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Original Articles

Can the Fine Wheat Bran be a Betaine Source in Poultry Diets?

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ABSTRACT

The effect of fine wheat bran (FWB) as a methyl donor source on performance, metabolism, body composition and blood traits of growing broilers was studied. Three hundred and twenty broilers from eight to 28 d of age, distributed in a randomized block design, with five treatments and eight replicates of eight animals each were used. The experimental diets were: NC, formulated with 72% of the Met+Cys requirement; Met, formulated with 85% of the Met+Cys equivalents by DL-methionine addition; Bet, formulated with 85% of the Met+Cys equivalents by anhydrous betaine addition; Fwb^-, formulated with 72% of the Met+Cys requirement and 20% FWB; and Fwb^+, formulated with 85% of the Met+Cys requirement and 20% FWB. Feed intake was reduced (p<0.05) by FWB inclusion but the feed conversion ratio was the same (p>0.05) between the positive control diets. Supplementation of DL-methionine and anhydrous betaine showed the same (p>0.05) metabolizability of nutrients. Treatments with higher DL-methionine levels (Met and Fwb^+) promoted more weight of feathers (p<0.05). Animals fed with FWB showed the lowest (p<0.05) body gains. In conclusion, FWB inclusion did not promote methyl radicals supply.

INTRODUCTION

In diets based on maize and soybean meal, methionine is often the first limiting amino acid for broilers. This amino acid plays a pivotal role in animal metabolism, because it is essential for optimum muscle accretion and for feather synthesis. In addition, methionine is an intermediate in transmethylation reactions, serving as the major methyl group donor in vivo (Nelson & Cox, 2012). However, methyl groups for remethylation of methionine can also be provided by betaine (Nelson & Cox, 2012). Therefore, the dietary supplementation of betaine may reduce the requirement for other methyl group donors, such as methionine and choline (Siljander-Rasi *et al.*, 2003).

Previous experiments have shown that it can replace the dietary DL-methionine supplementation for broilers, fed diets that had either an adequate or deficient methionine content (Siljander-Rasi *et al.*, 2003; El-Husseiny *et al.*, 2007). Recently, the betaine effects on nutrient digestibility were also reported (Ratriyanto *et al.*, 2017). Although betaine is available in purified form as a feed additive, e.g. anhydrous betaine, it is also present in large quantities in wheat and wheat products, like wheat bran, with contents ranging between 1,339 and 1,506 mg·100 g^{-1}, as demonstrated by Zeisel *et al.* (2003). Moreover, in comparison with other wheat by products, fine wheat bran (FWB) can be a good source of energy and protein as well, showing values of 19.56 MJ·kg^{-1} and 181.4 g·kg^{-1} for gross energy (GE) and crude protein (CP), on dry matter (DM) basis, respectively (Wesendonck *et al.*, 2013).

In this context, the FWB, although its high crude fiber content, could be a promising source of energy and betaine, and it may be used in poultry diets as a partial replacer of methionine. However, to our knowledge, no information was published about the availability of the betaine present in FWB of poultry diets. The objective of this paper was to investigate the FWB as a methyl donor source in diets for growing broilers using performance, metabolism and body composition as responses.

MATERIAL AND METHODS

All procedures involving animals were in accordance with Brazilian guidelines and reviewed and approved by the Ethics Committee of the Federal University of Rio Grande do Sul (protocol N° 22583). The Brazilian guidelines are based on Federal Law N°. 11794 of October 8, 2008.

Three hundred and twenty sexed male Cobb 500® broilers, from eight to 28 d of age were used in this study. Birds were distributed in a completely randomized design, with five treatments and eight replicates of eight animals each. The animals were housed in an environmentally controlled room and kept in metabolic cages. The experimental period was divided into an adaptation period (from 1 to 7 d in cages and from 5 to 7 d receiving experimental diets, with average body weight, BW, at 7 d of 179 ± 1,26 g) and a period with total excreta collection (20 d).

The study utilized five treatments, which consisted of diets formulated to meet the nutritional requirements proposed by Rostagno *et al.* (2011) for growing broilers (Table 1). Only the level of methionine

Table 1 - Feed formulations and chemical compositions of broiler diets ($g \cdot kg^{-1}$ as-fed basis).

	Treatments				
	NC	Met	Bet	Fwb$^-$	Fwb$^+$
Ingredients					
Maize	520.6	520.6	520.6	421.7	421.7
Soybean meal	348.9	348.9	348.9	291.8	291.8
Fine wheat brain	-	-	-	200.0	200.0
Oil	49.40	49.40	49.40	49.40	49.40
Inert (kaolin)	44.40	44.40	44.40	-	-
Dicalcium phosphate	16.50	16.50	16.50	14.70	14.70
Limestone	9.80	9.80	9.80	11.10	11.10
Salt	4.90	4.90	4.90	4.70	4.70
Starch	1.30	-	0.30	2.00	0.60
DL-methionine	1.00	2.30	1.00	0.30	1.70
Anhydrous betaine	-	-	1.00	-	-
L-lysine	0.70	0.70	0.70	1.50	1.50
L-threonine	1.10	1.10	1.10	1.40	1.40
Monensin 40%	0.25	0.25	0.25	0.25	0.25
Mineral mix[1]	0.80	0.80	0.80	0.80	0.80
Vitamin mix[2]	0.40	0.40	0.40	0.04	0.04
Chemical composition					
ME, $MJ \cdot kg^{-1}$	12.56	12.56	12.56	12.56	12.56
Crude protein	205.0	205.0	205.0	205.0	205.0
Calcium	85.00	85.00	85.00	85.00	85.00
Available P	40.00	40.00	40.00	40.00	40.00
Digestible Lys	11.70	11.70	11.70	11.70	11.70
Digestible Met+Cys	5.90	7.20	5.90	5.90	7.20
Digestible Trp	2.10	2.10	2.10	2.10	2.10
Digestible Thr	7.60	7.60	7.60	7.60	7.60
Digestible Arg	13.70	13.70	13.70	13.70	13.70
Choline	1.27	1.27	1.27	1.29	1.29
Na+K-Cl, $mEq \cdot kg^{-1}$	207.0	207.0	207.0	223.0	223.0

NC, formulated with 72% of the Met+Cys requirement; Met, 85% of the Met+Cys equivalents by adding DL-methionine; Bet, 85% of the Met+Cys equivalents by adding anhydrous betaine; Fwb$^-$, formulated with 72% of the Met+Cys requirement and 20% of fine wheat bran (FWB); Fwb$^+$, diet formulated with 85% of the Met+Cys requirement and 20% FWB.

[1]Content·kg^{-1} of product: Mn, 150.000 mg; Zn, 100.000 mg; Fe, 80.000 mg; Cu, 15.000 mg, I, 1.200 mg; Se, 700 mg.

[2] Content·kg^{-1} of product: vit.A, 23.200 IU; vit. D, 5.600 IU; vit. E, 52.000IU; vit. K, 6.000 mg; vit. B1, 6.000 mg; vit. B2, 18.000 mg; vit. B6, 9.000 mg; vit. B12, 40.000 µg; pantotenic acid, 44.000 mg; niacin, 132.000 mg; folic acid, 2.400 mg; biotin, 200.000 µg.

plus cysteine (Met+Cys) required, which was set at 85% of the requirement in the positive controls, with the objective to maximize the supplementation effects, was nutritionally modified. The negative control diet (NC) was formulated with 72% of the Met+Cys requirement. The remaining diets were formulated by using methionine, betaine or FWB as methyl sources: (i) Met, formulated with 85% of the Met+Cys equivalents, by adding 2.3 g·kg^{-1} of DL-methionine; (ii) Bet, 85% of the Met+Cys equivalents, by adding 1.0 g·kg^{-1} of anhydrous betaine; (iii) Fwb^{-}, diet formulated with 72% of the Met+Cys requirement and 20% FWB; (iv) Fwb^{+}, diet formulated with 85% of the Met+Cys requirement and 20% FWB. The betaine content in the FWB was 1.1%. Hence, the contribution of FWB to the diets Fwb^{-} and Fwb^{+} was 0.22% betaine. This excess was maintained because the bioavailability of betaine in FWB was not known. The metabolizable energy (ME) value of FWB (11.03 MJ·kg^{-1}) was obtained from a previous study (Bockor *et al.*, 2011). In order to achieve the same ME level in all diets, an inert material (kaolin) was included in the diets without FWB. The supplemental methionine equivalent by anhydrous betaine, was made based on the molar ratio of 1:1.27 betaine: methionine. Daily feed allowances were adjusted, considering a linear body weight gain (BWg) between eight and 28 d of age, according to growth curves proposed by Rostagno *et al.* (2011).

The daily total excreta collected from each experimental unit were weighed and frozen (-10°C) until the end of the experimental period. After, excreta were sampled (300 g·kg^{-1}) and dried in a force-ventilation oven at 60ºC for 72 h. The body composition evaluation was based on a comparative slaughter technique, in which two 8-d-old chicks, for replicate, were slaughtered to obtain initial body composition. At 28 d, four birds with similar BW to the pen average from each replicate, were euthanized to measure body composition. The birds underwent fasting for 6 h, to empty the gastrointestinal tract (GIT), and were then slaughtered and stored in a cold chamber. After slaughter, the feathers and GIT were weighted, and the carcasses were stored frozen (-15°C). Subsequently, each frozen carcass (including the heads and feet) was cut into strips using a band saw and was minced twice with a meat grinder (MCR22/G.Paniz), using a 5 mm die. The samples (250 g·kg^{-1}) were dried at 60°C for 72 h and were then ground with a ball mill (DL-ME/De Leo) for 90 s, or until a thin and homogeneous paste was obtained.

According to AOAC (1990) procedures, excreta and carcass samples were analyzed for DM (105ºC for 24 hours – method 930.15) and CP (according to Kjeldahl procedure, nitrogen * 6.25; using TECNAL model TE-036/2 – method 954.01). The GE was analyzed by bomb calorimetry (GE, model C2000 – IKA Werke GmbH and Co. KG, Staufen, Germany). In carcass samples crude fat (CF, by extraction with ethyl ether, Soxhlet procedure– method 920.39) was analyzed. The betaine content of the FWB was measured by liquid chromatography-electrospray ionization-isotope dilution mass spectrometry, according to the method described by Zeisel *et al.* (2003). High-performance liquid chromatography was used for amino acid analysis of the corn, soybean meal and FWB (Table 2).

From the calculation described by Sakomura & Rostagno (2016), the metabolizability coefficients (MC) of DM (MCDM), CP (MCCP) and GE (MCGE) were calculated, following the general equation: MC = 100 – [(nutrient excreted * 100) / nutrient intake]. The apparent metabolizable energy corrected for nitrogen (AMEn), was calculated by using the correction factor proposed by Hill & Anderson (1958).

Table 2 – Chemical composition of maize, soybean meal and fine wheat bran used in the experimental diets (as-fed basis).

	Feedstuffs		
	Fine wheat bran	Maize	Soybean meal
Composition, g·kg^{-1}			
Dry matter	875	875	888
Crude protein	161	75.6	469
Acid detergent fiber	83.2	33.8	80.7
Neutral detergent fiber	291	119	138
Betaine, mg 100g^{-1}	1100	-	-
Lysine	7.30	2.40	31.4
Methionine	1.90	1.30	3.70
Methionine + cysteine	6.50	2.60	12.6
Threonine	5.00	2.40	17.8
Arginine	12.2	3.60	36.5
Tryptophan	2.00	0.50	6.40
Isoleucine	5.40	2.50	20.7
Leucine	10.1	9.30	36.3
Histidine	4.10	2.20	12.8
Valine	7.50	3.60	22.0
Phenylalanine	6.30	3.60	24.0
Phenylalanine + Tyrosine	11.4	6.50	41.4
Glycine + Serine	15.5	6.50	45.5

The values of body DM, CP, GE and CF were applied to the average BW of the replicate, to obtain the average body composition of each replication at days eight and 28. Gains of GE, CP, CF and water (GEg, CPg, CFg, Wg, respectively) were determined by the difference between the final and the initial composition. The intake of AMEn was calculated for the total period. Feathers and GIT weight were expressed in g·kg^{-1}and in g·bird^{-1}.

A one-way analysis of variance was performed on all data, followed by Student-Newman-Keuls multiple-range test, at a significance level of $p<0.05$. The R software (R Development Core Team, 2016) was used for all statistical analyses.

RESULTS

Performance and metabolism of growing chickens are presented in Table 3. Feed intake was affected ($p<0.05$) by FWB inclusion. Birds receiving diets with FWB, consumed 1.7 and 4.1% less, i.e. treatments Fwb$^-$ and Fwb$^+$, respectively, than birds fed a diet with additional DL-methionine supplementation (Met). However, despite the lower feed intake, the group fed with FWB presented the same ($p>0.05$) feed conversion ratio (FCR) as those fed with additional DL-methionine and anhydrous betaine. Meanwhile, animals fed with the NC, showed a 6.1% increase ($p<0.05$) in FCR, compared with those fed with additional DL-methionine. Broilers fed with the NC showed the lowest ($p<0.05$) weight gain and final BW. However, because the negative control was formulated with 72% of the Met+Cys requirement, it provided 94.5% of the weight gain observed in the broilers fed with the diet containing DL-methionine supplementation, formulated with 85% of the Met+Cys requirement. Animals fed with FWB, showed the same final BW ($p>0.05$) as those fed with anhydrous betaine. The AMEn intake was higher ($p<0.05$) among birds fed with additional DL-methionine and anhydrous betaine than with the other dietary treatments.

Regarding metabolism variables, the MCDM was affected ($p<0.05$) by methionine deficiency, as well as by the FWB inclusion (Table 3), wherein birds receiving the negative control demonstrated a 3.3% lower DM metabolizability compared to the positive control with additional DL-methionine (Met). Likewise, broilers receiving FWB presented 3.6 and 3.5% lower MCDM, for treatments Fwb$^-$ and Fwb$^+$, respectively, than those receiving Met. There was no effect ($p>0.05$) between treatments Met and Fwb$^+$, for metabolizability of CP. For birds fed with anhydrous betaine and with Fwb$^-$, the MCCP did not differ ($p>0.05$) from the other treatments. Broilers fed with FWB showed the worse value ($p<0.05$) for the metabolizability of GE, with a mean value 4% less than those fed with the negative control, while birds fed with DL-methionine and anhydrous betaine showed the highest values in this variable. The dietary AMEn value was higher ($p<0.05$) for birds fed with DL-methionine supplementation but did not differ ($p>0.05$) from those with anhydrous betaine supplementation. The AMEn for the negative control was 4.5% lower ($p<0.05$) than the treatment with supplementation of DL-methionine.

Body composition and body gains of growing chickens are presented in Table 4. There was no difference ($p>0.05$) between treatments for body protein (mean of 186.2 g·kg^{-1}) and body fat (mean of 117 g·kg^{-1}) composition. However, body DM and body GE values were lower ($p<0.05$) in broilers fed with FWB compared to those that did not receive FWB. Broilers fed

Table 3 – Effect of different methionine levels on performance and metabolizability of nutrients in broilers fed diets supplemented or not with anhydrous betaine and fine wheat brain.

Variable	Treatments					RSE	*p*-value
	NC	Met	Bet	Fwb$^-$	Fwb$^+$		
Performance, kg							
Feed intake	1.805^a	1.801^a	1.794^a	1.770^{ab}	1.727^b	0.044	0.006
Weight gain	1.176^c	1.245^a	1.221^{ab}	1.210^{abc}	1.199^{bc}	0.029	0.001
FCR	1.535^b	1.447^a	1.470^a	1.463^a	1.440^a	0.037	0.001
Final BW	1.415^c	1.491^a	1.456^b	1.440^{bc}	1.431^{bc}	0.028	0.001
AMEn intake, kcal	5014^b	5208^a	5174^a	4976^b	4858^b	153.5	0.001
Metabolism, g·kg^{-1}							
MCDM	67.0^b	69.3^a	68.6^{ab}	66.8^b	66.9^b	1.49	0.003
MCCP	57.9^b	62.9^a	60.3^{ab}	59.5^{ab}	61.6^a	2.66	0.031
MCGE	74.8^b	77.3^a	76.6^a	71.7^c	71.9^c	1.41	0.000
AMEn, kcal·kg^{-1} DM	3060^c	3204^a	3185^{ab}	3127^{bc}	3120^{bc}	58.5	0.001

NC, formulated with 72% of the Met+Cys requirement; Met, 85% of the Met+Cys equivalents by adding DL-methionine; Bet, 85% of the Met+Cys equivalents by adding anhydrous betaine; Fwb$^-$, formulated with 72% of the Met+Cys requirement and 20% of fine wheat bran (FWB); Fwb$^+$, diet formulated with 85% of the Met+Cys requirement and 20% FWB.

RSE, residual standard error; FCR, feed conversion rate; BW, body weight; MCDM, metabolizability coefficient of the dry matter; MCCP, metabolizability coefficient of the crude protein; MCGE, metabolizability coefficient of the gross energy; EMAn, apparent metabolizable energy corrected by the nitrogen balance.

Mean values with different letters differ statistically according to Student-Newman-Keuls test ($p<0.05$).

Table 4 – Effect of different methionine levels on body composition, feathers and gastrointestinal tract (GIT) weight and body weight (BW) gains in broilers fed diets supplemented or not with anhydrous betaine and fine wheat brain.

Variables	Treatments					RSE	*p*-value
	NC	Met	Bet	Fwb$^-$	Fwb$^+$		
Body composition (without feathers)							
DM, g·kg^{-1}	332.8^a	333.5^a	331.5^a	322.0^b	322.4^b	8.38	0.011
CP, g·kg^{-1}	187.1	186.6	191.6	183.2	182.3	6.41	0.085
CF, g·kg^{-1}	116.9	121.9	118.0	114.8	115.1	6.21	0.174
GE, g·kg^{-1}	2152^a	2138^a	2131^a	2060^b	2041^b	69.0	0.007
Feathers and GIT							
Feathers, g·kg^{-1}	42.8	44.6	39.1	39.2	44.5	6.36	0.231
Feathers, g·bird^{-1}	60.5	66.5	56.9	56.1	63.6	8.91	0.120
GIT, g·kg^{-1}	23.4^b	28.8^{ab}	26.6^{ab}	39.1^a	29.8^{ab}	9.39	0.026
GIT, g·bird^{-1}	32.9^b	42.9^{ab}	38.8^{ab}	56.2^a	42.6^{ab}	13.57	0.025
Body gain							
CPg, g	249.9^{ab}	263.0^a	256.9^{ab}	245.7^b	245.5^b	11.6	0.018
EEg, g	145.4^b	160.8^a	151.5^{ab}	146.7^b	145.5^b	9.83	0.016
Wg, g	710	742	738	725	731	23.9	0.080
GEg, kcal	2791^{abc}	2928^a	2845^{ab}	2707^{bc}	2675^c	120.1	0.001

NC, formulated with 72% of the Met+Cys requirement; Met, 85% of the Met+Cys equivalents by adding DL-methionine; Bet, 85% of the Met+Cys equivalents by adding anhydrous betaine; Fwb$^-$, formulated with 72% of the Met+Cys requirement and 20% of fine wheat bran (FWB); Fwb$^+$, diet formulated with 85% of the Met+Cys requirement and 20% FWB.

RSE, residual standard error; DM, dry matter; CP, crude protein; CF, crude fat; GE, gross energy, GIT, gastrointestinal tract, Wg, water gain, E.Efficiency, energy retention efficiency.

Mean values with different letters differ statistically according to Student-Newman-Keuls test ($p<0.05$).

with higher DL-methionine levels (treatments Met and Fwb$^+$) showed a greater weight of feathers ($p<0.05$). Birds fed the diet with FWB addition and 72% of the Met+Cys requirement showed the highest ($p<0.05$) GIT weight, both in g·kg^{-1} as g·bird^{-1} assessment, while birds fed with the negative control, showed the lowest ($p<0.05$) corresponding values.

Water gain and energy efficiency were not affected ($p>0.05$) by the treatments (Table 4). Animals fed a diet with additional DL-methionine, showed the highest ($p<0.05$) gains of protein, fat and energy, among the treatments, but did not differ from those fed with anhydrous betaine. Broilers fed with FWB showed the lowest ($p<0.05$) protein, fat and energy gains, among treatments.

DISCUSSION

It has been demonstrated that anhydrous betaine can replace DL-methionine supplementation (Siljander-Rasi *et al.*, 2003; El-Husseiny *et al.*, 2007). This was also observed in the current trial, which showed that anhydrous betaine inclusion had the same metabolizability values as DL-methionine supplementation. Likewise, except for the final BW, birds fed with anhydrous betaine showed the same values for performance, body composition and body gains as those fed with DL-methionine. However, the Met was formulated with 85% of the Met+Cys requirements, with the broilers showing good performance values and, at 28 d, achieved the BW expected for male broilers (Rostagno *et al.*, 2011). Furthermore, betaine supplemented broilers with 1.0 g·kg^{-1} diet, showed performance results similar to those observed in 2.3 g·kg^{-1} DL-methionine supplemented broilers. Nonetheless, the main purpose of the current study was to investigate the impact of dietary FWB, as a betaine source, on growing chickens. Accordingly, the Bet treatment should perform as a second positive control, as indeed occurred. Although the chemical analysis showed that the FWB had a high betaine content (1,100 mg·100 g^{-1}), this experiment failed to significantly show the FWB as an effective methyl radical donor.

An explication for the low metabolizability of nutrients by broilers fed with FWB can be associated with the high CF content in this ingredient. According to other authors, increasing the dietary fiber level, increases the rate of feed passage, which might decrease nutrient utilization (Warpechowski, 1996; Krás *et al.*, 2013). Consequently, the lower nutrient utilization in broilers fed FWB can be linked to their low performance values, despite the diets formulated to contain similar nutrient contents, except for Met+Cys. The feed intakes were reduced in birds fed with FWB. Given the dietary fiber content in the FWB diets was higher than the other treatments, the results agree with those of Krás *et al.* (2013), in which the feed intake of broiler chicks was depressed, when exposed to a high fiber diet with wheat bran. The lower feed intake associated with the low nutrient utilization by birds fed with FWB, are associated with the relatively lower weight gain and final BW, among the birds.

The lower weight gain in broilers fed with the negative control deficient in Met+Cys, could be connected to a low protein synthesis. Protein synthesis is initiated universally, with the amino acid methionine (Nelson & Cox, 2012). When birds are fed a diet deficient in amino acid composition, or with a disbalance between amino acids, the protein synthesis is limited and the animal is unable to efficiently utilize the other dietary amino acid (McLeod, 1997). It is quite remarkable that the NC induced a reduction in the metabolizability coefficients of DM, CP and GE and this was accompanied by lower GIT weights in broilers receiving the negative control. This finding is scarcely reported but is probably related to the impairment of protein synthesis in the GIT (including enzymes). Studies have shown a reduced energy metabolizability in chickens fed a non-supplemented methionine diet (Nieto *et al.*, 1995) and a depressed protein digestion in broilers receiving the lower level of methionine in high-starch diets (Hartono *et al.*, 2014). Further, a methionine deficient diet (0.56% digestible Met+Cys), induced a lower growth of intestinal structures (villus height and width) in 21-d-old broilers, when compared to L- or DL-methionine supplemented diets (Shen *et al.*, 2015). It is also noticeable that the supplementation of methyl equivalents by anhydrous betaine (Bet) in the present experiment, was sufficient to overcome the observed effects in the NC broilers.

According to Fischer (1994), in conditions of nutrient deficiency, mainly of essential amino acids, body protein and fat are parameters that are affected. However, this response was not observed in the present trial, in which CP and CF body composition was not affected by the treatments. This can indicate that the methionine deficiency was insufficient to affect the protein or fat body composition. Broilers fed with high fiber (Fwb$^-$ and Fwb$^+$), showed the same CF body composition as those fed without dietary fibrous ingredients, which may indicate some capacity of FWB to donate methyl radicals through betaine. Zhan *et al.* (2006) reported a reduction in abdominal fat weight and an increase in breast meat yield in broilers supplemented with betaine. This occurred because betaine, which can donate a methyl radical, could improve the methionine availability for protein deposition. Furthermore, when betaine donates a methyl radical, the choline availability can also be improved.

Birds fed with FWB, showed the lower DM and GE body composition (Table 4), among the treatments. This result may be due to the higher fiber level and lower ME intake in the FWB diets. However, lower DM carcass composition is also observed in high fiber diets, when fat content is also depressed (Krás *et al.*, 2013). Nonetheless, although not significant, FWB fed broilers, presented the lower numbers for fat and protein composition, that additively contributed to the lower GE content.

It has been well documented that feathers are rich in sulfur amino acids (Garcia & Batal, 2005). Thereby, treatments rich in methionine can improve feathers synthesis. In contrast to the other treatments, birds fed with any DL-methionine supplementation (Met and Fwb$^+$) showed the most weight of feathers. In accordance, Garcia Neto *et al.* (2000) reported that the effect of betaine on feathers weight was less than that of methionine. These authors also observed that methionine supplementation was particularly beneficial in increasing total feather growth of birds, while betaine appears to assist in sparing methionine for the growth of the primary feathers.

Measurements in the present study, confirm that intake of high dietary fiber diets causes a significant expansion of the GIT, as confirmed in other studies (Jørgensen *et al.*, 1996). Broilers fed an FWB diet, deficient in Met+Cys content, showed a heavier GIT. These changes could have an impact on energy metabolism once visceral organs have a high rate of energy expenditure relative to their size (Pekas & Wray, 1991).

Treatments with FWB decreased body protein gain. Lopes *et al.* (2011) found that increasing the fibrous ingredients in poultry diets, promotes lower body protein retention. The changes in body protein gains were accompanied by changes in body CF and GE gains. This result may be due to the higher fiber content of the FWB diets and, consequently, a greater heat production in digestion and, also, to the greater expenses of feeding activity (Butzen *et al.*, 2015). According to Warpechowski (1996), the non-digestible fiber components can alter the thermal effect of the feed, because it can reduce the net energy-to-ME ratio.

Although birds fed with FWB showed a lower DM body composition, the water gain was not affected by the treatments. Considering the osmotic effects of betaine, an increased water retention could be expected. Betaine is accumulated in cell organelles and its osmoprotective effect assists cells exposed to osmotic and ionic stress, protecting enzymes associated with cell membranes from inactivation by inorganic ions (Klasing *et al.*, 2002).

In this trial, the energy efficiency was the same between all treatments. In accordance, Jørgensen *et al.* (1996) reported that the increasing levels of dietary fiber in the broilers diets, promoted similar values of retained energy expressed as per kg of DM intake.

CONCLUSIONS

Although the dietary inclusion of FWB, which is rich in betaine, improved the FCR in growing broilers, other expected effects from supplying methyl radicals were not observed under the evaluated conditions. These expected observations were probably suppressed by the negative effects of the high-fiber FWB on feed intake and energy use.

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