

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/327619726>

# Sources of Betaine as methyl group donors in broiler diets

Article · June 2018

---

CITATIONS

0

READS

133

6 authors, including:



Rita de Cassia Alves Pereira  
Brazilian Agricultural Research Corporation (EMBRAPA)

70 PUBLICATIONS 366 CITATIONS

[SEE PROFILE](#)



José Fernando Machado Merten  
University of São Paulo

98 PUBLICATIONS 1,198 CITATIONS

[SEE PROFILE](#)



Flavio Alves Longo  
NOVUS do Brasil

49 PUBLICATIONS 572 CITATIONS

[SEE PROFILE](#)



Leonardo Willian de Freitas  
Universidade Federal de Mato Grosso (UFMT)

22 PUBLICATIONS 157 CITATIONS

[SEE PROFILE](#)

Some of the authors of this publication are also working on these related projects:



Nutritional value of mealworm larvae meal-fed broiler chickens [View project](#)



Measurement of true ileal calcium digestibility in foods and requirement of calcium digestible for broiler chicken [View project](#)

## Sources of Betaine as Methyl Group Donors in Broiler Diets

### Research Article

R. Pereira<sup>1\*</sup>, J.F.M. Menten<sup>1</sup>, F.A. Longo<sup>2</sup>, M.B. Lima<sup>1</sup>, L.W. Freitas<sup>1</sup> and K.C. Zavarize<sup>1</sup>

<sup>1</sup> Department of Animal Science, University of São Paulo, Piracicaba, Brazil  
<sup>2</sup> Research and Development, Btech, Valinhos/SP, Brazil

Received on: 22 Jul 2017  
 Revised on: 22 Aug 2017  
 Accepted on: 15 Sep 2017  
 Online Published on: Jun 2018

\*Correspondence E-mail: [rafaelapereira@usp.br](mailto:rafaelapereira@usp.br)  
 © 2010 Copyright by Islamic Azad University, Rasht Branch, Rasht, Iran  
 Online version is available on: [www.ijas.ir](http://www.ijas.ir)

### ABSTRACT

Betaine is donor of methyl groups and can partially replace methionine in diets for this purpose. The objective of this study was to evaluate three sources of betaine in partial substitution of methionine supplement in broiler diets. The Cobb-500 broiler chickens were allocated in a completely randomized experimental design with 5 treatments and 7 replicates of 49 birds each. The positive control treatment consisted of standard level of digestible methionine, the negative control was the digestible methionine reduced by 17% and the other three treatments consisted of the negative control diet supplemented with natural betaine (95%) or hydrochloride betaine (72%), HCl 1 and HCl 2 that had the same composition, and were obtained from two different manufacturers. The performance was evaluated from 7 to 21 days, 7 to 35 days and 7 to 43 days. At 43 days, the carcass and carcass parts (breast, thigh+drumstick, liver and abdominal fat) were determined and an economic analysis of each diet was performed. The performance results of the negative control were similar to the other treatments; however, there was a reduction in breast meat yield of birds fed the negative control compared to positive control and betaine HCl 2. The birds from the negative control had the lowest breast meat yield and the highest thigh + drumstick yield. The chickens fed betaine HCl 2 had greater amount of breast in the carcass (42.85 vs. 41.17%) and the cost of production of breast was reduced (US\$1.941 vs. US\$ 2.042) compared to the negative control. There was not difference between treatments in carcass yield, percentage of liver and abdominal fat. There was a tendency to higher abdominal fat deposition in carcass in animals fed with the negative control. As a conclusion, the inclusion of betaine in the diets of chickens containing restricted levels of methionine is economically feasible, reducing the cost of breast meat production.

**KEY WORDS** breast yield, broiler chickens, carcass traits, methyl groups.

### INTRODUCTION

Betaine which is derived from the amino acid glycine has three reactive methyl groups and is naturally synthesized in large amounts by some plants and microorganisms (Eklund *et al.* 2005). In the past, sugar beet has been the main source of betaine, a by-product of the sugar industry (Lever and Slow, 2010), but recently several synthetic sources of betaine are also available on the market to economically replace methionine. The commercially available sources of

betaine are anhydrous (natural) betaine, betaine monophosphate and betaine hydrochloride. Methionine, choline and betaine are the main sources of methyl groups ( $\text{CH}_3$ ) in animal diets (Ratriyanto *et al.* 2009). Among these three compounds, only betaine can act directly as a methyl group donor (Kettunen *et al.* 2001). The methyl groups of choline become available when the molecule is oxidized to betaine in a two-step enzymatic reaction. Methyl groups are necessary for the synthesis of various physiologically essential compounds, such as methionine, carnitine, creatine, phos-

pholipids, adrenal hormones, RNA and DNA (Santana *et al.* 2014). The action of betaine as a donor of methyl groups in remethylation permits methionine to be directed toward protein synthesis, reducing the action of this amino acid on methyl group donation (Metzler-Zebeli *et al.* 2009). Betaine transfers a methyl group *via* the enzyme betaine-homocysteinemethyl transferase (BHMT) to become dimethylglycine (Alirezaei *et al.* 2011). Methionine is one the most limiting amino acids, playing crucial role in body protein synthesis, and therefore it would be beneficial to spare its function as a methyl donor (Sun *et al.* 2008). It has been shown that choline must first be activated and then converted to betaine before the methyl groups are liberated to methylation cycles (Sun *et al.* 2008). In contrast, betaine contains three methyl groups in this structure and donates these in several metabolic reactions, therefore allowing it to be used as an effective compound to spare dietary methionine as methyl donor group (Alirezaei *et al.* 2011).

Betaine significantly improved the breast meat yield and growth performance of broilers, however, other studies did not show effect on chickens performance (Pereira *et al.* 2010).

The addition of betaine in a diet deficient in methionine can significantly improve antioxidant defenses and meat quality, decreasing lipid peroxidation in the breast muscle of broiler chickens (Alirezaei *et al.* 2011). Extensive studies with pigs, ducks, laying hens and broiler chickens (Leng *et al.* 2016) suggested that betaine supplementation was effective in reducing body fat deposition.

In addition, betaine has been shown to protect cells from osmotic stress and allow them to continue regular metabolic activities in conditions that would normally inactivate the cell (Hamidi *et al.* 2010). He *et al.* (2015) demonstrated that dietary betaine may to be a potential nutritional strategy to prevent heat stress related depression in performance and carcass characteristics of broilers.

The strategy of partial replacement of synthetic methionine with betaine is of interest in poultry production since it permits to reduce the feed costs. Therefore, the objective of the present study was to evaluate the effects of three sources of betaine, a natural betaine and two synthetic sources as partial replacement of supplemental methionine in diets of broiler chickens.

## MATERIALS AND METHODS

### Housing and feeding

All the procedures used in this experiment were approved by the institutional animal care and use committee of the College of Agriculture “Luiz de Queiroz”, University of Sao Paulo.

A total of 1715 day-old male Cobb-500 broiler chicks were raised in floor pens from zero to 43 days of age. The environmental conditions were controlled for each phase (starter, grower and finisher). A completely randomized design consisting of five treatments and seven replicates was adopted with 49 birds per experimental unit. The positive control treatment consisted of standard level of digestible methionine, the negative control was the digestible methionine reduced by 17% and the other three treatments consisted of the negative control diet supplemented with natural betaine (95%) or hydrochloride betaine (72%), HCl 1 and HCl 2. During the pre-experimental phase (0 to 7 days), all birds received same basal diet based on corn grain and soybean meal according to the ingredient composition and nutritional specifications the Brazilian Tables (Rostagno *et al.* 2005). After the pre-experimental phase, the birds were weighted and assigning to the experimental diets (Tables 1, 2 and 3). The 5 treatments consisted of: 1) positive control group: contained the standard levels of digestible methionine (0.505 g/kg from 7-21 d, 0.484 g/kg from 21-35 d and 0.453 g/kg from 35-43 d); 2) negative control diet, in which: the digestible methionine level was reduced by 17% in all rearing phases; 3) negative control + natural betaine (Manufacturer Betafin S1, Finnfeeds, Finland); 4) negative control + betaine HCl 1 (synthetic source; Manufacturer Nanchang Lifeng Industry and Trading Co., Ltd., China) and 5) negative control + betaine HCl 2 (synthetic source; Manufacturer Skystone Feed Co., Ltd., China). Natural betaine was extracted from the beet at 95% purity. The chemical formula of betaine is  $(CH_3)_3N-CH_2COO^-$  and its molecular weight is 117.15. Betaine HCl is a synthetic product (95% purity of betaine+HCl). Its chemical formula is  $(CH_3)_3N-CH_2COO^- + HCl$  and its molecular weight is 153.61. This betaine source contained 72% of pure betaine on a weight basis. The two sources of synthetic betaine (HCl 1 and HCl 2) with the same composition were provided by two different manufacturers. All sources of betaine were purchased in the local market.

There are indication (Eklund *et al.* 2005) that the capacity of betaine to donate methyl groups corresponds to 20% of the digestible methionine in the diet. Thus, the practical recommendation in the 20% in the limit of substitution of betaine for methionine. A 17% reduction was adopted considering around  $600 \text{ g kg}^{-1}$  as a minimum for the methionine + cysteine to lysine ratio.

Ratriyanto *et al.* (2009) described that values lower than  $600 \text{ g kg}^{-1}$  on this ratio can contribute to a cysteine deficiency, what was not corrected or supplied by a betaine supplementation. Around 800 to 900 g of DL-methionine was replaced with about 600 to 800 g of the betaine source per ton of feed.

**Table 1** Composition of the diets (kg/ton) during the starter phase (7-21 days)

Ingredients	Control+	Control-	Natural betaine	HCl betaine
Corn	604.62	601.74	604.46	603.72
Soybean meal	339.22	341.05	339.43	339.56
Soybean oil	17.44	18.60	17.64	17.89
Limestone	8.90	8.90	8.90	8.90
Dicalcium phosphate	17.81	17.81	17.81	17.81
Inert (caolin)	-	0.87	-	-
Salt	3.91	3.91	3.91	3.59
Sodium bicarbonate	1.45	1.45	1.45	1.91
Betaine source	-	-	0.64	0.85
DL-methionine	2.16	1.28	1.28	1.28
L-lysine HCl	1.53	1.53	1.53	1.53
Vitamin premix <sup>1</sup>	1.00	1.00	1.00	1.00
Mineral premix <sup>2</sup>	0.50	0.50	0.50	0.50
L-threonine	0.28	0.28	0.28	0.28
Coccidiostat <sup>3</sup>	0.55	0.55	0.55	0.55
Halquinol (60%)	0.03	0.03	0.03	0.03
Cholinechloride (60%)	0.58	0.58	0.58	0.58
Total (kg)	1000	1000	1000	1000
<b>Calculated composition</b>				
Men (kcal/kg)	2980	2980	2980	2980
Crude protein (CP) (%)	20.65	20.65	20.65	20.65
Digestible Lys (%)	1.113	1.113	1.113	1.113
Digestible Met + cys (%)	0.790	0.703	0.703	0.703
Digestible Met (%)	0.505	0.419	0.419	0.419
Digestible Thr (%)	0.723	0.723	0.723	0.723
Calcium (%)	0.88	0.88	0.88	0.88
Available P (%)	0.44	0.44	0.44	0.44
Potassium (%)	0.79	0.79	0.79	0.79
Sodium (%)	0.21	0.21	0.21	0.21

<sup>1</sup> Premix DSM nutritional products, composition per kg ration: vitamin A: 10000 IU; vitamin D<sub>3</sub>: 3000 IU; vitamin E: 40 IU; vitamin K<sub>3</sub>: 3.0 mg; vitamin B<sub>1</sub>: 2 mg; vitamin B<sub>2</sub>: 6 mg; vitamin B<sub>6</sub>: 4 mg; vitamin B<sub>12</sub>: 20 µg; Nicotinic acid: 40 mg; Pantothenic acid: 12 mg; Biotin: 0.15 mg; Folic acid: 1 mg and Selenium: 0.25 mg.

<sup>2</sup> Premix DSM nutritional products, composition per kg ration: Manganese: 80 mg; Iron: 50 mg; Zinc: 50 mg; Copper: 10 mg; Cobalt: 1 mg and Iodine: 1 mg.

<sup>3</sup> Coxistac 12%.

From 7-21 d, 21-35 d and 35-42 d the inclusion of natural betaine was 640, 620 and 580 g/ton of feed, respectively and, the inclusion of betaine hydrochloride was 850, 830 and 780 g/ton of feed for each rearing phase. This replacement follows the recommendation of the suppliers for betaine to methionine ingredient ratio of each betaine sources (1.37 and 1.03 for natural and synthetic sources, respectively). This calculation was done by considering the molecular weight of each molecule, an efficacy of the betaine as donor methyl groups compared to methionine and the betaine concentration of each source.

**Performance, yields of the carcass and cuts and diet cost**  
 Performance traits (live weight, weight gain, feed intake, feed conversion ratio and livability) were evaluated at 21, 35 and 42 days. On day 42, three birds from each experimental unit close to average pen weight ( $\pm 3\%$ ) were selected for carcass evaluation. The selected chicks were transported to the experimental slaughterhouse for carcass weight determination by stunning, bleeding, scalding, pluc-

king, evisceration, and cooling in a chiller. The fasting period before slaughter was approximately 10 h. Carcass yield (CY), breast meat yield (BY) and thigh + drumstick yield (TDY) as well as liver and abdominal fat percentages were determined.

The process of carcass and cut yields evaluation consisted of individual weighing of the animals before slaughter (live weight) and after fasting for about 10 h. Carcass yield was calculated as the ratio between hot carcass weight (without viscera, feet, and neck) and live weight. Breast yield and TDY were calculated as the ratio between their respective weights and cold carcass weight (without viscera, feet, and neck). Liver and abdominal fat percentages were determined as the ratio between their weights and hot carcass weight (without viscera, feet, and neck).

#### Statistical analysis

The data were analyzed by ANOVA and statistical difference measured by Duncan's test using the General Linear Model (GLM) procedure of [SAS \(2006\)](#).

**Table 2** Composition of the diets (kg/ton) during the grower phase (21-35 days)

Ingredients	Control+	Control-	Natural betaine	HCl betaine
Corn	640.83	639.87	640.93	640.19
Soybean meal	302.44	302.61	302.42	302.56
Soybean oil	20.81	21.60	20.96	21.20
Limestone	8.38	8.38	8.38	8.38
Dicalcium phosphate	16.18	16.18	16.18	16.18
Inert (caolin)	-	0.84	-	-
Salt	4.24	4.24	4.24	3.95
Sodium bicarbonate	0.51	0.51	0.51	0.96
Betaine source	-	-	0.62	0.83
DL-methionine	2.10	1.25	1.25	1.25
L-lysine HCl	1.82	1.81	1.81	1.82
Vitamin premix <sup>1</sup>	0.80	0.80	0.80	0.80
Mineral premix <sup>2</sup>	0.50	0.50	0.50	0.50
L-threonine	0.35	0.35	0.35	0.35
Coccidiostat <sup>3</sup>	0.55	0.55	0.55	0.55
Halquinol (60%)	0.03	0.03	0.03	0.03
Cholinechloride (60%)	0.46	0.46	0.46	0.46
Total (kg)	1000	1000	1000	1000
<b>Calculated composition</b>				
Men (kcal/kg)	3050	3050	3050	3050
Crude protein (CP) (%)	19.31	19.26	19.30	19.30
Digestible Lys (%)	1.049	1.049	1.049	1.049
Digestible Met + Cys (%)	0.755	0.671	0.671	0.671
Digestible Met (%)	0.484	0.400	0.400	0.400
Digestible Thr (%)	0.682	0.682	0.682	0.682
Calcium (%)	0.81	0.81	0.81	0.81
Available P (%)	0.41	0.41	0.41	0.41
Potassium (%)	0.73	0.73	0.73	0.73
Sodium (%)	0.20	0.20	0.20	0.20

<sup>1</sup> Premix DSM nutritional products, composition per kg ration: vitamin A: 8000 IU; vitamin D<sub>3</sub>: 2400 IU; vitamin E: 32 IU; vitamin K<sub>3</sub>: 2.4 mg; vitamin B<sub>1</sub>: 1.6 mg; vitamin B<sub>2</sub>: 4.8 mg; vitamin B<sub>6</sub>: 3.2 mg; vitamin B<sub>12</sub>: 16 µg; Nicotinic acid: 32 mg; Pantothenic acid: 7.2 mg; Biotin: 0.12 mg; Folic acid: 0.8 mg and Selenium: 0.2 mg.

<sup>2</sup> Premix DSM nutritional products, composition per kg ration: Manganese: 80 mg; Iron: 50 mg; Zinc: 50 mg; Copper: 10 mg; Cobalt: 1 mg and Iodine: 1 mg.

<sup>3</sup> Coxistac 12%.

## RESULTS AND DISCUSSION

### Performance

No significant differences in the performance results were observed between the treatments groups during the rearing phases (7-21, 7-35, and 7-42 days) (Tables 4, 5 and 6). These results were in agreement with those of [Pereira et al. \(2010\)](#) who did not find statistical difference between the negative control compared to the positive control. The basal level of digestible amino acids used in this study was based on the dietary specifications published in the Brazilian Tables ([Rostagno et al. 2005](#)).

For example, the recommended digestible methionine requirement by the chicks in the period 7-21 days, is 4.5 g (Table 4). In this study, due to greater feed intake of the birds, the digestible methionine intake was 6.2 g in the same period; a 17% reduction of the dietary methionine resulted in an intake of 5.1 g in the period, a value still greater than the requirement encountered in the Brazilian Table. Thus, this could explain, at least in part, the unexpected lack of effect of the methionine reduction observed. Also, the good environmental and health conditions, without any kind of challenge, may have contributed to the high

her feed intake as well as better efficiency of use of nutrients.

The results of this study disagree with the findings reported by [He et al. \(2015\)](#), who showed that betaine exerts positive effects in improving growth performance and meat qualities in chickens under heat stress.

[Amerah and Ravindran \(2015\)](#) reported that betaine supplementation reduced the impact of coccidia challenge and positively affected nutrient digestibility and the feed conversion ratio in chickens.

### Yield of carcass and cuts and diet cost

The analysis of the slaughter results in this research showed a reduction in breast yield of chickens receiving the negative control diet compared to the treatments positive control and betaine HCl 2 ((P<0.05), Table 7), indicating a possible inadequate supply of amino acids for the treatment in which digestible methionine was reduced by 17% and without betaine supplementation. Different mechanisms can be used by the animal to cope with a marginally deficient amino acids supply, such as a reduction in growth rate and a change in the composition of growth ([Conde-Aguilera et al. 2013](#)).

**Table 3** Composition of the diets (kg/ton) during the finisher phase (35-43 days)

Ingredients	Control+	Control-	Natural betaine	HCl betaine
Corn	671.22	670.32	671.32	670.63
Soybean meal	274.13	274.29	274.41	274.24
Soybean oil	22.14	22.89	22.28	22.51
Limestone	7.97	7.97	7.97	7.97
Dicalcium phosphate	14.65	14.65	14.65	14.65
Inert (caolin)	-	0.79	-	-
Salt	4.24	4.24	4.24	3.95
Sodium bicarbonate	0.14	0.14	0.14	0.14
Betaine source	-	-	0.58	0.78
DL-methionine	1.90	1.10	1.10	1.10
L-lysine HCl	1.93	-	-	-
Vitamin premix <sup>1</sup>	0.60	0.60	0.60	0.60
Mineral premix <sup>2</sup>	0.50	0.50	0.50	0.50
L-threonine	0.35	0.35	0.35	0.35
Choline chloride (60%)	0.23	0.23	0.23	0.23
Total (kg)	1000	1000	1000	1000
<b>Calculated composition</b>				
Men (kcal/kg)	3100	3100	3100	3100
Crude protein (CP) (%)	18.27	18.27	18.27	18.27
Digestible Lys (%)	0.992	0.992	0.992	0.992
Digestible Met + Cys (%)	0.714	0.634	0.634	0.634
Digestible Met (%)	0.453	0.374	0.374	0.374
Digestible Thr (%)	0.645	0.645	0.645	0.645
Calcium (%)	0.75	0.75	0.75	0.75
Available P (%)	0.37	0.37	0.37	0.37
Potassium (%)	0.69	0.69	0.69	0.69
Sodium (%)	0.19	0.19	0.19	0.19

<sup>1</sup> Premix DSM nutritional products, composition per kg ration: vitamin A: 6000 IU; vitamin D<sub>3</sub>: 1800 IU; vitamin E: 24 IU; vitamin K<sub>3</sub>: 1.8 mg; vitamin B<sub>1</sub>: 1.2 mg; vitamin B<sub>2</sub>: 3.6 mg; vitamin B<sub>6</sub>: 2.4 mg; vitamin B<sub>12</sub>: 12 µg; Nicotinic acid: 24 mg; Pantothenic acid: 7.2 mg; Biotin: 0.09 mg; Folic acid: 0.6 mg and Selenium: 0.15 mg.

<sup>2</sup> Premix DSM nutritional products, composition per kg ration: Manganese: 80 mg; Iron: 50 mg; Zinc: 50 mg; Copper: 10 mg; Cobalt: 1 mg and Iodine: 1 mg.

<sup>3</sup> Coxistac 12%.

**Table 4** Live weight (LW) at 21 days, weight gain (WG), feed intake (FI), feed conversion (FCR), and livability (LB) of birds in different treatments

Treatments	21 days		7-21 days		
	LW (g)	WG (g)	FI (g)	FCR	LB (%)
Control+	1019	826	1233	1.492	100
Control-	1031	837	1231	1.471	98.83
Natural betaine	1023	828	1245	1.504	99.42
Betaine HCl 1	1016	822	1230	1.497	99.13
Betaine HCl 2	1022	828	1224	1.478	99.13
P-value	0.63	0.74	0.74	0.3	0.64
SEM	6.91	10.62	10.62	0.01	0.41

SEM: standard error of the means.

**Table 5** Live weight (LW) at 35 days, weight gain (WG), feed intake (FI), feed conversion (FCR), and livability (LB) of birds in different treatments from 7 to 35 days

Treatments	35 days		7-35 days		
	LW (g)	WG (g)	FI (g)	FCR	LB (%)
Control+	2363	2166	3642	1.682	100
Control-	2371	2174	3666	1.686	98.57
Natural betaine	2361	2166	3640	1.680	99.14
Betaine HCl 1	2358	2161	3599	1.665	98.56
Betaine HCl 2	2348	2150	3578	1.664	99.13
P-value	0.94	0.94	0.36	0.37	0.17
SEM	20.09	19.72	33.29	0.009	0.44

SEM: standard error of the means.

**Table 6** Live weight (LW) at 42 days, weight gain (WG), feed intake (FI), feed conversion (FCR), and livability (LB) of birds in different treatments from 7 to 42 days

Treatments	42 days		7-42 days		
	LW (g)	WG (g)	FI (g)	FCR	LB (%)
Control+	2976	2765	5040	1.823	99.12
Control-	2983	2778	5088	1.834	98.25
Natural betaine	2997	2794	5057	1.809	98.25
Betaine HCl 1	2975	2769	5014	1.811	98.54
Betaine HCl 2	2959	2748	4975	1.811	98.54
P-value	0.94	0.95	0.62	0.77	0.81
SEM	33	40	52	0.01	0.57

SEM: standard error of the means.

There was not difference between treatments in carcass yield, percentage of liver and abdominal fat (Table 7). Although % abdominal fat was not statistically different, it should be noted that the birds supplemented with betaine tended to have less fat than the negative control.

Abdominal fat is a key indicator used to judge total body fat (Chen *et al.* 2011). The deposition of abdominal fat was 13% higher in birds from the negative control compared to those fed diets with betaine. This result may be due to an indirect effect of betaine on the synthesis of carnitine, which is responsible for the transfer of long-chain fatty acids to the inner membrane of the mitochondria where beta-oxidation occurs (De Ridder and Van Dam, 1975). L-carnitine is an amine compound biosynthesized primary in the liver from the amino acids, lysine and methionine. Farrokhyan *et al.* (2014) showed that the supplementation of gembifrozil and L-carnitine in the diet of broilers influence lipid redistribution, intramuscular fat and carcass quality and may result in improved carcass quality. Consequently, betaine may reduce fat deposition in animals. Leng *et al.* (2016) showed that although the betaine supplementation did not affect growth performance of broilers, it was effective in reducing abdominal fat deposition in a dose-dependent manner, which was probably caused by a combination of a decrease in fatty acid synthesis and an increase in beta-oxidation.

The birds from the negative control resulted in the lowest BY and highest in the TDY ( $P<0.05$ ). This higher TDY may be explained by the high deposition of fat in these cuts. Chickens fed the positive control and betaine HCl 2 diets deposited more breast meat in the carcass (Table 7) and breast meat production costs were also lower for these animals (Table 8). These data suggested that 17% of replacement of digestible methionine by betaine did not impair the function of the protein synthesis. In animal nutrition, betaine is widely discussed as a carcass modifier due to its lipotropic and growth-promoting effects (Eklund *et al.* 2005).

A higher deposition of breast meat in carcasses of chickens fed diets supplemented with betaine has also been observed in the studies of Steve-Garcia and Mack (2000), Waldroup *et al.* (2006) and Zhan *et al.* (2006), irrespective of the percent reduction in methionine.

These current results indicate that carcass traits, such as BY and TDY, may be affected in broilers fed diets containing reduced levels of methionine and supplemented with betaine.

In agreement with earlier study, Coma *et al.* (1995) suggested that supplementing betaine can reduce protein turnover rate resulting in higher nitrogen retention which, in turn, has a positive effect on carcass leanness.

Carcasses with less fat can be resulted of use of betaine sparing methionine, leaving more of the available essential amino acid (methionine) for protein synthesis. In this case, better use of dietary nutrients would leave fewer amino acids for deamination and decreased synthesis into adipose tissue (Wallis, 1999).

The positive effect of betaine on lean meat probably was not related to higher water deposition on meat. Barbosa *et al.* (2009) reported that the betaine dietary did not cause higher drip loss in the meat (osmoprotection function).

The lower BY in chickens of the negative control group compared to the betaine HCl 2 treatment resulted in a higher cost per kg of breast meat produced (Tables 7 and 8), even when considering the lower feed cost due to lower supplementation with DL-methionine. The cost reduction per kg of breast meat obtained with the betaine HCl 2 treatment in relation to the negative control was about 5% ( $P<0.05$ ).

Intermediate costs were observed for the other treatments, with the absence of a significant difference. In view of the high value attributed to breast meat compared to other cuts, there is interest in increasing BY through nutrition (Leclercq, 1998), or at least that the nutritional strategies adopted do not compromise breast meat deposition in chickens.

**Table 7** Carcass yield (CY), breast meat yield (BY), thigh + drumstick yield (TDY), and liver (L) and abdominal fat (AF) percentage of the chickens at slaughter (43 days of age)

Treatments	CY (%)	BY (%)	TDY (%)	L (%)	AF (%)
Control+	70.61	42.72 <sup>a</sup>	31.07 <sup>b</sup>	2.35	2.51
Control-	70.91	41.17 <sup>b</sup>	32.25 <sup>a</sup>	2.36	2.62
Natural betaine	70.87	42.12 <sup>ab</sup>	31.68 <sup>ab</sup>	2.28	2.33
Betaine HCl 1	71.26	42.12 <sup>ab</sup>	31.71 <sup>ab</sup>	2.27	2.25
Betaine HCl 2	70.93	42.85 <sup>a</sup>	31.26 <sup>b</sup>	2.32	2.35
P-value	0.75	0.05	0.03	0.72	0.17
SEM	0.33	0.43	0.26	0.05	0.13

The means within the same column with at least one common letter, do not have significant difference (P&gt;0.05).

SEM: standard error of the means.

**Table 8** Cost of feed consumed per chicken (US\$/chicken), per kg of chicken (US\$/kg) and per kg of breast meat (US\$/kg) according to treatment

Treatments	US\$/chicken	US\$/kg of broiler	US\$/kg of breast
Control+	1.774	0.596	1.977 <sup>ab</sup>
Control-	1.774	0.596	2.042 <sup>a</sup>
Natural betaine	1.776	0.592	1.987 <sup>ab</sup>
Betaine HCl 1	1.759	0.591	1.973 <sup>ab</sup>
Betaine HCl 2	1.745	0.590	1.941 <sup>b</sup>
P-value	0.7	0.2	0.1
SEM	0.048	0.005	0.03

The means within the same column with at least one common letter, do not have significant difference (P&gt;0.05).

SEM: standard error of the means.

Although the negative control treatment did not compromise performance, the results obtained demonstrated a negative effect of this treatment in some characteristics of the birds. The negative control promoted lower BY and higher cost per kg of breast produced as well as a tendency of higher accumulation of abdominal fat in broilers. These findings can be explained by a deficiency in methionine and the absence of other sources of methyl group donors such as betaine.

## CONCLUSION

The chickens supplemented with synthetic betaine HCl 2 in the diet had higher breast meat yield and lower cost per kg of breast meat in relation to the negative control. The chickens fed diets with synthetic betaine HCl 1 and natural betaine had similar carcass characteristic and cost of diets. There was no decrease of performance of chickens receiving the negative control diet, in which digestible methionine level was reduced by 17%, compared to the positive control diet. It is possible to use different sources of betaine as replacement of 17% digestible methionine in broiler chickens diets without compromising performance and carcass traits.

## ACKNOWLEDGEMENT

We are most grateful to ESALQ for use of the building.

## REFERENCES

Alirezai M., Jelodar G., Niknam P., Ghayemi Z. And Nazifi S. (2011). Betaine prevents ethanol-induced oxidative stress and

reduces total homocysteine in the rat cerebellum. *J. Physiol. Biochem.* **67**, 605-612.

Amerah A.M. and Ravindran V. (2015). Effect of coccidia challenge and natural betaine supplementation on performance, nutrient utilization, and intestinal lesion scores of broiler chickens fed suboptimal level of dietary methionine. *Poult. Sci.* **94**, 673-680.

Barbosa N.A.A. (2009). Avaliação de aditivos em dietas de frangos de corte. MS Thesis. Faculdades de Ciências Agrárias e Veterinária, Universidade Estadual Paulista, Jaboticabal, Brazil.

Chen J., Wang M., Kong Y., Ma H. and Zou S. (2011). Comparison of the novel compounds creatine and pyruvate on lipid and protein metabolism in broiler chickens. *Animal.* **5**, 1082-1089.

Coma J., Carrion D. and Zimmerman D.R. (1995). Use of plasma urea nitrogen as a rapid response criterion to determine the lysine requirement of pigs. *J. Anim. Sci.* **73**, 472-481.

Conde-Aguilera J., Cobo-Ortega C., Tesseraud S., Lessire M., Mercier Y. and Van Milgen J. (2013). Changes in body composition in broilers by a sulfur amino acid deficiency during growth. *Poult. Sci.* **92**, 1266-1275.

De Ridder J.J.M. and Van Dam K. (1975). Control of choline oxidation by rat-liver mitochondria. *Biochim. Biophys. Acta.* **408**, 112-122.

Eklund M., Bauer E., Wamatu J. and Mosenthin R. (2005). Potential nutritional and physiological functions of betaine in livestock. *Nutr. Res. Rev.* **18**, 31-48.

Farrokhyan P., Bouyeh M., Lartey F. and Seidavi A.R. (2014). The effects of dietary L-carnitine and gemfibrozil on performance, carcass characteristics, cholesterol and triglycerides in broiler chicks. *Avian Biol. Res.* **7**, 160-166.

Hamidi H., Jahanian R. and Pourreza J. (2010). Effect of dietary betaine on performance, immunocompetence and gut contents osmolarity of broilers challenged with a mixed coccidial infec-

tion. *Asian J. Anim. Vet. Adv.* **5**, 193-201.

He S., Zhao S., Dai S., Liu D. and Bokhari S.G. (2015). Effects of dietary betaine on growth performance, fat deposition and serum lipids in broilers subjected to chronic heat stress. *Anim. Sci. J.* **86**, 897-903.

Kettunen H., Tiihonen K., Peuranen S., Saarinen M.T. and Remus J.C. (2001). Dietary betaine accumulates in the liver and intestinal tissue and stabilizes the intestinal epithelial structure in healthy and coccidia-infected broiler chicks. *Comp. Biochem. Physiol.* **130**, 759-769.

Leclercq B. (1998). Specific effects of lysine on broiler production: comparison with threonine and valine. *Poult. Sci.* **77**, 118-123.

Leng Z., Fu Q., Yang X., Ding L., Wen C. and Zhou Y. (2016). Increased fatty  $\beta$ -oxidation as a possible mechanism for fat-reducing effect of betaine in broilers. *Anim. Sci. J.* **87**, 1005-1010.

Lever M. and Slow M. (2010). The clinical significance of betaine, an osmolyte with a key role in methyl group metabolism. *Clin. Biochem.* **43**, 732-744.

Metzler-Zebeli B.U., Eklund M. and Mosenthin R. (2009). Impact of osmoregulatory and methyl donor functions of betaine on intestinal health and performance in poultry. *Worlds Poult. Sci. J.* **65**, 419-441.

Pereira P.W.Z., Menten J.F.M., Racanicci A.M., Traldi A.B., Silva C.S. and Rizzo P.V. (2010). Avaliação de complexo enzimático e betaina natural em rações para frangos de corte criados em aviário comercial. *R. Bras. Zootec.* **39**, 2230-2236.

Ratriyanto A., Mosenthin R., Bauer E. and Eklund M. (2009). Metabolic, osmoregulatory and nutritional functions of betaine in monogastric animals. *Asian-Australas. J. Anim. Sci.* **22**, 1461-1476.

Rostagno H.S., Teixeira A., Donzele J.L., Gomes P.C., De Oliveira R.F.M., Lopes D.C., Ferreira A.J.P. and Toledo Barreto S.L. (2005). Brazilian tables for poultry and swine: Composition of feedstuffs and nutritional requirements. MS Thesis. Universidade Federal de Viçosa, Minas Gerais, Brazil.

Santana M.H.M., Costa F.G.P., Ludke J.V. and Figueiredo Júnior J.P. (2014). Interações nutricionais entre aminoácidos sulfurosos, colina e betaina para aves. *Arch. Zootec.* **63**, 69-83.

SAS Institute. (2006). SAS<sup>®</sup>/STAT Software, Release 9.1. SAS Institute, Inc., Cary, NC. USA.

Steve-Garcia A.E. and Mack S. (2000). The effect of DL-methionine and betaine on growth performance and carcass characteristics in broilers. *Anim. Feed Sci. Technol.* **87**, 85-93.

Sun H., Yang W.R., Yang Z.B., Wang Y., Jiang S.Z. and Zhang G.G. (2008). Effects of betaine supplementation to methionine deficient diet on growth performance and carcass characteristics of broilers. *Am. J. Anim. Vet. Sci. J.* **3**, 78-84.

Waldroup P.W., Motl M.A., Yan F. and Fritts C.A. (2006). Effects of betaine and choline on response to methionine supplementation to broiler diets formulated to industry standards. *J. Appl. Poult. Res.* **15**, 58-71.

Wallis I.R. (1999). Dietary supplements of methionine increase breast meat yield and decrease abdominal fat in growing broiler chickens. *Australasian J. Exp. Agric.* **39**, 131-141.

Zhan X.A., Li J.X., Xu Z.R. and Zhao R.Q. (2006). Effects of methionine and betaine supplementation on growth performance, carcass composition and metabolism of lipids in male broilers. *Br. Poult. Sc.* **47**, 576-580.