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PERFORMANCE OF BROILERS FED DIFFERENT DIETARY CHOLINE SOURCES AND LEVELS

DESEMPENHO DE FRANGOS DE CORTE SUPLEMENTADOS COM DIFERENTES FONTES E NÍVEIS DE COLINA NA DIETA

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Abstract
Two experiments were carried out to evaluate the bioequivalence of a commercial phosphatidylcholine source (Biocholine™) as an alternative to choline chloride and the choline requirements of broilers of a fast-growth strain. In Experiment I, 672 broilers were fed four Biocholine™ levels (0, 100, 200, or 300 mg/kg) and three choline levels (200, 400, or 600 mg/kg) supplied as choline chloride between 4 and 28 days (d) of age. In Experiment II, 462 broilers received diets supplemented with 0, 200, 400, 600 or 800 mg/kg choline as choline chloride. In both experiments, diets were based on white rice, soybean meal, and corn gluten. In Experiment I, birds fed choline chloride presented higher feed intake than those fed Biocholine™. Both choline supplements linearly improved feed conversion ratio (FCR) between 15 and 28 d, but the curves presented different slopes, showing that one unit (U) of Biocholine™ was equivalent to 2.52 U of choline supplied as choline chloride. In Experiment II, the supplementation of choline had a quadratic effect on weight gain (WG) but did not affect FCR. Choline requirements for WG were determined as 778, 632, and 645 mg/kg for 1-7, 1-35, and 1-42 d of age, respectively.
Keywords: bioequivalence; broiler; fatty liver; nutritional requirements; perosis.

Resumo
Dois experimentos foram conduzidos para avaliar a bioequivalência de uma fonte comercial de fosfatidilcolina (Biocholine®) como alternativa ao cloreto de colina e as exigências de colina de frangos de rápido desempenho. No Experimento I, 672 frangos foram alimentados com quatro níveis de Biocholine® (0, 100, 200 ou 300 mg/kg) e três níveis de colina (200, 400 ou 600 mg/kg) supridas pelo cloreto de colina entre 4 e 28 dias de idade. No Experimento II, 462 frangos receberam dietas suplementadas com 0, 200, 400, 600 ou 800 mg/kg de colina através do cloreto de

colina. Em ambos os experimentos, as dietas foram à base de arroz branco, farelo de soja e glúten de milho. No Experimento I, as aves alimentadas com cloreto de colina apresentaram maior consumo de ração em relação àquelas alimentadas com Biocholine®. Ambas as fontes de colina melhoraram linearmente a conversão alimentar entre 15 e 28 dias, mas as curvas apresentaram diferentes inclinações, mostrando que uma unidade de Biocholine® foi equivalente a 2,52 unidades de colina suprida na forma de cloreto de colina. No Experimento II, a suplementação de colina apresentou efeito quadrático sobre o ganho de peso sem afetar a conversão alimentar. As exigências de colina para ganho de peso foram 778, 632 e 645 mg/kg para as fases de 1-7, 1-35 e 1-42 dias de idade, respectivamente.
Palavras-chave: bioequivalência; frango de corte; fígado gorduroso; exigência nutricional; perose.

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Introduction

The nutrient choline has three essential metabolic functions: it is a component of membrane phospholipids[1]; it participates in lipid liver metabolism, preventing fat accumulation in the liver[2]; and it is a precursor of acetylcholine[3]. In addition, choline prevents perosis or chondrodystrophy in poultry[4], and it can be oxidized to betaine to donate methyl groups[5], sharing this function with methionine, which, according to Zeisel[6], consumes the most choline in the body. Studies have shown that the nutritional requirements of choline and methionine are interdependent, i.e., increasing the supply of one of these nutrients reduces the requirement of the other[7]. Similarly, higher dietary betaine levels reduce choline requirements[8]. Choline is present in the feedstuffs as free choline or in the form of complexes, including phosphocholine, glycerophosphocholine, sphingomyelin, or phosphatidylcholine[9], which is the main form of choline in plant feedstuffs[10]. In the body, phosphatidylcholine is responsible for removing lipids from the liver as it is essential for the synthesis of very low-density lipids (VLDL)[11], which transport fat to the peripheral tissues. Phosphatidylcholine also accounts for 35% of cell membrane phospholipids[1].

Due to its importance, choline is commonly supplemented in broiler diets as choline chloride. However, this product is highly hygroscopic, and may result in losses of water-soluble vitamin added to premixes because it increases free water content in the mixture, resulting in higher reactive potential. In addition, this causes operational problems in the feed mill as the product may form lumps[12].

Choline nutritional requirements of broilers are based on studies carried out decades ago[13,14], and therefore, they need to be updated as there have been significant changes in diet formulation and particularly in bird performance since then. Waldroup et al.[15] observed improvement in the Feed Convertion Rate (FCR) and in the breast yield to 42, 49, and 56 days with 1000 mg/kg of choline supplementation on a diet containing between 1193 (1 to 14 days) and 925 mg/kg of choline (42 to 56 day), without changing the Weight Gain (WG) and the carcass yield. Similar results were obtained by Pompeu et al.[16], who observed a difference only for FCR at 21 days, with linear response for this variable with choline supplementation up to 400 mg/kg in a basal diet containing

1367 mg/kg of choline. Swain and Johri[17] used a diet containing 1300 mg/kg of choline and found no effect of supplementation on performance at 42 days.

The objectives of this study were to evaluate the bioequivalence of a commercial phosphatidylcholine source (Biocholine™) as an alternative to choline chloride, and to determine choline requirements of broilers of a fast-growth strain. The incidence of perosis and fatty liver was also investigated.

Material and Methods

All the experimental procedures were approved by the Committee of Ethics on the Use of Animals of the Federal University of Rio Grande do Sul, under protocol No 24156.

Two experiments were performed. In experiment I, the bioequivalence between the commercial product Biocholine™ (phosphatidylcholine source, low-hygroscopicity plant extract based on *Trachyspermum amni*, *Citrullus colocynthis*, *Achyranthus aspera*, and *Azadirachta indica*) and choline chloride was evaluated. As none of the birds showed any deficiency symptoms at the choline levels employed, Experiment II was carried out to determine choline requirements of fast-growing broilers.

In both experiments, birds were housed in an environmentally-controlled room in 1-m^2 pens on wood-shavings litter. Pens were equipped with nipple drinkers and tube feeders. Water and feed were supplied ad libitum. An artificial lighting program of 24.00 hours of light was adopted.

In Experiment I, 672 one-day-old male broilers were housed in 56 pens, at a density of 12 birds/pen, and in Experiment II, 462 one-day-old male broilers were housed in 42 pens, at a density of 11 birds/pen. In both experiments, Cobb 500™ broilers were used. Birds were vaccinated at the hatchery against Marek’s disease, infectious bronchitis, fowl pox, and infectious bursal disease. At the start of the experimental period, birds were uniformly distributed in the pens according to body weight (average initial weight of 117±2,7 g in Experiment I and 47±1 g in Experiment II).

The following feeding phases were applied: 4-21 and 22-28 days (d) of age (Experiment I) and 1-21, 22-35, and 36-42 d of age (Experiment II). In both experiments, feedstuffs containing low choline levels were used in order to evaluate the effect of supplementation. Diets were based on white rice, corn gluten (600 g/kg crude protein), and gummed or de-gummed soybean meal in Experiments I and II, respectively (Table 1). Choline content of white rice was obtained from the USDA tables[10]; the content of soybean isolate protein was supplied by the manufacturer (Solae Company™), and of the remaining feedstuffs was obtained from the NRC for pigs[18]. Aiming at preventing severe choline deficiency symptoms in Experiment I, birds were fed up to four d of age a diet based on corn and soybean meal formulated to supply the nutritional levels proposed by Rostagno et al.[19] for this phase. This diet contained 1300 mg/kg choline. As no choline deficiency symptoms were observed in Experiment I, choline values of the basal diets supplied in Experiment II were reduced by using soy protein isolate and de-gummed soybean meal. All experimental diets were supplied in the meal form and contained equal energy and nutrient levels calculated to supply the nutritional requirements proposed by Rostagno et al.[19], except for choline.

Table 1. Ingredient composition and nutritional levels of the experimental diets (on as-is basis)

	Experiment I		Experiment II		
Ingredients (g/kg)	Starter 4-21 days	Grower 22-28 days	Starter 1-21 days	Grower 22-35 days	Finisher 36-42 days
White rice	632.7	634.5	644.3	696.4	720.7
Soybean meal 450 g/kg[1]	228.3	230.3	143.5	109.4	107.5
Corn gluten 600 g/kg	77.0	59.5	115.5	100.0	72.0
Soy protein isolate	-	-	30.0	30.0	30.0
Soybean oil	12.6	30.4	11.0	14.8	23.6
Limestone	8.2	7.6	11.4	9.4	8.1
Dicalcium phosphate	17.4	14.9	20.6	16.1	14.0
Salt	4.8	4.6	1.0		
L-lysine	3.7	3.5	4.8	4.3	4.1
DL-methionine	2.6	2.3	1.6	1.4	1.7
L-threonine	1.2	0.9	0.8	0.7	0.9
Mineral premix[2]	1.0	1.0	0.7	0.7	0.7
Vitamin premix[3]	0.5	0.5	0.5	0.5	0.5
Na bicarbonate	-	-	5.5	6.7	6.7
K chloride	-	-	3.6	4.4	4.5
Monensin 400 g/kg	-	-	0.25	0.25	0.25
Corn starch[4]	10.0	10.0	5.0	5.0	5.0
Nutritional values					
ME[5] (kcal/kg)	3050	3150	3082	3150	3200
CP[6], analyzed (g/kg)	200.0	189.1	217.1	203.0	176.9
Ca (g/kg)	8.2	7.3	9.4	7.6	6.6
Available P (g/kg)	3.9	3.4	4.4	3.5	3.1
Na (g/kg)	2.1	2.0	2.4	2.3	2.3
Dig. Lys (g/kg)	11.7	10.8	11.4	10.2	9.7
Dig. Met (g/kg)	5.9	5.4	5.4	4.9	4.8
Dig. Met+Cys (g/kg)	8.5	7.9	8.2	7.4	7.1
Dig. Thr (g/kg)	7.6	7.0	7.4	6.6	6.3
Dig. Trp (g/kg)	2.1	2.1	2.1	1.9	1.8
Dig. Arg (g/kg)	12.7	11.9	12.1	11.0	10.5
DEB[7] (mEq/kg)	140	140	180	180	180

[1]Gummed and de-gummed soybean meal in Experiments I and II, respectively.
[2]Composition (per kg): 150,000 mg Mn; 100,000 mg Zn; 80,000 mg Fe; 15,000 mg Cu; 1,200 mg I; 700 mg Se.
[3]Composition (per kg): 23,200,000 UI vitamin A; 5,600,000 UI vitamin D; 52000 mg vitamin K; 6000 mg vitamin B_1; 18,000 mg vitamin B_2; 9,000 mg vitamin B_6; 132,000 mg niacin; 44,000 mg pantothenic acid; 2,400 mg folic acid, 200,000 µg biotin; 40,000 µg vitamin B_{12}.
[4]In Experiment I: treatments with Biocholine™ (BC) - 1% starch; 0.99% starch and 0.01% BC; 0.98% starch and 0.02% BC; 0.97% starch and 0.03% BC for 0, 100, 200, and 300 mg BC/kg; treatments with choline chloride (CL) - 0.957% starch and 0.043% CL; 0.913% starch and 0.087% CL; 0.870% starch and 0.130% CL for 200, 400, and 600 mg choline/kg supplied by CL.
In Experiment II 0.5% starch; 0.041% CL and 0.459% starch; 0.083% CL and 0.417% starch; 0.123% CL and 0.377% starch; 0.166% CL and 0.334% starch for 0, 200, 400, 600, and 800 mg choline/kg supplied by CL.
[5]Metabolizable energy.
[6]Crude protein.
[7]Dietary electrolyte balance (Na + K – Cl).

In Experiment I, seven treatments were applied, consisting of the comparison of four Biocholine™ levels (0, 100, 200, and 300 mg/kg) with three choline levels (200, 400, and 600 mg/kg), with eight replicates per treatment. In Experiment II, five treatments, consisting of choline levels of 0, 200, 400, 600, or 800 mg/kg diet, were applied, with eight replicates each, except for the level of 0 mg/kg, which had 10 replicates. The choline levels used in Experiment II were selected considering the calculated choline values of the basal diets, to include both the linear and the plateau section of the performance response curves, based on the study of Lima[7], who found choline requirement between 872 and 1013 mg/kg for broilers from 1 to 21 days. Choline requirements were determined by calculating the maximum point of weight gain (WG) equations and considering the cumulative periods in order to prevent the interference of the previous treatment. In Experiment II, choline was supplemented as choline chloride at 600 g/kg, discounting the participation of chlorine, which accounts for 251.8 g/kg of the molecule of choline chloride. In both experiments, choline supplements were included at the expense of corn starch. A single basal diet was manufactured for each phase into which the supplements were added.

The product Biocholine™, white rice, gummed and de-gummed soybean meal, corn gluten, and soy protein isolate were submitted to the Department of Nutrition of North Carolina University for the analysis of choline according to the methodology described by Koc et al.[20]. Choline chloride was analyzed by ion chromatography[21]. Crude protein content of the basal diets was determined according to the method No 984.13 of the AOAC[22] adapted by Prates[23].

Although in the report issued by the manufacturer Biocholine™ contains 16.7 g/kg phosphatidylcholine, the analysis showed only 2.9 g/kg. The choline values analyzed in the diets of both experiments were also lower than those reported in feedstuff tables (Table 2). Dietary betaine content was also analyzed due to its choline-sparing effect[24]. However, the analysis results showed low betaine levels, and therefore, it is assumed that they did not influence choline requirements in the present study.

Table 2. Choline and betaine levels in the feedstuffs and in the experimental diets

Item	Choline, analyzed (mg/kg)	Choline, calculated[1] (mg/kg)
White rice	30	58
Corn gluten	141	330
De-gummed soybean meal	1593	2794
Gummed soybean meal	2267	2794
Soy protein isolate	1318	1110
Starter diet, T1, Experiment I[2]	547	700
Grower diet, T1, Experiment I[2]	549	700
Starter diet, T1, Experiment II[2]	304	600
Grower diet, T1, Experiment II[2]	249	510
Finisher diet, T1, Experiment II[2]	243	500

[1]Values based on the tables of the NRC (1998), USDA (2008), and Solae Company™.

[2] Formulation values based on choline and betaine values in the feedstuffs.

In both experiments, body weight (BW), feed intake (FI), and perosis incidence were weekly evaluated, and WG and feed conversion rate (FCR) corrected for mortality were calculated. Mortality and environmental temperature were daily measured.

At the end of the experiments, birds were euthanized by stunning using electronarcosis and jugular vein excision. Livers were then collected, immediately frozen at -86 °C for subsequent freeze-drying and ether extract (EE) determination by the method No 920.39 of the AOAC[22]. In

Experiment I, one bird per replicate was euthanized, totaling 56 birds, and in Experiment II, five birds fed 0, 400, and 800 mg choline/kg of diet were euthanized, totaling 15 birds.

Each experiment was individually analyzed using the software program Statgraphics Plus 5.1™. Data were submitted to analysis of variance and analyses of regression for each choline source. In Experiment I, analyses of contrasts were performed (no choline supplementation *vs*. Biocholine™; no choline supplementation *vs*. choline chloride; and Biocholine™ *vs*. choline chloride). When F was significant, means were compared by the LSD test.

Results and Discussion

The different forms of choline present in the feedstuffs (free choline, glycerophosphocholine, phosphocholine, phosphatidylcholine, and sphingomyelin)[(9)] may hinder choline quantification and may explain differences in the results of analyses carried out in different laboratories as well as justify the differences between calculated and analyzed values obtained in the present study. For instance, white rice choline values varied between 58 and 1003 mg/kg depending on the table used[(10,18)]. Therefore, we decided to apply analyzed values due to the variations in the nutritional composition of feedstuffs.

In both experiments, the mortality rate was as expected, below 3%, and it was not influenced by choline sources or levels. In Experiment I, WG and FCR were not influenced by choline sources or levels in none of the studied phases, as determined by the analysis of variance (Table 3). However, the contrast analysis showed that Biocholine™ provided better FCR between 15 and 28 d, when compared to birds that did not receive supplementation, proving to be an alternative for the reduction of production costs. Besides that, in the same period and during the total experimental period, birds supplemented with Biocholine™ presented lower FI in relation to choline chloride (Table 3).

While some researchers did not find any influence of choline supplementation on FCR[(24,25)], other studies demonstrated that choline had a positive effect on this parameter[(15,26,27)]. This may be explained by differences in sulfur amino acid levels because choline did not affect FCR only in studies where diets contained high levels of these amino acids, as in the present study.

According to the regression analyses, which are used to compare choline sources, both supplements linearly improved FCR between 15 and 28 d (Table 5). However, the slopes of the equations were different ($P<0.04$, Figure 1), and the ratio between angle coefficients showed that one unit of the product Biocholine™ was equivalent to 2.52 units of pure choline as supplied by choline chloride. Biocholine™ is an alternative to choline chloride for dietary choline supplementation, as Normative Instruction No 46 of the Brazilian Ministry of Agriculture[(28)] that regulates organic food production banned the use of vitamins obtained by chemical synthesis, such as choline chloride, since 2013. The knowledge on the bioequivalence of Biocholine™ allows it to be efficiently used in organic food production systems.

In the present study, birds fed Biocholine™, which is a phosphatidylcholine source, presented better FCR responses (Figure 1) compared with those fed choline chloride. These results are similar to the ones obtained by Rodelas et. al.[29], which indicate that biocholine associated with herbal vitamins C and E supplementation enhanced the overall feed efficiency of the broilers between 8 and 42 days. Calderano et al.[30] found no performance differences between the biocholine or choline chloride supplementation for broilers. According to Cheng et al.[31], the bioavailability and utilization of different choline esters varies, which may explain the higher efficiency of phosphatidylcholine. In addition, choline in the form of chloride can be broken down by the intestinal flora, being transformed in trimethylamine and immediately excreted[32]. On the other hand, there is no[33] or low[34] degradation of phosphatidylcholine in the gastrointestinal tract.

Table 3. Performance of male broilers fed diets supplemented with different choline sources and levels (Experiment I)

Supplemental choline level (mg/kg)	Period (days)		
	4-14	15-28	4-28
	Weight gain (g)		
0	409	1106	1517
200	415	1141	1558
400	413	1143	1550
600	407	1142	1552
100 - Biocholine™	422	1117	1533
200 - Biocholine™	418	1128	1546
300 - Biocholine™	407	1104	1511
P	0.21	0.42	0.47
SEM	13	47	52
	Feed conversion ratio (g/g)		
0	1.351	1.531	1.480
200	1.366	1.507	1.468
400	1.370	1.463	1.437
600	1.377	1.472	1.457
100 - Biocholine™	1.369	1.466	1.439
200 - Biocholine™	1.387	1.480	1.454
300 - Biocholine™	1.385	1.468	1.445
P	0.95	0.13	0.56
SEM	0.068	0.054	0.049
	Feed intake (g)		
0	552	1670abc	2245
200	567	1716c	2286
400	558	1671abc	2227
600	578	1677bc	2259
100 - Biocholine™	570	1637ab	2206
200 - Biocholine™	579	1666abc	2245
300 - Biocholine™	564	1620a	2183
P	0.62	0.03	0.14
SEM	32	53	73

Contrast	Mean	P-value
FCR 15-28 days (g:g) – no supplementation vs. Biocholine™	1.531 vs. 1.471	<0.05
FI 15-28 days (g:g) – Biocholine™ vs. choline chloride	1641 vs. 1688	<0.05
FI 4-28 days (g:g) – Biocholine™ vs. choline chloride	2211 vs. 2258	<0.05

Means followed by different letters in the same column are statistically different by the LSD test at 0.05 probability level. Average initial weight (4 d): 117 g.

Table 4. Performance of male broilers fed diets supplemented with different choline levels (Experiment II)

Supplemental choline level (mg/kg)	Period (days)			
	1-7	1-21	1-35	1-42
	Weight gain (g)			
0	122	887	2202	2986
200	132	942	2338	3105
400	127	929	2295	3100
600	127	898	2193	3000
800	127	912	2233	3015
SEM	8	44	91	91
	Feed conversion ratio (g/g)			
0	1.256	1.307	1.459	1.523
200	1.246	1.313	1.453	1.520
400	1.253	1.302	1.444	1.510
600	1.258	1.339	1.483	1.532
800	1.301	1.324	1.466	1.523
SEM	0.106	0.036	0.032	0.027
	Feed intake (g)			
0	154	1159	3209	4546
200	164	1236	3396	4721
400	163	1210	3312	4637
600	159	1200	3249	4596
800	165	1207	3273	4594
SEM	11	52	123	151

Average initial weight (1 day): 47 g.

Table 5. Regression equations using calculated choline values (Experiment I)

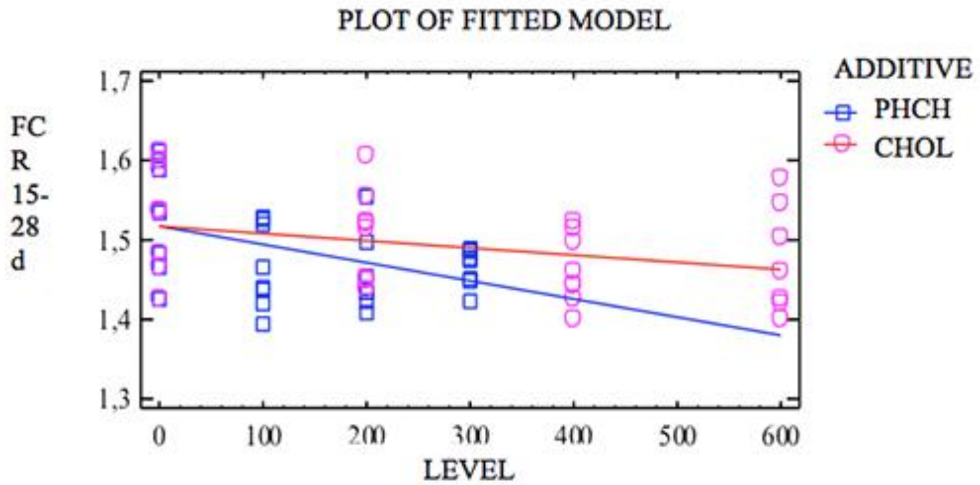
Parameter	Equation	Effect	P-value
WG[1], 4-14 d	For Biocholine™, $Y = 409.698 + 0.1725 \times$ choline level $- 0.0006 \times$ choline level^2, $R^2 = 0.192$.	Quadratic	0.016
WG, 4-28 d	For Biocholine™, $Y = 1503.17 + 0.6117 \times$ choline level $- 0.002 \times$ choline level^2, $R^2 = 0.185$.	Quadratic	0.020
FCR[2], 15-28 d	For choline chloride, $Y = 1.6012 - 0.0001 \times$ choline level, $R^2 = 0.147$.	Linear	0.033
	For Biocholine™, $Y = 1.5105 - 0.0002 \times$ choline level, $R^2 = 0.157$.	Linear	0.027
FI[3], 4-14 d	For Biocholine™, $Y = 551.531 + 0.2878 \times$ choline level $- 0.0008 \times$ choline level^2, $R^2 = 0.199$.	Quadratic	0.028
FI, 15-28 d	For Biocholine™, $Y = 1681.16 - 0.184 \times$ choline level, $R^2 = 0.129$.	Linear	0.043

[1] WG: Weight gain.
[2] FCR: Feed conversion rate.
[3] FI: Feed intake.

Table 6. Regression equations using calculated choline values (Experiment II)

Parameter			
WG[1], 1-7d	$Y = 106.669 + 0.0611 \times$ choline level $- 0.00004 \times$ choline level^2, $R^2 = 0.133$.	Quadratic	0.055
WG, 1-35 d	$Y = 2100.17 + 0.6018 \times$ choline level $- 0.0005 \times$ choline level^2, $R^2 = 0.100$.	Quadratic	0.053
WG, 1-42 d	$Y = 2858.31 + 0.6969 \times$ choline level $- 0.0005 \times$ choline level^2, $R^2 = 0.128$.	Quadratic	0.026

[1] WG: Weight gain.



Equation for choline chloride: FCR 14 to 28 days = 1.51814 - 0.0000908354 x choline level (mg/kg) (P<0.02). Equation for Biocholine™: FCR 14 to 28 days = 1.51814 - 0.000228921 x Biocholine™ level (P<0.003). R^2 = 0.156. PHCH: Biocholine™. CHOL: Choline chloride.

Figure 1. Comparison between Biocholine™ and choline chloride regression curves for feed conversion rate (FCR) during the period of 14-28 days (Experiment I).

The observed FCR improvement caused by Biocholine™ may be related to better dietary fat absorption. According to Rioux et al.(35) and LeBlanc et al.(36), the supplementation of phosphatidylcholine in the diet of rats increased bile juice flow, phosphatidylcholine content in the bile juice, and bile cholesterol content. These factors indicate that phosphatidylcholine has an indirect emulsifying role in fat digestion. Huang et al.(37) observed that the replacement of 250 g/kg soybean oil by soy lecithin improved fat digestibility in the starter phase and WG and FCR of one- to 42-d-old broilers. Raber et al.(38) verified that the inclusion of 5 g/kg soy lecithin in broiler diets increased the percentage of dietary metabolizable fat. Zhang et al.(39) demonstrated that the administration of lysophosphatidylcholine improved broiler WG in the starter phase and fatty acid digestibility and apparent metabolizable energy between 35 and 38 d of age.

In Experiment II, FCR and FI were not influenced by choline levels in none of the evaluated phases. On the other hand, choline supplementation caused quadratic effect in WG during 1-35 and 1-42 (P<0.05, Table 6). The absence of WG response in the first week may be attributed to the choline reserves in the yolk, which contains approximately 6800 mg of choline/kg(10). Choline requirements for WG were determined as 778, 632, and 645 mg/kg for the ages of 1-7, 1-35, and 1-42 d, respectively (Table 6). These values are close to those obtained with purified diets, of 600(14,40) to 720 mg/kg(8), but much lower than those determined in studies using practical diets. The WG regression equation was not significant for the phase of 1-21 d, allowing us to conclude that the choline level of the basal diet was sufficient to supply the birds' requirements.

However, the regression equation for the period of 1-35 d shows that choline requirements increased after the third week. Viola et al.(41) also observed higher nutritional requirements during the phase of 21-28 d in broilers, which was attributed by the authors to the high lean growth rate at this age. Pesti et al.(42) supported the theory that performance parameters are not adequate to evaluate choline requirements in poultry because when choline-deficient diets are offered, birds utilize other sources, such as methionine, betaine, and cystine, to supply this deficiency. Studies have shown that when dietary choline level is increased, methionine requirement is reduced and vice versa, which is called the methionine-sparing effect(7,26). Studies using diets with low methionine levels (3.8 and 3.2 g/kg) determined choline requirements of 1200(14) and 1910 mg/kg(10) in starter broilers.

Changes in the genetic potential should also be taken into account when comparing the nutritional requirements of broilers. In fast-growing poultry, the increase in muscle cell volume (hypertrophy) seems to be more important for growth than the increase in the number of cells (hyperplasia)(42). Considering that the increase in internal volume in these cells is proportionally higher than the increase in cell surface membrane, and taking into account the participation of choline in cell membranes(1) as well as its role as precursor of acetylcholine(43), it is plausible to suppose that choline requirements of broilers of different sizes is similar.

Broiler genetic improvement has focused on increasing both FI capacity and body growth rate. As modern broilers have high FI, their choline intake has also increased; therefore, their choline requirement per unit of WG may have decreased. In a recent study feeding diets based on corn and soy protein concentrate for Cobb 500™ broilers, choline requirement at standard methionine levels (5.9 g/kg) was determined as 1013 mg/kg for the period of 1-21 d of age(7), which value is intermediate between the studies mentioned in this article and the present study. According to Briz and Pérez(44), diets based on corn and soybean contain approximately 1350 mg choline/kg and do not require supplementation(25), even at low dietary methionine levels(17).

There was no difference (P>0.05) in liver fat percentage among choline sources or levels in none of the experiments. Average fat values determined on dry matter basis were 176.8 g/kg (experiment I) and 177.7 g/kg (experiment II), and are consistent with those reported in literature(16). Fatty liver is caused by the lack of methyl groups in the diet, and not merely by choline deficiency(45). The supply of methyl groups was adequate in the present study because the diet contained adequate methionine levels, and due to the dietary inclusion of soybean meal and corn gluten, which contain the molecule S-methylmethionine (SMM) that is analogous to S-adenosylmethionine(46). Lipstein et al.(47) found that broilers fed practical (1942 mg choline/kg) and semi-purified (268 mg choline/kg) diets presented similar lipid deposition in the liver.

No symptoms of perosis were observed in none of the experiments. The results of literature studies on the effect of choline on perosis are contradictory. Pesti et al.(48) fed broilers a basal diet with no choline and 4.3 g/kg methionine and found high incidence of perosis when the diet was supplemented with 150-600 mg choline/kg. According to Fritz et al.(49), 1900 mg choline/kg of diet are required to prevent that disorder. On the other hand, a study demonstrated that 450 mg choline/kg can dramatically reduce perosis(47) in broilers, and Ryu et al.(50), using a diet containing 750 mg choline/kg did not observe any signs of perosis in broilers up to 18 d of age. Manganese deficiency also causes perosis in broilers(51), but in all the studies mentioned above, Manganese dietary levels were adequate. Other factors, in addition of the deficiency of those nutrients, may influence the incidence of perosis in broilers. For instance, Rizk et al.(52) demonstrated that broilers reared on litter showed lower incidence of perosis than those reared in battery cages.

Conclusions

Comparing regression curves for feed conversion rate (FCR) during the period of 14-28 days, the utilization of the product Biocholine™ improved this variable compared with choline chloride. Based on this response, the calculated equivalence between these two products is one unit of Biocholine™ for 2.52 units of choline supplied as chloride (Experiment I).

The supplementation of choline chloride has a quadratic effect on WG, but did not influence FCR (Experiment II). Choline requirements for WG, based on the analyzed choline content of feedstuffs, is 778, 632, and 645 mg/kg for the phases of 1-7, 1-35, and 1-42 d of age, respectively.

Dietary choline levels of 304 mg/kg in the starter phase, 249 mg/kg in the grower phase, and 243 mg/kg in the finisher phase do not cause perosis or fatty liver in broilers (Experiment II).

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