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Dietary Supplementation of Alternative Methionine and Choline Sources in the Organic Broiler Production in Brazil

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ABSTRACT

The objective of this study was to evaluate the use of natural and alternative sources of methionine and choline which can be allowed to use in organic livestock systems to feed broilers produced in Brazil. Seven hundred and twenty one-d-old male Cobb broilers were randomly allocated to four treatments with six replicates of 24 birds each. The treatments consisted in substituting the commonly used DL-methionine 99% by a vegetable source of methionine and cholinechloride 60% by alternative source of choline in the form of phosphatidylcholine. The following treatments were evaluated: I) feed with DL-methionine 99% and choline chloride 60%, II) feed with an vegetable methionine source and choline chloride 60%, III) feed with DL-methionine 99% and choline as phosphatidylcholine, and IV) feed with vegetable methionine source and choline as phosphatidylcholine. Daily weight gain, body weight, feed intake, feed conversion ratio, and mortality were evaluated for the periods of 1 to 21 and 1 to 42 days of age. During both periods, broilers fed the vegetable methionine source presented lower daily gain and lower body weight. When only choline chloride was substituted by the alternative choline source, broiler performance was not different compared with that of the control group. The group fed the diet with substitution of both DL-methionine 99% and choline chloride 60% by natural sources presented lower daily weight gain, final body weight, and feed intake. Further research on alternative nutrient sources are required for the development of the organic production chain.

INTRODUCTION

According to Ubabef (2014), the Brazilian production of chicken meat was 12.30 million tones. In this context, Brazil is the third largest producer and the first exporter of this product in the world.

In part, this gigantism of poultry industry is driven by the availability of feedstuffs. This accessibility is a direct reflection of a very well structured supply chain, which allows producers to take advantage of the natural potential of the country to produce grains, such as corn and soybeans.

According to Bonaudo *et al*. (2010) and Demattê Filho & Marques (2011), assessing the diversity of the Brazilian meat supply chain, determined that the poultry production follows an industrial model, constantly seeking to reduce costs and presenting a strong coordination between agents, which generate a very cohesive agro-industrial structure, and consequently, outstanding competitiveness.

Nevertheless, despite the many companies producing regular chicken meat in Brazil, only one applies organic production methods, and in a very small scale (Korin2012). One of the strongest barrier for the expansion of this production model is the price of the final product-, which is usually two to three fold higher than regular ones. According to Buainain *et al*. (2007) the cost of this production model, depending on the product and the season, it can be200% more expensive than that achieved by conventional industrial systems.

Differently fromthe conventional system, the organic system does not have a well-structured supply chain, resulting in several limitations, such as low availability of grains, especially of corn and soybeans, both in terms of quantity and quality, throughout the year (Korin, 2012).

Additionally, the standards imposed by the rules of organic production, such as forbidding the use of synthetic amino acids, negatively affecting production efficiency by preventing the expression of the maximum production potential of broilers. Organic broilers are produced, processed, distributed, and marketed under specific norms, which are verified and certified by third-party bodies or through voluntary participatory certification process (Brasil, 2011). The rules for organic broiler production in Brazil are currently established by Act n. 10.831 (Brasil, 2003) and its normative instructions (NI), especially NI n. 46 and NI n.17, which specify the rules for the production of livestock and crops under organic systems (Brasil, 2011). Considering the lack of information on the nutritional and financial viability of organic production, this paper focuses on the attempt to replace synthetic sources of amino acids and vitamins commonly used for poultry by natural sources. This study aimed at identifying natural amino acid sources that may be allowed by organic livestock production regulations and certification bodies, and that may provide positive results in organic chicken production.

MATERIALS AND METHODS

The study was conducted at the experimental facilities of Korin Agropecuária Ltd., located in the municipality of Ipeúna, state of São Paulo, Brazil, between February and April of 2012. Seven hundred and twenty one-d-old male Cobb broilers were vaccinated at the hatchery against Marek's disease, avian fowlpox, infectious bursal disease, and coccidiosis. Average initial body weight was 46.04g ± 0.89g.

Birds were housed in a conventional open-sided masonry chicken house (8.0m x 30.0m), with concrete floors, 3.0m ceiling height and rood covered with clay tiles. Gas hoods were used for brooding in the first experimental week. The experimental housed was equipped with four fans for air circulation, and the thermal comfort range and lighting program followed the recommendations of the genetic strain manual. Average minimum and maximum temperatures of 22.3°C and 31.4°C (average of 26.8°C) were recorded. The experiment lasted 42 days.

The chicks were allocated to 24 pens (1.0m x 2.5m) of 30 birds each, thereby complying with the requirement of a maximum density of 12 birds/m². The experimental diets were based on corn and soybean meal, formulated according to the nutritional recommendations of Rostagno *et al*.,(2011), with no inclusion of coccidio stats, antibiotics, or animal byproducts. A 5-phasefeeding strategy was applied, and included pre-starter (1-7 days of age), starter (8-21 days), grower I (22-30 days), grower II (31-37 days), and finisher (38-42 days) periods. Feed and water were available *ad libitum*.

The treatments consisted of four diets: a control diet, containing the conventional synthetic amino acid DL-methionine 99% and choline chloride 60%; a diet where synthetic methionine was replaced by a vegetable methionine source, primarily extracted from soybeans, which, according to the manufacturer, has 99% methionine content; a diet where choline chloride was replaced by a natural choline source in the form of phosphatidylcholine, a product of the company Techno feed marketed under the trade name Biocholine Powder®; and a diet where both synthetic DL-methionine and choline chloride were replaced by their respective natural sources (Table 1). The ingredients and the nutritional composition of the experimental diets are shown in Tables 4, 5 and 6.

Both alternative products were included at the levels recommended by the manufacturers. Natural choline was added to the diets at the ratio 1/3 of

Table 1 – Description of the abbreviations of treatments.

Treatments	Abbreviations	Source of methionine	Source of choline
Control	Control	99% DL-methionine	choline chloride at 60%
Alternative methionine source	VM	vegetable methionine	choline chloride at 60%
Alternative choline source	AC	99% DL-methionine	choline as phosphatidylcholine
Alternative methionine and choline sources	VMAC	vegetable methionine	choline as phosphatidylcholine

Treatments evaluated in the article: Dietary supplementation of alternative methionine and choline in the production of organic broilers in Brazil.

choline chloride 60%, and vegetable methionine was included at the same ratio as 99% DL-methionine.

Live performance parameters were evaluated for the periods 1 to 21 and 1 to 42 days of age. Body weight gain (BW) was calculated as the difference between final weight and initial weight for each investigated period. Birds were weighed per pen (experimental unit). Feed intake was calculated as the difference between the total feed supplied and feed residues remaining at the end of each feeding phase. Feed conversion rate (FCR) was calculated as the ratio between total feed intake and weight gain, corrected for mortality, as proposed by Sakomura & Rostagno (2007). Mortality (MO) was daily recorded. The production efficiency index was calculated using the following equation: PEI = (BW* (100 - MO))/Age*FCR)*100.The evaluation of the production cost took into consideration the price of feedstuffs and a carcass yield of 75%. The price of the final product was obtained in large supermarket chains.

Broilers were distributed in a completely randomized design, consisting of four treatments with six replicates of 30 broilers each, totaling 180 birds per treatment. Data were statistically analyzed using Sisvar software (Ferreira, 2008),and the differences between treatments were compared by the Student-Newman Keuls test (SNK) at5% significance levels. Data on mortality was subjected to log (x + c) transformation prior the statistical tests.

RESULTS AND DISCUSSION

Broilers fed the vegetal source of DL-methionine (VM) presented lower daily weight gain feed intake ($p<0.05$) during both evaluated periods compared with the control group (Table 2). When only choline chloride was replaced by alternative source of choline chloride 60% (AC), performance variables no performance differences were detected relative to the control group during the same periods (Table 2). The substitution of both synthetic DL-methionine 99% and choline chloride60% (VMAC) resulted in lower daily weight gain, final body weight, and feed intake compared with the control group (Table 2). In addition, broilers in the groups fed VM presented worse feed conversion ratio ($p<0.05$) than the AC and the control groups considering the entire experimental period (1-42 days). No differences in mortality were observed among groups in any of the periods.

Based on the information that both products contained the same percentage of methionine, the lower performance observed when synthetic DL-methionine 99% was replaced by the methionine of vegetable source (VM) was possibly due to differences in the absorption of both sources of amino acids. Partridge *et al*. (1985) reported that amino acid absorption depends on their source, and that the absorption of synthetic amino acids is more efficient than amino acids available in vegetal sources. The same authors highlighted that free (synthetic) amino

Table 2 – Performance parameters (mean ± standard error): average daily gain (ADG) final body weight (FBW), feed intake (FI), feed conversion ratio (FCR), and mortality (MO) of broilers fed the experimental diets during the periods of 1 to 21 and 1 to 42 days.

Treatment	ADG (g)	FBW (g)	FI (g)	FCR	MO*
			1 to 21 days of age		
Control	38.14 ± 1.02a	844.92 ± 21.31a	1271.00 ± 33.52a	1.57 ± 0.04a	3.33 ± 1.72a
VM	32.65 ± 0.69b	730.68 ± 14.63b	1149.14 ± 9.51b	1.65 ± 0.02a	3.88 ± 2.18a
AC	37.63 ± 1.01a	833.89 ± 21.23a	1323.95 ± 33.76a	1.64 ± 0.04a	6.66 ± 3.10a
VMAC	33.06 ± 0.41b	738.38 ± 9.02b	1163.21 ± 23.54b	1.63 ± 0.01a	8.33 ± 1.42a
CV (%)	5.74	5.39	5.38	5.77	12.24
			1 to 42 days of age		
Control	59.24 ± 0.81a	2488.16 ± 34.09a	4727.95 ± 99.32a	1.91 ± 0.02b	4.44 ± 2.04a
VM	52.26 ± 0.69b	2195.18 ± 29.03b	4573.51 ± 54.64b	2.07 ± 0.00a	8.33 ± 2.54a
AC	60.94 ± 1.00a	2559.83 ± 42.32a	4831.79 ± 54.05a	1.89 ± 0.02b	8.88 ± 3.29a
VMAC	51.53 ± 0.73b	2164.51 ± 30.68b	4494.49 ± 67.12b	2.06 ± 0.00a	11.66 ± 1.42a
CV (%)	3.58	3.58	3.75	2.17	11.8

Means followed by the same letter in the same column are not statistically different at $p<0.05$ by by the SNK test.

Control = feed containing 99% DL-methionine and choline chloride 60%; VM = feed containing vegetable methionine and choline chloride 60%, AC = feed containing 99% DL-methionine and choline in the form of phosphatidylcholine; VMAC = feed containing vegetable methionine and choline in the form of phosphatidylcholine.

* Values transformedlog (x+c)

CV = Coefficientofvariation

Table3 – Effect of treatments on production efficiency index (PEI), feed costs, and the cost of production of 100 kg of meat.

Treatments	EPI	*Cost	**Feed intake	***Cost to produce 100 kg of meat	****Average price of chicken meat / Kg
Control	295.60a	R$ 64.60	254.66	R$ 164.51	R$ 9.00
VM	230.75b	R$ 64.80	276.71	R$ 179.31	R$ 13.77
AC	293.79a	R$ 64.60	252.00	R$ 162.79	R$ 9.00
VMAC	220.93b	R$ 64.80	274.60	R$ 177.94	R$ 13.77

Means followed by the same letter in the same column are not statistically different at p<0.05by the SNK test.

Control = feed containing 99% DL-methionine and choline chloride 60%; VM = feed containing vegetable methionine and choline chloride 60%, AC = feed containing 99% DL-methionine and choline in the form of phosphatidylcholine; VMAC = feed containing vegetable methionine and choline in the form of phosphatidylcholine.

* Cost of 100 kg of feed, considering the cost of the raw materials obtained in the state of São Paulo in 2012.

** Feed intake required to produce 100 kg of meat

*** Cost to produce 100 kg of meat, considering only feed and a carcass yield of 75%.

**** Sales price in the market of organic and antibiotic-free chickens.

acids are more available to animals, resulting in a faster absorption.

The absorption of the plant methionine source used in the present study was possibly lower than that of synthetic methionine, and considering that methionine is the first limiting amino acid in broiler nutrition, this resulted in worse performance. In contrast, when only choline chloride 60% was substituted by AC, broiler performance is not statistically influenced. This is probably due to the fact that broilers are able to synthesize choline when its precursors are available in adequate amounts (Lisboa *et al.*, 2014). In addition,

Table 4 – Ingredients and calculated nutritional composition of the experimental diets fed during the initial and grower phases

Ingredients (%)	Initial (1-7 days of age)				Growth (8-21 days of age)			
	Control	VM	AC	VMAC	Control	VM	AC	VMAC
Corn	58,34	58,34	58,38	58,38	56,83	56,83	56,86	56,86
Full fat soybean	---	---	---	---	12,60	12,60	12,60	12,60
Soybean meal	38,40	38,40	38,40	38,40	27,50	27,50	27,50	27,50
Dicalcium phosphate	1,10	1,10	1,10	1,10	1,00	1,00	1,00	1,00
Limestone	1,00	1,00	1,00	1,00	0,95	0,95	0,95	0,95
NaCl	0,38	0,38	0,38	0,38	0,40	0,40	0,40	0,40
Mineral/vitamin premix[1]	0,28	0,28	0,28	0,28	0,28	0,28	0,28	0,28
L-lysine HCl	0,13	0,13	0,13	0,13	0,10	0,10	0,10	0,10
DL-methionine 99	0,27	---	0,27	---	0,26	---	0,26	---
Alternative methionine	---	0,27	---	0,27	---	0,26	---	0,26
L-threonine	0,03	0,03	0,03	0,03	0,02	0,02	0,02	0,02
Choline chloride 60%	0,06	0,06	---	---	0,055	0,055	---	---
Phosphatidylcholine	---	---	0,02	0,02	---	...	0,018	0,018
Phytase	0,01	0,01	0,01	0,01	0,01	0,01	0,01	0,01
Total	100	100	100	100	100	100	100	100
Metabolizable energy (Kcal/Kg)	2.946	2.946	2.946	2.946	3.080	3.080	3.080	3.080
Crude protein (%)	23,00	23,00	23,00	23,00	22,49	22,49	22,49	22,49
Calcium (%)	0,88	0,88	0,88	0,88	0,83	0,83	0,83	0,83
Sodium (%)	0,18	0,18	0,18	0,18	0,18	0,18	0,18	0,18
Fosforo (%)	0,58	0,58	0,58	0,58	0,56	0,56	0,56	0,56
Total de lysine (%)	1,33	1,33	1,33	1,33	1,28	1,28	1,28	1,28
Total methionine + cystine (%)	1,00	1,00*	1,00	1,00*	0,97	0,97*	0,97	0,97*
Total methionine (%)	0,63	0,63*	0,63	0,63*	0,61	0,61*	0,61	0,61*
Total threonine (%)	0,90	0,90	0,90	0,90	0,87	0,87	0,87	0,87
Choline (PPM)	1.409	1.409	1.409*	1.409*	1.360	1.360	1.360*	1.360*

Control = feed containing 99% DL-methionine and choline chloride 60%; VM = feed containing vegetable methionine and choline chloride 60%, AC = feed containing 99% DL-methionine and choline in the form of phosphatidylcholine; VMAC = feed containing vegetable methionine and choline in the form of phosphatidylcholine

[1] Supplemented per kilogram of premix: Fe 7200mg; Cu 1440mg; Mn 14,40g; Zn 10,80g; I 180mg; Se 36mg; Vit A 1200000IU; Vit D3 300000IU; Vit E 3000IU; Vit K3 436,80mg; Vit B1 288mg; Vit B2 720mg; Niacin 4000mg; Pantothenic acid 1560mg; Vit B6 384mg; Folic acid 200mg; Vit B12 1880mg.

* Calculated in the agreement with the security level available for manufacturer.

Table5 – Ingredients and calculated nutritional composition of the experimental diets fed during the fattening I and fattening II phases

Ingredients (%)	Fattening I (22-30 days of age)				Fattening II (31-37 days of age)			
	Control	VM	AC	VMAC	Control	VM	AC	VMAC
Corn	58,37	58,37	58,40	58,40	64,87	64,87	64,90	64,90
Full fat soybean	24,70	24,70	24,70	24,70	18,80	18,80	18,80	18,80
Soybean meal	13,90	13,90	13,90	13,90	13,50	13,50	13,50	13,50
Dicalcium phosphate	0,90	0,90	0,90	0,90	0,90	0,90	0,90	0,90
Limestone	0,80	0,80	0,80	0,80	0,80	0,80	0,80	0,80
NaCl	0,40	0,40	0,40	0,40	0,40	0,40	0,40	0,40
Mineral/vitamin premix[2]	0,26	0,26	0,26	0,26	0,26	0,26	0,26	0,26
L-lysine HCl	0,24	0,24	0,24	0,24	0,17	0,17	0,17	0,17
DL-methionine 99	0,28	---	0,28	---	0,23	---	0,23	---
Alternative methionine	---	0,28	---	0,28	---	0,23	---	0,23
L-threonine	0,09	0,09	0,09	0,09	0,02	0,02	0,02	0,02
Choline chloride 60%	0,050	0,050	---	---	0,045	0,045	---	---
Phosphatidylcholine	---	---	0,016	0,016	---	---	0,015	0,015
Phytase	0,01	0,01	0,01	0,01	0,01	0,01	0,01	0,01
Total	100	100	100	100	100	100	100	100
Metabolizable energy (Kcal/Kg)	3.250	3.250	3.250	3.250	3.250	3.250	3.250	3.250
Crude protein (%)	21,00	21,00	21,00	21,00	19,03	19,03	19,03	19,03
Calcium (%)	0,74	0,74	0,74	0,74	0,73	0,73	0,73	0,73
Sodium (%)	0,18	0,18	0,18	0,18	0,184	0,184	0,184	0,184
Fosforo (%)	0,54	0,54	0,54	0,54	0,51	0,51	0,51	0,51
Total de lysine (%)	1,28	1,28	1,28	1,28	1,10	1,10	1,10	1,10
Total methionine + cystine (%)	0,95	0,95*	0,95	0,95*	0,86	0,86*	0,86	0,86*
Total methionine (%)	0,61	0,61*	0,61	0,61*	0,54	0,54*	0,54	0,54*
Total threonine (%)	0,87	0,87	0,87	0,87	0,73	0,73	0,73	0,73
Choline (PPM)	1.260	1.260	1.260*	1.260*	1.160	1.160	1.160*	1.160*

Control = feed containing 99% DL-methionine and choline chloride 60%; VM = feed containing vegetable methionine and choline chloride 60%, AC = feed containing 99% DL-methionine and choline in the form of phosphatidylcholine; VMAC = feed containing vegetable methionine and choline in the form of phosphatidylcholine

[2]Supplemented per kilogram of premix: Fe 6000mg; Cu 1200mg; Mn 12g; Zn 9000mg; I 150mg; Se 36mg; Vit A 1000000IU; Vit D3 250000IU; Vit E 2500IU; Vit K3 364mg; Vit B1 240mg; Vit B2 600mg; Niacin 3200mg; Pantothenic acid 1400mg; Vit B6 320mg; Folic acid 160mg; Vit B12 1560mcg.

* Calculated in the agreement with the security level available for manufacturer.

one of the main roles of choline is to donate methyl radicals, as well as methionine (Silva *et al.*, 2010). These features may explain the inferior live performance when both synthetic methionine and choline were replaced in the diet (VMAC)compared with broilers consuming the diet where choline chloride 60% (AC) was replaced.

Although the use of the vegetal methionine source resulted in inferior performance compared with synthetic DL-methionine 99%, it is a valuable ingredient to be used in organic production systems where synthetic amino acids are not allowed.

Based on production data and also on the observations of the company's technical team (Korin, 2012), the use of the vegetal source of methionine for broilers and egg layers managed in organic systems presented superior results when compared with birds not supplemented with the vegetal methionine source. New research on the bioavailability of nutrients from natural sources may promote further improvements of the production indices.

The emergence of new sources of choline, as well as the possibility to add vegetable sources of methionine to broiler diets, are a breakthrough in the search for strengthening the production chain of organic chickens. Their use improves production efficiency and reduces the need for incorporating new protein sources in feeding strategies. Such aspects will contribute to reduce production costs and to minimize the excretion of minerals by birds, particularly nitrogen and phosphorus.

Financially, the lower productive efficiency index is compensated by the market value of organic animal products, which is generally much higher than that obtained by conventional products (Table 3). According with Demattê Filho (2014), the production of organic broilers at commercial scale only succeeds due to price differences, since there is a reduction in yield.

Table 6 – Ingredients and calculated nutritional composition of the experimental diet fed during the Final phases

Ingredients (%)	Final (38-42 days of age)			
	Control	VM	AC	VMAC
Corn	66,65	66,65	66,68	66,68
Full fat soybean	22,00	22,00	22,00	22,00
Soybean meal	8,90	8,90	8,90	8,90
Dicalcium phosphate	0,70	0,70	0,70	0,70
Limestone	0,80	0,80	0,80	0,80
NaCl	0,40	0,40	0,40	0,40
Mineral/vitamin premix[3]	0,26	0,26	0,26	0,26
L-lysine HCl	0,06	0,06	0,06	0,06
DL-methionine 99	0,18	---	0,18	---
Alternative methionine	---	0,18	---	0,18
Choline chloride 60%	0,035	0,035	---	---
Phosphatidylcholine	---	---	0,012	0,012
Phytase	0,01	0,01	0,01	0,01
Total	100	100	100	100
Metabolizable energy (Kcal/Kg)	3.310	3.310	3.310	3.310
Crude protein (%)	18,05	18,05	18,05	18,05
Calcium (%)	0,68	0,68	0,68	0,68
Sodium (%)	0,18	0,18	0,18	0,18
Fosforo (%)	0,48	0,48	0,48	0,48
Total de lysine (%)	0,96	0,96	0,96	0,96
Total methionine + cystine (%)	0,79	0,79*	0,79	0,79*
Total methionine (%)	0,48	0,48*	0,48	0,48*
Total threonine (%)	0,68	0,68	0,68	0,68
Choline (PPM)	1.073	1.073	1.073*	1.073*

Control = feed containing 99% DL-methionine and choline chloride 60%; VM = feed containing vegetable methionine and choline chloride 60%, AC = feed containing 99% DL-methionine and choline in the form of phosphatidylcholine; VMAC = feed containing vegetable methionine and choline in the form of phosphatidylcholine

[3]Supplemented per kilogram of premix: Fe 7500mg; Cu 1500mg; Mn 15g; Zn 11,25g; I 187,50mg; Se 45mg; Vit A 1250000IU; Vit D3 306250IU; Vit E 2500IU; Vit K3 455mg; Vit B1 300mg; Vit B2 750mg; Niacin 4000mg; Pantothenic acid 1750mg; Vit B6 400m; Folic acid 200mg; Vit B12 1950mcg.

* Calculated in the agreement with the security level available for manufacturer.

The emergence of alternative products, such as those evaluated in the present study, demonstrates the gradual development of the supply chain for the commercial production of chickens in organic systems. Such technological development are promising, and will make organic poultry production more attractive to the farmers and more accessible to the population.

CONCLUSIONS

The replacement of choline chloride 60% by choline in the form of phosphatidylcholine did not affect broiler performance. However, the substitution of DL-methionine 99% by a vegetable source of methionine resulted in worse performance.

The bioavailability of natural nutrients, especially of natural methionine sources, with respect to its ability to donate methyl radicals, should be further investigated in order to better evaluate its role in organic production.

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