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Partial replacement of DL-Methionine and methionine hydroxy analogue with betaine in diets for broiler chickens

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Abstract

The study evaluated the effects on the growth performance and carcass characteristics of broiler chickens of DL-Methionine (DL-Met) and methionine hydroxy analogue (MHA) supplementation and their partial replacement with betaine (Bet). Two experiments were performed from day 1 to 21 and from day 22 to 42. Broiler chickens were assigned to six treatments, in a completely randomized design and a 2 × 2 + 2 factorial arrangement, consisting of two Met sources (DL-Met and MHA), with or without Bet, and two negative controls. The six treatments consisted of i) NC-DLM: negative control for DL-Met with 7% reduction in DL-Met; ii) NC-MHA: negative control for MHA with 7% reduction of MHA, iii) DLM_{100}: without Bet, supplemented exclusively with DL-Met reaching 100% of requirements, iv) MHA_{100}: without Bet supplemented exclusively with MHA reaching 100% of requirements, v) DLM_{93}+Bet: DL-Met at 93% of recommended level plus betaine; vi) MHA_{93}+Bet: MHA at 93% of recommended level plus betaine. Growth from day 1 to 21 was not altered by methionine source or betaine supplementation. However, the negative controls had numerically less weight gain and feed conversion ratio (FCR) . Between days and 42 there were no effects on growth. Betaine could partially replace methionine without altering the growth of broiler chickens. In addition, the partial replacement of DL-Met with betaine increased body crude protein content. However, its use increased body fat content regardless of methionine source.

Keywords: amino acid, carcass composition, growth, methyl source
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Introduction

Methionine is one of the more limiting amino acids in animal nutrition, with an important function in protein synthesis. It is also a source of methyl groups, which are important in metabolism and must be supplied in the diets as they cannot be synthesized (Sun *et al*., 2008; Nutautaitė *et al*., 2020). The usual supplemental sources of methionine are DL-Met and MHA (Payne *et al*., 2006), which is chemically different from DL-Met because it has a hydroxyl group at the asymmetric carbon atom, whereas DL-Met has an amino group. This chemical difference lowers the bioavailability of MHA compared with DL-Met (Rehman *et al*., 2019; Sauer *et al*., 2008).

The methyl groups found in betaine may provide benefits by sparing methionine as a methyl donor and permitting methionine to be directed towards protein synthesis (Sun *et al*., 2008; Nutautaitë *et al*., 2020). Betaine is a natural compound that is synthesized by a variety of plants and organisms and is important for synthesis of methionine and carnitine (Shakeri *et al*., 2019). On a molecular weight basis, betaine contains about 3.75 times more methyl groups than methionine, and have been shown to counteract the partial deficiency of labile methyl groups in corn-soybean-based diets (Rama Rao *et al*., 2011; Sun *et al*., 2008).

Betaine also acts in lipid metabolism, being associated with enhanced synthesis of methylated compounds such as carnitine and creatine in the liver and muscle. By increasing the concentration of

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carnitine in the liver, it can facilitate fatty acid oxidation and reduce the amount of long-chain fatty acids to be stored in adipose tissue (Chen *et al*., 2018), thus altering carcass characteristics (Leng *et al*., 2016).

Reported effects of betaine on broiler chicken carcasses are conflicting (Fu *et al*., 2016). Some studies observed lower fat deposition in broilers supplemented with betaine (He *et al*., 2015; Leng *et al*., 2016). However, others reported increased fat accumulation (Garcia Neto *et al*., 2000; Zhan *et al*., 2006). According to Sun *et al.* (2008), Bet and methionine in marginally methionine-deficient diets could lead to an equivalent growth response in broiler chickens, and Bet could spare a small portion of the methionine. However, the effect of Bet supplementation on feed efficiency may depend on diet composition, dietary level of methionine and level of Bet supplementation, breed, and rearing environment (Sun *et al*., 2008; Chen *et al*., 2018).

Thus, partial replacement of methionine sources with betaine was hypothesized to alter body composition while maintaining the growth rate of broiler chickens. It was further hypothesized that this effect depended on the methionine source. Thus, the aim of this study was to evaluate the effects of betaine supplementation in diets with reduced DL-Met or MHA for effects on growth, carcass traits and body composition of broiler chickens at various ages.

Materials and methods

This study was performed at the poultry research centre of Western Paraná State University, Unioeste, Marechal Cândido Rondon, PR, Brazil. All the experimental procedures followed the rules established by the National Council for the Control of Animal Experimentation in Brazil and had been approved by the Animal Use Ethics Committee of the university, under number 25/2014.

Two trials were initiated using one-day-old male broiler chickens (Cobb 500) obtained from a commercial hatchery. In both trials, birds were placed in the same curtain-sided house and raised in pens measuring 1.76 m^2 with concrete floors that were covered with wood shavings. The pens were equipped with tubular feeders and nipple drinkers, and the chickens received feed and water ad libitum. For trial 1, 660 one-day-old broiler chickens were weighed individually (mean weight = 45.38 ± 0.57 g) and assigned to a completely randomized design in a 2 x 2 + 2 factorial arrangement with five replicates (pens) of 22 broiler chickens. The treatments consisted of two methionine sources, two betaine supplementation levels (with or without Bet), and two negative controls (reduced for DL-methionine or MHA). The diets consisted of i) NC-DLM: a negative control with 7% less DL-Met, ii) NC-MHA: a negative control with 7% less MHA, iii) DLM_{100}: a DL-Met supplemented diet, iv) MHA_{100}: a MHA supplemented diet, and v) diet 1 supplemented with betaine; and vi) diet 2 supplemented with betaine. Both DL-Met and MHA were included based on their equimolar equivalence of 1.125 (Visentini *et al.*, 2005). Betaine hydrochloride 95% (Btech, Valinhos, SP, Brazil) was used at 0.5 g/kg of diet. All the necessary adjustments when changing the level of dietary inclusion of DL-Met, MHA, and/or betaine were made at the expense of corn. These corn and soybean meal-based diets (Table 1) were formulated to meet or exceed the requirements proposed by Rostagno *et al*. (2011), except for the negative control diets. The chicks were fed these diets until they reached 21 days old when the birds and feeders were weighed to calculate the average feed intake, body weight gain (BWG) and feed conversion ratio (FCR). Mortality was recorded daily and used to correct FCR according to Sakomura & Rostagno (2016).

In trial 2, 480 one-day-old broiler chickens were raised in similar conditions and fed a common diet until they were 21 days old. Then the birds were weighed individually (mean weight = 729.96 ± 11.50 g) and assigned to a completely randomized design with six treatments and five replications of 16 birds. Treatments were similar to those in trial 1, except for the nutritional composition of the diets, which, following Rostagno *et al*. (2011), was adjusted to be appropriate to the finishing period (Table 2).

At day 42, the broiler chickens and feeders were weighed and the growth performance was determined. Three birds per pen were selected (pen average weight ± 5%), fasted for eight hours, weighed and slaughtered. One of these birds was immediately stored at -20 °C for further chemical analysis. The two remaining birds were scalded, plucked, and eviscerated. The head, neck, and feet of each were removed and the hot carcass was weighed. The carcasses were assembled as breast, wings, and legs according to commercial practices, weighed, and then analysed as percentages of the hot carcass.

After 30 days, the frozen birds were thawed at room temperature, ground, dried (65 °C, 72 hours), and analysed according to AOAC (1990) for dry matter (DM) (method 93.01), crude protein (CP) (method 981.10), and total fat (method 920.85).

Table 1 Ingredients and calculated composition (g/kg as-fed) of experimental diets for broiler chickens from day 1 to 21 with different sources of methionine

Ingredient	DLM_{100}	NC-DLM_{93}	DLM_{93}+Bet	MHA_{100}	NC-MHA_{93}	MHA_{93}+Bet
Corn, 78.8 g CP/kg	574.2	574.9	574.4	573.5	574.2	573.
Soybean meal, 450 g CP/kg	369.0	369.0	369.0	369.0	369.0	369.0
Soybean oil	18.0	18.0	18.0	18.0	18.0	18.0
Monocalcium phosphate	14.7	14.7	14.7	14.7	14.7	14.7
Limestone	12.0	12.0	12.0	12.0	12.0	12.0
Salt	5.3	5.3	5.3	5.3	5.3	5.3
Premix[1]	2.2	2.2	2.2	2.2	2.2	2.2
L-Lysine (78%)	1.7	1.7	1.7	1.7	1.7	1.7
L-Threonine (99%)	0.4	0.4	0.4	0.4	0.4	0.4
DL-Methionine (98%)	2.6	1.9	1.9	0.0	0.0	0.0
Methionine hydroxy analogue	0.0	0.0	0.0	3.3	2.6	2.60
Betaine	0.0	0.0	0.5	0.0	0.0	0.5
Composition						
Metabolizable energy, MJ/kg	12.35	12.35	12.34	12.34	12.34	12.33
Crude protein, g/kg	218	218	217	216	216	216
Digestible lysine, g/kg	12	12	12	12	12	12
Digestible methionine + cysteine, g/kg	8.5	7.9	7.9	8.5	7.9	7.9
Digestible threonine, g/kg	7.8	7.8	7.8	7.8	7.8	7.8
Betaine, g/kg	0.0	0.0	0.4	0.0	0.0	0.4
Calcium, g/kg	8.6	8.6	8.6	8.6	8.6	8.6
Available phosphorus, g/kg	4.4	4.4	4.4	4.4	4.4	4.4
Sodium. g/kg	2.2	2.2	2.2	2.2	2.2	2.2

DLM_{100}: DL-Methionine supplemented, NC-DLM_{93}: negative control supplemented with DL-Met at 93% of DLM_{100}, DLM_{93}+Bet: DLM_{93} supplemented with Betaine; MHA_{100}: methionine hydroxy analogue (MHA) supplementation; MHA_{93}: negative control supplemented with 93% of MHA, MHA_{93}+Bet: MHA_{93} supplemented with betaine

[1] Vitamin A: 10,000 IU, vitamin D_3: 2,500 IU, vitamin E: 20,000 UI, vitamin K_3: 2,500 mg, vitamin B_1: 1,800 mg, vitamin B_2: 6,000 mg, vitamin B_{12}: 16,000 mcg, vitamin B_6: 2,600 mg, vitamin B_3: 40,000 mg, vitamin B_5: 12,000 mg, vitamin B_7: 65 mg, vitamin B_9: 1,000 mg, iron: 50 g, copper: 9 g, zinc: 60 g, manganese: 70 g, iodine: 1 g, selenium: 0.3 g, choline chloride: 0.5 g, salinomycin: 0.55 g, virginiamycin: 0.05 g

For both trials, the data were subjected to analysis of variance and the residuals were evaluated to ascertain the homogeneity of variance among the treatment groups. The treatment means were compared using Tukey's test, with the exception of the negative controls, which were compared with the other treatments with a Dunnett's test. The significance threshold was a probability of 0.05. All statistical procedures were performed using the general linear model procedure of SAS version 9.1 (SAS Institute Inc., Cary, North Carolina, USA). The model was:

$$y_{ijk} = \mu + B_i + M_j + BM_{ij} + e_{ijk}$$

where: y_{ijk} = observation,
μ = overall mean,
B_i = effect of betaine,
M_j = effect of methionine source,
BM_{ij} = effect of interaction between betaine and methionine source, and
e_{ijk} = experimental random residual error.

Table 2 Ingredients and calculated composition (g/kg as-fed) of experimental diets for broiler chickens from day 22 to 42 with different methionine sources

Ingredient	DLM_{100}	NC- DLM_{93}	DLM_{93}+Bet	MHA_{100}	NC- MHA_{93}	MHA_{93}+Bet
Corn, 78.8 g CP/kg	583.0	583.7	583.2	582.5	583.2	582.7
Soybean meal, 450 g CP/kg	332.5	332.5	332.5	332.5	332.5	332.5
Soybean oil	52.9	52.9	52.9	52.9	52.9	52.9
Monocalcium phosphate	11.6	11.6	11.6	11.6	11.6	11.6
Limestone	10.2	10.2	10.2	10.2	10.2	10.2
Salt	4.2	4.2	4.2	4.2	4.2	4.2
Premix[1]	2.2	2.2	2.2	2.2	2.2	2.2
L-Lysine (78%)	0.9	0.9	0.9	0.9	0.9	0.9
L-Threonine (99%)	0.3	0.3	0.3	0.3	0.3	0.3
DL-Methionine (98%)	2.2	1.5	1.5	0	0	0
Methionine hydroxy analogue	0	0	0	2.7	2.0	2.0
Betaine	0	0	0.5	0	0	0.5
Composition						
Metabolizable energy, MJ/kg	13.39	13.39	13.39	13.39	13.39	13.39
Crude protein, g/kg	200.7	200.4	200.4	199.4	199.4	199.4
Digestible lysine, g/kg	10.5	10.5	10.5	10.5	10.5	10.5
Digestible methionine + cysteine, g/kg	7.7	7.1	7.1	7.7	7.1	7.1
Digestible threonine, g/kg	7.0	7.0	7.0	7.0	7.0	7.0
Betaine, g/kg	0.0	0.0	0.04	0.0	0.0	0.04
Calcium, g/kg	7.2	7.2	7.2	7.2	7.2	7.2
Available phosphorus, g/kg	3.7	3.7	3.7	3.7	3.7	3.7
Sodium. g/kg	1.8	1.8	1.8	1.8	1.8	1.8

DLM_{100}: DL-Methionine supplemented, NC-DLM_{93}: negative control supplemented with DL-Met at 93% of DLM_{100}, DLM_{93}+Bet: DLM_{93} supplemented with Betaine; MHA_{100}: methionine hydroxy analogue (MHA) supplementation; MHA_{93}: negative control supplemented with 93% of MHA, MHA_{93}+Bet: MHA_{93} supplemented with betaine

[1] Vitamin A: 10,000 IU, vitamin D_3: 2,500 IU, vitamin E: 20,000 UI, vitamin K_3: 2,500 mg, vitamin B_1: 1,800 mg, vitamin B_2: 6,000 mg, vitamin B_{12}: 16,000 mcg, vitamin B_6: 2,600 mg, vitamin B_3: 40,000 mg, vitamin B_5: 12,000 mg, vitamin B_7: 65 mg, vitamin B_9: 1,000 mg, iron: 50 g, copper: 9 g, zinc: 60 g, manganese: 70 g, iodine: 1 g, selenium: 0.3 g, choline chloride: 0.5 g, salinomycin: 0.55 g, virginiamycin: 0.05 g

Results and Discussion

The weight gain of broiler chickens from day 1 to day 21 was not affected (P >0.05) by the source of methionine or betaine inclusion. However, according to Dunnett's test, all the treatments, namely DLM_{100}, MHA_{100}, DLM_{93}+Bet, and MHA_{93}+Bet, produced greater weight gain than the negative controls NC-DLM and NC-MH). Likewise, no interaction effect was detected (P >0.05). Nor was there an effect of either betaine or methionine source on feed intake (Table 3). According to Dunnett's test, broiler chickens receiving the treatments MHA_{100} and DLM_{93}+Bet had a smaller FI compared with NC-MHA. Finally, no treatment main effects or interaction effects on FCR were detected (P >0.05). However, the treatments DLM_{100}, MHA_{100}, DLM_{93}+Bet, and MHA_{93}+Bet had better FCR compared with NC-DLM and NC-MHA.

Table 3 Performance of broiler chickens receiving DL-Methionine or methionine hydroxy analogue partially replaced by betaine from day 1 to 21

Treatment	Weight gain, g/d	Feed intake, g/d	Feed conversion ratio
NC-DLM_{93}	35.07	55.98	1.60
NC-MHA_{93}	35.09	56.17	1.60
DLM_{100}	37.49*[#]	54.62	1.46*[#]
MHA_{100}	37.24*[#]	55.63[#]	1.49*[#]
DLM_{93}+Bet	37.74*[#]	55.46[#]	1.47*[#]
MHA_{93}+Bet	36.97*[#]	54.64	1.48*[#]
SE	0.27	0.20	0.01
P-value			
Betaine (Bet)	0.976	0.857	0.933
Methionine source (Met)	0.338	0.832	0.131
Bet x Met	0.625	0.245	0.298

MHA: methionine hydroxy analogue, DLM_{100}: DL-Methionine supplemented, NC-DLM_{93}: negative control supplemented with DL-Met at 93% of DLM_{100}, DLM_{93}+Bet: DLM_{93} supplemented with Betaine; MHA_{100}: methionine hydroxy analogue (MHA) supplementation; NC-MHA_{93}: negative control supplemented with 93% of MHA, MHA_{93}+Bet: MHA_{93} supplemented with betaine
Means followed by an * differ from the NC-DLM_{93} by Dunnett's test at 5% significance
Means followed by # differ from the NC-MHA_{93} by Dunnet's t test at 5% significance

In terms of growth performance from day 22 to day 42 (Table 4), WG, FI and FCR were not affected (P >0.05) by the treatments, with mean values of 80.2 g/d, 141.93 g/d, and 1.77 g/g, respectively.

Table 4 Performance of broiler chickens receiving DL-Methionine or methionine hydroxy analogue partially replaced with betaine from day 22 to 42

Treatment	Weight gain	Feed intake	Feed conversion ratio
NC-DLM_{93}	78.20	138.8	1.78
NC-MHA_{93}	80.29	141.7	1.77
DLM_{100}	79.83	140.1	1.76
MHA_{100}	80.68	143.6	1.78
DLM_{93}+Bet	81.79	143.9	1.76
MHA_{93}+Bet	80.42	143.5	1.78
SE	0.35	0.6	0.01
P-value			
Betaine (Bet)	0.286	0.176	0.713
Methionine source (Met)	0.735	0.267	0.065
Bet x Met	0.169	0.152	0.956

MHA: methionine hydroxy analogue, DLM_{100}: DL-Methionine supplemented, NC-DLM_{93}: negative control supplemented with DL-Met at 93% of DLM_{100}, DLM_{93}+Bet: DLM_{93} supplemented with Betaine; MHA_{100}: methionine hydroxy analogue (MHA) supplementation; NC-MHA_{93}: negative control supplemented with 93% of MHA, MHA_{93}+Bet: MHA_{93} supplemented with betaine

When the birds were harvested at day 42, carcass and wing yields were not affected (*P* >0.05) by the treatments (Table 5). However, breast yield was influenced by methionine source (*P* =0.02) with the broiler chickens receiving DL-Met having a higher breast yield than those with MHA. When compared with the negative controls, there was no effect of betaine or methionine supplementation. However, the leg yield of birds receiving treatment DLM_{100} was numerically less than both negative controls, and the leg yield of those treated with MHA_{93}+Bet was lower than NC-MHA.

Table 5 Carcass and cuts yield (%) of broiler chickens receiving DL-Methionine or methionine hydroxy analogue partially replaced with betaine from day 22 to 42

Item	Carcass	Breast	Legs	Wings
NC-DLM_{93}	72.07	34.78	29.15	10.57
NC-MHA_{93}	72.37	33.54	32.13	10.36
DLM_{100}	74.73	35.19	29.92*#	10.39
MHA_{100}	72.36	34.78	31.30	10.21
DLM_{93}+Bet	72.04	36.43	30.86	10.30
MHA_{93}+Bet	74.18	32.11	29.67#	9.63
SE	0.472	0.483	0.264	0.126
Betaine effect				
Without betaine	73.55	34.99	30.61	10.30
With betaine	73.11	34.27	30.27	9.97
Methionine source effect				
DL-Methionine	73.39	35.81[a]	30.39	10.35
Methionine hydroxy analogue	73.27	33.45[b]	30.49	9.92
P-value				
Betaine (Bet)	0.654	0.485	0.556	0.237
Methionine source (Met)	0.899	0.031	0.874	0.138
Bet x Met	0.151	0.068	0.185	0.396

MHA: methionine hydroxy analogue, DLM_{100}: DL-Methionine supplemented, NC-DLM_{93}: negative control supplemented with DL-Met at 93% of DLM_{100}, DLM_{93}+Bet: DLM_{93} supplemented with Betaine; MHA_{100}: methionine hydroxy analogue (MHA) supplementation; NC-MHA_{93}: negative control supplemented with 93% of MHA, MHA_{93}+Bet: MHA_{93} supplemented with betaine
Within an effect, means having similar superscripts were not different with probability P =0.05 by Tukey's test
Means followed by an * differ from the NC-DLM_{93} by Dunnett's test at 5% significance
Means followed by # differ from the NC-MHA_{93} by Dunnet's t test at 5% significance

There was no Bet x Met interaction (P >0.05) for body fat content (Table 6). However, the main effect of betaine supplementation indicated that broiler chickens that received betaine had higher body fat content than the birds that were not supplemented with this additive. Dunnett's test indicated that broiler chickens supplemented with betaine had a higher fat content than those in the negative control treatments.

The Bet x Met interaction affected (P <0.01) for body CP content, such that the birds receiving DL-Met_{93}+Bet had higher values than those that were subjected to the other treatments (DL-Met_{100}, MHA_{100}, DL-Met_{93}+Bet, and MHA_{93}+Bet). When the treatments were compared with the negative control diets, the CP content in the treatment DL-Met_{100} was lower than in the NC-DLM (P <0.05) and the CP content of birds receiving the DL-Met_{93}+Bet treatment was greater than those that received the NC-MHA diet.

The source of methionine had no effect on the growth performance in the two trials, since the diet was supplemented on an equimolar basis, which was consistent with other studies conducted on broiler chickens (Yodseranee & Bunchasak, 2012; Kluge *et al*., 2016). However, the efficacy of MHA for replacing DL-Met varied among studies. Payne *et al*. (2006) reported that the observed efficacy of MHA was 79% for weight gain and 81% for FCR on an equimolar basis, which was lower than the 88% efficacy that they had estimated. Ullrich *et al*. (2019) also found no differences in the weight gain of broiler chickens fed MHA or DL-methionine from day 1 to day 34.

Carcass yield was not impaired by the negative control diets, which can be attributed to the low level of methionine restriction. Nor was it influenced by methionine source or betaine supplementation. The higher breast yield in broilers receiving DL-Met than those that received MHA was consistent with the results of Esteve-Garcia and Llauradó (1997), who reported the relative efficacy of DL-methionine hydroxy analogue free acid relative to DL-methionine was 51% and suggested that breast yield might be a more sensitive parameter for testing the efficacy of various methionine sources than growth or feed conversion efficiency. Chen *et al.* (2018) evaluated a basal diet with DL-Met according to broiler chicken requirements plus betaine, and obtained a higher breast yield with the inclusion of 1000 mg Bet/kg compared with the basal diet, with no differences at the inclusion levels of 250 and 500 mg Bet/kg.

Table 6 Body composition of broiler chickens receiving DL-Methionine or methionine hydroxy partially replaced with betaine from day 22 to 42

Treatment	Fat, %	Crude Protein, %
NC-DLM93	12.83	21.44
NC-MHA93	11.45	21.21
DLM100	11.13	20.34*[b]
MHA100	11.93	20.76[b]
DLM93+Bet	13.84[#]	22.48[#a]
MHA93+Bet	13.72[#]	20.82[b]
SE	0.246	0.167
Betaine effect		
Without betaine	11.53[b]	20.55
With betaine	13.78[a]	21.65
Methionine source effect		
DL-Methionine	12.49	21.40
Methionine hydroxy analogue	12.83	20.79
P-value		
Betaine (Bet)	<0.001	<0.001
Methionine source (Met)	0.404	0.008
Bet x Met	0.270	<0.001

MHA: methionine hydroxy analogue, DLM_{100}: DL-Methionine supplemented, NC-DLM_{93}: negative control supplemented with DL-Met at 93% of DLM_{100}, DLM_{93}+Bet: DLM_{93} supplemented with Betaine; MHA_{100}: Methionine hydroxy analogue (MHA) supplementation; NC-MHA_{93}: negative control supplemented with 93% of MHA, MHA_{93}+Bet: MHA_{93} supplemented with betaine
Within an effect, means with similar superscripts were not different with probability P =0.05 by Tukey's test
Means followed by an asterisk * differ from the NC-DLM_{93} by Dunnett's test at 5% significance
Means followed by hashtag # differ from the NC-MHA_{93} by Dunnet's t test at 5% significance

The increase in body fat content with betaine supplementation was an unexpected result, since betaine may increase fat metabolism and reduce the availability of long chain fatty acids for storage in adipose tissue (He *et al*., 2015; Chen *et al*., 2018). However, Met-replacing and fat-distribution effects of betaine differ in the literature (Yang *et al*., 2017). Zhan *et al*. (2006) reported a higher plasma concentration of free fatty acids and a trend toward higher fat deposition in the breast of broilers fed a methionine-deficient diet supplemented with betaine. Garcia Neto *et al*. (2000) also found higher fat deposition in the carcass of broilers fed betaine instead of methionine as the main source of methyl groups. One possible explanation for these differing results was documented in trials with pigs (Suster *et al*., 2004; Dunshea *et al*., 2009), where a reduction in the total amount of body fat was observed as well as changes the distribution of adipose tissues in the body. However, in the present study only total body fat percentage was evaluated.

Betaine favours skeletal muscle growth by increasing the expression of myogenic regulatory factors such as myogenic determination factor 1 and myogenin, thereby enhancing metabolic pathways associated with protein synthesis (Chen *et al*., 2018). However, the reasons for the higher amounts of fat in MHA treatments and higher body protein in broilers fed DLM_{93}+Bet are not completely understood. Sun *et al*. (2008) evaluated the partial replacement of DL-Met at 75% of broiler chicken requirements plus 500 mg

Bet/kg and recorded a higher fat and protein content in the breast of supplemented broiler chickens at day 42 compared with the control diet. They also found elevated serum levels of growth hormone and insulin-like growth factor-1 (IGF-1) and reported that these metabolites were positively related to feed efficiency, plus increased protein content at that level of betaine supplementation.

Conclusion

The source of methionine did not influence the growth from day 1 to 21 or from day 22 to 42 or carcass yield at day 42 of broiler chickens. Betaine could partially replace methionine without altering the growth performance of broiler chickens. The partial replacement of DL-Met with betaine increased body crude protein. However, its use increased body fat content, regardless of methionine source.

Authors' contributions

LS, CS and JB collected the data. BSV and ASA, conducted the statistical analyses, and collaborated in interpretation of the results. LS and LDC wrote the initial draft of this manuscript and finalized the manuscript. RVN, SET and PSC developed the original hypothesis, designed the experiments, and collaborated in interpreting the results. The authors have read and approved the finalized manuscript.

Conflict of interest declaration

The authors declare there is no conflict of interest.

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