values of $x < R$; ECCI, encapsulated choline chloride intake. [2] The 95% confidence interval of the supplemental choline chloride requirement (mg/kg of diet).[3] *SEM*, standard error of the means.

Lima (2012) estimated that chickens require 1013 mg CC/kg in 21-d Cobb 500 male broilers that fed a basal corn-soybean meal diet with standard methionine level (5.9 g/kg). Farina *et al.* (2017)

reported that choline requirements for Cobb 500 broilers, based on the BWG, is 778, 632, and 645 mg/kg for the phases of 1-7, 1-35, and 1-42 days of age, respectively. There is a dearth of research investigating the influence of dietary choline concentration and choline requirement of poultry; thus, more studies are needed.

Conclusion

It is concluded that the ECC at the level of 1500 mg/kg in the corn and isolated soybean protein diet can be substituted for the commercial type, without an adverse effect on growth and lipids concentrations in serum and liver of broilers at 1 to 21 days of age. Encapsulation technology decreased moisture absorption of CC to nearly 70% (Aami Azghadi *et al*., 2019). Regarding the hygroscopic property of CCC, ECC can improve the mixing and storage time of this vital ingredient in feed and premixes by increasing its resistance to caking (Aami Azghadi *et al*., 2019). These technological improvements to ECC directly affect the availability of other vitamins and minerals when it is included in premixes. Based on broken-line regression analysis, the choline requirements for BWG, carcass weight, and breast weight are 1335.1, 1371.6 and 1353.3 mg ECC/kg diet, respectively.

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References

Aami Azghadi M, Kermanshahi H, Golian A, Kadkhodaee R & Vakili SA. 2019. Method of production and assessment of an encapsulated choline chloride and its effects on growth performance and serum lipid indices in broilers. Poultry Science Journal, 7: 77-86. DOI: 10.22069/PSJ.2019.16155.1402

Aviagen International. 2014. Nutrition Specifications Manual: Ross 308. Aviagen Ltd., Scotland, UK.

Baker DH & Sugahara M. 1970. Nutritional investigation of the metabolism of glycine and its precursors by chicks fed a crystalline amino acid diet. Poultry Science, 49: 756-760. DOI: 10.3382/ps.0490756

Calderano AA, Nunes RV, Rodrigueiro RJB & César RA. 2015. Replacement of choline chloride by a vegetal source of choline in diets for broilers. Ciência Animal Brasileira, 16: 37-44. DOI: 10.1590/1089-6891v16i127404

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

Dilger RN, Garrow TA & Baker DH. 2007. Betaine can partially spare choline in chicks but only when added to diets containing a minimal level of choline. Journal of Nutrition, 137: 2224-2228. DOI: 10.1093/jn/137.10.2224

Duncan DB. 1955. Multiple range and multiple F tests. Biometrics, 11: 1-42. DOI: 10.2307/3001478

Emmert JL & Baker DH. 1997. A chick bioassay approach for determining the bioavailable choline concentration in normal and overheated soybean meal, canola meal and peanut meal. Journal of Nutrition, 127: 745-752. DOI: 10.1093/jn/127.5.745

Farina G, Kessler ADM, Ebling PD, Marx FR, César R & Ribeiro AML. 2017. Performance of broilers fed different dietary choline sources and levels. Ciência Animal Brasileira, 18. DOI: 10.1590/1089-6891v18e-37633

Folch J, Lees M & Sloane Stanley GH. 1957. A simple method for the isolation and purification of total lipides from animal tissues. Journal of Biological Chemistry, 226: 497-509.

Fouladi P, DoustNobar RS & Ahmadzade A. 2008. Effect of Choline Chloride Supplement and Canola Oil on the Broiler Chick's Liver Cholesterol and Triglyceride Contents. Research Journal of Poultry Sciences, 2: 67-71. DOI: rjpscience.2008.58.62

Harms RH & Russell GB. 2002. Betaine does not improve performance of laying hens when the diet contains adequate choline. Poultry Science, 81: 99-101. DOI: 10.1093/ps/81.1.99

Hassan RA, Attia, YA & El-Ganzory EH. 2005. Growth, carcass quality and serum constituents of slow growing chicks as affected by betaine addition to diets containing 1. Different levels of choline. International Journal of Poultry Science, 4: 840-850. DOI: 10.3923/ijps.2005.840.850

Igwe IR, Okonkwo, CJ, Uzoukwu UG & Onyenegecha CO. 2015. The effect of choline chloride on the performance of broiler chickens. Annual Research & Review in Biology, 8: 1. DOI: 10.9734/ARRB/2015/19372

Jukes TH. 1940. Effect of choline and other supplements on perosis. Journal of Nutrition, 20: 445-458. DOI: 10.1093/jn/22.5.445

Jukes TH, Oleson JJ & Dornbush AC. 1945. Observations on monomethylaminoethanol and dimethylaminoethanol in the diet of chicks. The Journal of Nutrition, 30: 219-223. DOI: 10.1093/jn/30.3.219

Khose KK, Manwar SJ, Gole MA, Ingole RS & Potdar GG. 2017. Influence of Herbal Choline as a Replacement of Synthetic Choline Chloride in Broiler Diets on Serum Biochemical Profile. Chemical Science Review and Letters, 6: 2387.

Khosravinia H, Chethen PS, Umakantha B & Nourmohammadi R. 2015. Effects of lipotropic products on productive performance, liver lipid and enzymes activity in broiler chickens. Poultry Science Journal, 3: 113-120. DOI: 10.22069/PSJ.2015.2648

Lima MB. 2012. Modelos matemáticos para predição das exigências da colina para frangos de corte [dissertation]. Piracicaba (SP): Universidade de São Paulo. Portuguese. Available from: http://www.teses.usp.br/teses/disponiveis/11/11139/tde-16082012-103634/pt-br.php.

Menten JF, Pesti GM & Bakalli RI. 1997. A new method for determining the availability of choline in soybean meal. Poultry Science, 76: 1292-1297. DOI: 10.1093/ps/76.9.1292

Messman MA, Weiss WP & Albrecht KA. 1996. In situ disappearance of individual proteins and nitrogen from legume forages containing varying amounts of tannins. Journal of Dairy Science, 79: 1430-1435. DOI: 10.3168/jds.S0022-0302(96)76501-3

Molitoris BA & Baker DH. 1976. Assessment of the quantity of biologically available choline in soybean meal. Journal of Animal Science, 42: 481-489. DOI: 10.2527/jas1976.422481x

NRC. 1994. Nutrient Requirements of Poultry. 9th ed. Natl. Acad. Press, Washington, DC.

Pesti GM, Harper AE & Sunde ML. 1980. Choline/methionine nutrition of starting broiler chicks. Three models for estimating the choline requirement with economic considerations. Poultry Science, 59: 1073-1081. DOI: 10.3382/ps.0591073

Rao AM, Hatcher JF & Dempsey RJ. 2001. Does CDP-choline modulate phospholipase activities after transient forebrain ischemia? Brain Research, 893: 268-272. DOI: 10.1016/S0006-8993(00)03280-7

Rizki G, Arnaboldi L, Gabrielli B, Yan J, Lee, GS, Ng, RK & Maher JJ. 2006. Mice fed a lipogenic methionine-choline-deficient diet develop hypermetabolism coincident with hepatic suppression of SCD-1. Journal of Lipid Research, 47: 2280-2290. DOI: 10.1194/jlr.M600198-JLR200

Robbins KR, Saxton AM & Southern LL. 2006. Estimation of nutrient requirements using broken-line regression analysis. Journal of Animal Science, 84: 155-165. DOI: 10.2527/2006.8413_supplE155x

SAS (Statistical Analysis System). 2003. SAS/STAT® 9.1 User's Guide. SAS Institute Inc. Cary, North Carolina.

Selvam R, Saravanakumar M, Suresh S, Chandrasekeran CV & Prashanth DS. 2018. Evaluation of polyherbal formulation and synthetic choline chloride on choline deficiency model in broilers: implications on zootechnical parameters, serum biochemistry and liver histopathology. Asian-Australasian Journal of Animal Sciences, 31: 1795. DOI: 10.5713/ajas.18.0018

Swain BK & Johri TS. 2000. Effect of supplemental methionine, choline and their combinations on the performance and immune response of broilers. British Poultry Science, 41: 83-88. DOI: 10.1080/00071660086457

Tao FT, Thurber JS & Dye DM. 1984. High□ performance liquid chromatographic determination of acetylcholine in a pharmaceutical preparation. Journal of Pharmaceutical Sciences, 73: 1311-1313. DOI: 10.1002/jps.2600730935

Vilstrup P. 2001. Microencapsulation of food ingredients. England: Leatherhead Publishing.

Waldroup PW & Fritts CA. 2005. Evaluation of separate and combined effects of choline and betaine in diets for male broilers. International Journal of Poultry Science, 4: 442-448. DOI: 10.3923/ijps.2005.442.448

Wen ZG, Tang J, Hou SS, Guo YM, Huang W & Xie M. 2014. Choline requirements of white Pekin ducks from hatch to 21 days of age. Poultry Science, 93: 3091-3096. DOI: 10.3382/ps.2014-03994

Workel HA. 2005. Quality and nutritional aspects of choline chloride. Krmiva: Časopis o hranidbi životinja, proizvodnji i tehnologiji krme, 47: 101-106.

Zeisel SH & Blusztajn JK. 1994. Choline and human nutrition. Annual Review of Nutrition, 14: 269-296. DOI: 10.1146/annurev.nu. 14.070194.001413

Zeisel SH, daCosta KA, Youssef M & Hensey S. 1989. Conversion of dietary choline to trimethylamine and dimethylamine in rats: dose-response relationship. The Journal of Nutrition, 119: 800-804. DOI: 10.1093/jn/119.5.800