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RESEARCH PAPER

Bioavailability of different methionine sources for broilers from 1 to 21 days old

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Abstract

C.P. Sangali, L.D.G. Bruno, R.V. Nunes, A.R. de Oliveira Neto, P.C. Pozza, J.R. Henz, F.C.N. Giacobbo, and E. Berwanger. 2015. Bioavailability of different methionine sources for broilers from 1 to 21 days old. Cien. Inv. Agr. 42(1): 35-43. This trial aimed to evaluate the bioavailability of DL-2-hydroxy-4-(methyl) butanoic acid (DL-HMBA) and a poly-herbal ingredient (PHI) in relation to DL-methionine (DLM) on broilers. A sample of 1100 broiler chickens of the Cobb 500 strain, both male and female, were fed either a basal diet without industrial methionine supplementation or a basal diet that was supplemented with three DL-HMBA levels (0.170, 0.340, and 0.511%) or three DLM levels (0.111, 0.221, and 0.332%) in an amount equivalent to 65% of the respective DL-HMBA levels (on a product weight basis) and three levels of PHI (0.111, 0.221, and 0.332%) in amounts equivalent to the used DLM levels from 1 to 21 days of age. The simultaneous linear regression analysis (slope ratio) revealed a relative bioavailability of DL-HMBA of 39% compared to that of DLM for weight gain and 44% for feed conversion. However, the performance data from birds that were supplemented with PHI did not significantly adjust to simultaneous regression designs; therefore, it was not possible to determine the PHI bioavailability in relation to the DLM. Considering together all of the studied parameters, the relative bioavailability of DL-HMBA was 42% compared to the DLM on a product basis.

Key words: DL-2-hydroxy-4-methylthio butanoic acid, DL-methionine, Poly-herbal ingredient, Sulfur amino acids.

Introduction

The traditional diets for broilers are mainly formulated on corn and soybean meals to supplement protein needs; however, these diets do not

meet the requirements for methionine and sulfur amino acids, which are usually adjusted using commercial methionine.

The most common sources of methionine in the supplements that are available to the poultry industry are DL-methionine (DLM) and DL-2-hydroxy-4-(methyl) butanoic acid (DL-HMBA).

Recently, however, some natural sources of this amino acid have become available, including poly-herbal ingredient – PHI (Narayanswamy and Bhagwat, 2010). According to the manufacturers of these products, DLM is a pure powder product with greater than 99% purity, while DL-HMBA is liquid and has 88% active compounds. In addition, there is powdered PHI, which contains the dipeptides and oligopeptides of methionine, as well as the precursors and intermediates of this amino acid.

In addition to these physical differences, there are also known biochemical and metabolic differences among methionine sources that may contribute to the differences in intestinal absorption in birds (Maenz and Engele-Schaan, 1996ab; Drew *et al.*, 2003). Thus, it is crucial to estimate the real methionine values given to DL-HMBA and PHI compared to DLM because these values are important prerequisites for deciding which source of methionine should be used, also considering the costs of the ingredients that are used in diet formulation and animal production (Brugalli, 2003).

There have already been many discussions concerning the relative bioavailability of methionine from different sources, as well as a considerable number of experiments comparing various sources, mainly DL-HMBA and DLM, conducted in birds (Esteve-Garcia and Llauro, 1997; Hoehler *et al.*, 2005a; Elwert *et al.*, 2008). Furthermore, the correct statistical design to evaluate the bioavailability of different sources of essential nutrients has been under debate (Littell *et al.*, 1997; Kratzer and Littell, 2006; Piepho, 2006). A standardized method of statistical analysis would make comparisons of experiments assessing different sources of nutrients easier and more accurate (Littell *et al.*, 1997).

Thus, the objective of this study was to evaluate the relative bioavailability of DL-HMBA and PHI compared to DLM in diets for male broilers from 1 to 21 days old.

Material and methods

The trial was carried out at the Poultry Section of the Center for Experimental Stations of the Western Paraná State University - UNIOESTE, Campus Rondon-PR, in October, 2011.

A total of 1,100 Cobb 500 broiler chicks, males and females, were used from 1 to 21 days old, whose initial body weight was on average 46 ± 04 g. The birds were subdivided into 50 boxes, each 1.00×1.35 m. The experimental design was completely randomized in a 3×3 factorial design (three sources of methionine \times three supplementation levels) and an additional treatment without supplemental methionine (basal diet), with five replications and 22 birds per experimental unit for a total of 16.30 birds m^{-2} .

All diets were in mashed form and were based on corn, soybean meal, meat, bone meal and offal (Table 1). The basal diets contained starch, which was replaced with crystalline amino acids to formulate the different treatment diets. An analysis of the amino acid and protein contents of the experimental diets (Llames and Fontaine, 1994; Fontaine *et al.*, 1998) confirmed the calculated values. Thus, these values were used in further statistical analysis.

Three increasing DLM (0.111, 0.221 and 0.332%), DL-HMBA (0.170, 0.340 and 0.511%) and PHI (0.111, 0.221 and 0.332%) levels were added to the basal diet to achieve 33, 66 and 100% of the methionine levels utilized by the poultry integrator company. Three DLM levels were added, always considering 65% of the DL-HMBA amount; thus, a 65:100 ratio (product basis) was maintained. For PHI, three increasing levels were used to replace DLM, always one to one (product basis); therefore, a 100:100 ratio was maintained.

The elaboration processes that were applied to produce all of the diets that were used in this study were to produce first the basal diets (with-

Table 1. Basal diet composition (% as is).

Ingredients	%
Corn	58.350
Soybeans meal	28.000
Meat meal	2.760
Poultry viscera meal	3.820
Soybean oil	3.780
L-lysine (Hcl)	0.559
L-threonine	0.138
Common salt	0.311
Limestone	0.800
Dicalcium phosphate	0.100
Sodium bicarbonate ¹	0.100
Mycotoxin adsorbent	0.250
Mineral vitamin supplement ²	0.400
Inert ³	0.622
Nutrient levels (calculated)	
Apparent metabolizable energy (kcal kg ⁻¹)	3,139
Crude protein (%)	21.00
Calcium (%)	0.840
Available phosphorus (%)	0.420
Digestible methionine (%)	0.295
Methionine + cystine (%)	0.570
Digestible lysine (%)	1.260
Digestible threonine (%)	0.820
Digestible tryptophan (%)	0.200
Digestible valine (%)	0.880
Digestible arginine (%)	1.260
Digestible isoleucine (%)	0.800

¹Added to the diet to maintain a 210 meg kg⁻¹ electrolyte balance.

²Vitamins, minerals and additives Supplementation per kg diet: vitamin A - 10,000 IU, vitamin D3 - 2,500 IU, vitamin E - 20.83 IU, vitamin K3 - 1.67 mg, vitamin B1 - 2.08 mg; vitamin B2 - 5.42 mg; vitamin B6 - 2.92 mg; vitamin B12 - 15 mg; Folic acid - 1.00 mg; nicotinic acid - 35.00 mg; Pantothenic acid - 12.05 mg; choline - 278.44 mg, Biotin - 0.07 mg; Iron - 45 mg; copper - 8 mg; Zinc - 110 mg; organic zinc - 40 mg; iodine - 0.8 mg; Selenium - 0.4 mg; organic selenium - 0.1 mg; manganese - 75 mg.

³Used to correct the additions of DL-HMBA, DLM and PHI. The DL-HMBA, PHI and DLM replaced the inert content of basal diets to formulate diets supplemented with methionine sources.

out any methionine sources) and the higher-level methionine sources DLM, DL-HMBA and PHI (Table 1). The intermediate levels for every source were produced by mixing the basal diet with the higher level of every methionine source, which

was performed in different percentages to achieve the desired methionine level. DLM treatment 2 was produced by mixing 66% of treatment 1 (basal diet) with 33% of treatment 4 (higher level of DLM), and DLM treatment 3 was performed by adding 33% of the basal diet with 66% of treatment 4. The same procedure was performed for every studied source.

Diet and water were provided *ad libitum* throughout the entire experimental period; to achieve this, tubular feeders and water nipple drinkers were used.

An electric system was used for heating based on 127-W infrared lamps. A lamp was placed in each cage, whose height and drive were regulated according to the birds' growth. This management aimed at maintaining temperature and humidity as close to the ranges of thermal well-being. The used lighting program was constant, with 24 h of light (natural and artificial).

The temperatures were recorded daily using thermometers and averaged between 27.1 °C and 32.2 °C for the period from 1-7 days, between 26.3 °C and 30.2 °C for the period from 8-14 days, and between 22.8 °C and 28.1 °C during the 15-21-day period.

The evaluated parameters were feed intake, weight gain feed conversion and production factor. For performance, the final weight was an average based on the average weight of a bird box (22 birds).

The weight gain was calculated in grams as the difference between the birds' initial and the final weights in each experimental unit. Feed conversion was obtained by dividing the average feed consumption by the average weight gain of the birds in each experimental unit. The production factor, or the productive efficiency index, was determined at 21 days, according to Equation 1.

$$PF = \frac{\text{weight gain (Kg)} \times \text{availability}}{\text{age (days)} \times \text{feed conversion}} \times 100 \dots\dots(1)$$

The relative bioavailability values of DL-HMBA and PHI in relation to DLM for variables of performance were determined by simultaneous linear regression design or slope-ratio according to Equation 2, as suggested by Littell *et al.* (1997), using the general linear model (GLM) procedure from the Statistical Analysis Systems Institute software package (SAS, 1996, Version 6.11, Institute Inc., Cary, NC, USA).

$$y = a + (b^1x^1 + b^2x^2 + b^3x^3) \dots\dots\dots(2),$$

where y = performance criterion; a = performance that was obtained with the basal diet (y-intercept); b¹, b², and b³ = regression coefficients of the lines for DLM, DL-HMBA and PHI, respectively; and x¹, x², and x³ = dietary levels of DLM, DL-HMBA and PHI, respectively.

According to Littell *et al.* (1997), the bioavailability values of DL-HMBA and PHI in relation to DLM are provided by the regression coefficient ratios (b²/b¹ and b³/b¹).

To evaluate the production factor values, an overall analysis was carried out with all of the treatments to perform the residual mean square to test the factorial and perform the Dunnett test at 5% with an additional treatment. Tukey’s test at 5% probability was used to compare production of treatments factors in a factorial design.

Results and discussion

The performance was improved by adding DL-HMBA and DLM in relation to the basal diet-fed broilers (Table 2), suggesting that the aim of such basal diet, deficiency in Met + Cys, was achieved according to Lemme *et al.* (2002), who suggested that this is essential for detecting any differences in the bioavailability of dietary methionine sources. Weight gain and feed conversion were 701.45 g and

1.50 kg kg⁻¹, respectively, for the birds that were fed the basal diet, whereas the maximal responses with DLM-65 and DL-HMB addition were, respectively, 808.69 g and 769.72 g for weight gain and 1.37 kg kg⁻¹ and 1.40 kg kg⁻¹ for feed conversion (Table 2). This result shows that there was 15.29 and 9.73% improvement in gain and 8.43 and 6.27% in feed conversion, respectively, for both DLM-65 and DL-HMB. These results differed from those previously reported by Payne *et al.* (2006) and Viana *et al.* (2009), who observed similar performance responses in birds that were supplemented with increasing levels of DL-HMB or DLM in an amount equivalent to 65% according to the amount of each level of DL-HMB.

The birds that were supplemented with PHI at the same amounts as the DLM levels showed the worst performance. For these birds, maximal responses of only 693.34 g and 1.49 kg kg⁻¹, respectively, for weight gain and feed conversion were observed. This result shows a 1.16% worsening for weight gain and an only 0.47% improvement for feed conversion compared to those of the not-supplemented broilers (Table 2).

Table 2. Broilers performance, from 1 to 21 days old, when fed diets that were supplemented with different methionine sources.

Treatment	Feed intake (g)	Weight gain (g)	Feed conversion (g g ⁻¹)
1 – Basal	1051 abcd	701 bc	1.50 ab
2 – DLM 1	1118 a	791 a	1.41 cd
3 – DLM 2	1098 ab	801 a	1.37 d
4 – DLM 3	1112 a	809 a	1.38 d
5 – HMBA 1	1095 ab	756 b	1.45 bc
6 – HMBA 2	1090 ab	770 a	1.42 cd
7 – HMBA 3	1080 abc	770 a	1.40 cd
8 – PHI 1	1017 cd	663 c	1.53 a
9 – PHI 2	1008 d	667 c	1.51 a
10 – PHI 3	1034 cd	693 bc	1.49 ab
P value	<.0001	<.0001	<.0001
CV (%)	3.06	4.14	2.41

CV: coefficient of variation; DLM: DL-methionine, HMBA: DL-2-hydroxy-4-(methyl) butanoic acid; PHI: poly-herbal ingredient.
a, b, c: Means in columns followed by different letters differ by the Tukey test. Each value represents the mean of five replicates, with 22 birds per pen (110 birds per treatment).

The lowest performance that was observed in the birds that were supplemented with PHI is likely related to the imbalance of amino acids in the diet (Albino *et al.*, 1999). This imbalance was caused by a deficiency of methionine and indicates that the additional source (PHI) was not enough to meet the amino acid requirements. It should be highlighted that when broilers have a diet deficient in Met + Cys, protein synthesis becomes limited, which results in the inefficient use of the other amino acids. This result can be observed in this study, in the birds that were fed the basal diet or supplemented with PHI.

The birds' performance response to increasing levels of methionine sources followed a linear trend (except for DLM), so that the asymptote is not reached with the highest level of each source (Figures 1 and 2). According to Littell *et al.* (1997), the most accurate way to describe the performance responses in the studied birds to the increasing levels of methionine would be by a simultaneous or slope-ratio linear regression model. This model provides a way to determine unbiased estimates concerning bioavailability among test substances (DL-HMB and PHI) and the reference substance (DLM).

Thus, a simultaneous linear regression analysis showed a relative bioavailability for DL-HMB of 39% (Figure 1) DLM for weight gain and 44% for feed conversion (Figure 2) on a product basis. Nevertheless, Hoehler *et al.* (2005b) applied simultaneous exponential regression and obtained values of mean relative bioavailability from DL-HMB to DLM of 63% for weight gain and 67% for feed conversion in five experiments with broilers in four different countries.

The performance data from the broilers that were supplemented with PHI were also analyzed by simultaneous regression; however, there was no significant adjustment to the SAS GLM; therefore, it was not possible to determine the PHI bioavailability in relation to the DLM.

According to the studied bioavailability values for weight gain and feed conversion, the average bioavailability of DL-HMB was 42% compared to that of DLM. However, some studies (Lemme *et al.*, 2002; Jansman *et al.* 2003; Hoehler *et al.*, 2005b; Payne *et al.*, 2006) have obtained average values of bioavailability concerning DL-HMB in relation to DLM that ranged from 57 to 65%. These variations of bioavailability values are common and essential because they are estimated using biological assays.

There are several possible reasons for the lower bioavailability of DL-HMBA in relation to DLM. Physical and chemical differences between DLM and DL-HMBA can be directly related to the efficiency by which each substance is used by birds as a source of methionine. DLM is a pure product with a 99% activity of methionine, while DL-HMBA is composed of 12% water and 88% active compounds (Boebel and Baker, 1982). Of the 88% active substances, 65% are in monomeric form, and the remaining 23% are dimers and oligomers (Boebel and Baker, 1982). Some authors (Van Weerden *et al.*, 1992) have suggested a lower bioavailability of DL-HMBA polymers compared to that of pure DL-HMBA. Pure DL-HMBA showed a 66% relative bioavailability, while polymers have shown a 49% bioavailability compared to DLM on product basis (Van Weerden *et al.*, 1992). Hasseberg (2002) and Mitchell and Lemme (2008) also suggested that the poor use of DL-HMBA polymeric forms is a major reason for its lower bioavailability in relation to DLM.

Another potential reason for the lowest DL-HMBA bioavailability is that both the D and L isomers should be transformed in L-methionine before use (Hasseberg, 2002; Barbi *et al.*, 2004; Rombola *et al.*, 2008), but only the D isomers of DLM must be transformed. On the other hand, before any conversion of L isomers, methionine sources must be absorbed. The body has specific mechanisms that are responsible for nutrient absorption, and there seem to be some differences in the effectiveness of these mechanisms in absorbing different sources

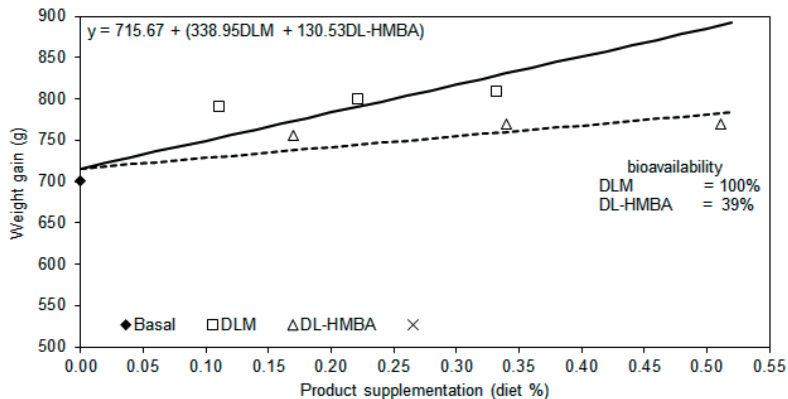


Figure 1. Bioavailability of DL-2-hydroxy-4-(methyl) butanoic acid (DL-HMBA) in relation to DL-Methionine (DLM) based on broiler weight gain.

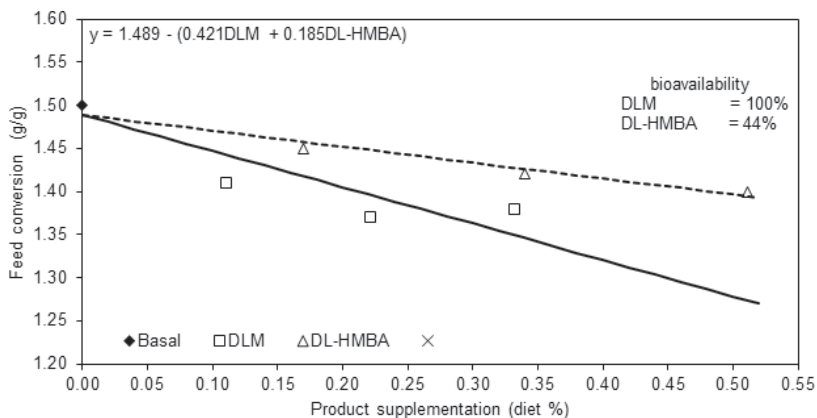


Figure 2. Bioavailability of DL-2-hydroxy-4-(methyl) butanoic acid (DL-HMBA) in relation to DL-Methionine (DLM) based on broiler feed conversion.

of methionine. Maenz and Engele-Schaan (1996b) have previously reported different mechanisms of active transport to uptake L-methionine (Na + dependent) and L-HMBA (H + dependent). These authors reported that the affinity by the carrier and the maximum speed of transport were higher for L-methionine than for L-HMBA. Therefore, differences in the transport mechanism between both sources of methionine can lead to differences concerning the transported quantity.

Several studies have also suggested a possible interaction between the gastrointestinal tract and DL-HMBA molecules. Maenz and Engele-Schaan (1996a) reported a significant conversion of DL-HMBA in non-absorbable subproducts during

their passage through the gastrointestinal tract. Furthermore, Lingens and Molnar (1996) have indicated that methionine absorption in broilers was significantly higher when given as DLM compared to HMBA. This result indicates a definite relationship between the availability of dietary methionine sources and its absorption into muscle tissue. Although these scientific studies have suggested that DL-HMBA absorption is reduced, there has been no clear explanation for this reduction. However, the interaction between the gastrointestinal tract and DL-HMBA was very clear in Drew *et al.* (2003). These authors reported that more than 10% of 3H-DL-HMBA activity remained in the distal ileum of conventional chickens, while only 4.7% remained in the distal ileum of germ-free chickens.

Moreover, there was no difference in the 3H-DLM residual amounts (3.0 vs 3.7%, respectively) in the distal ileum of conventional chickens or in those ones that are free of germs. Therefore, it seems that DL-HMBA is absorbed and metabolized by the intestinal microflora during its passage through the gastrointestinal tract. It seems that gut microflora have a significant impact on DL-HMBA availability in a broiler.

There are a considerable number of studies that compare DL-HMBA with DLM. However, there are few studies that compare the natural sources of methionine with DLM or studies that have investigated the possible metabolic loss of methionine activity from natural sources in poultry metabolism.

The values that were obtained for the production factor (Table 3) were higher ($P \leq 0.05$) in the diets with DLM and DL-HMB in all of the studied levels of supplementation when compared to those of the control treatment. The comparison among treatment averages in a factorial design showed no significant interaction ($P > 0.05$) for the production factor. However, there was no effect of the source or level, but the highest values were observed in diets with DLM and in the highest levels of supplementation.

Table 3. Mean values of production factor at 21 days old.

Treatment	Sources of methionine			Averages
	DLM	HMBA	PHI	
Basal (B)	223			
B + Level 1	267 ¹	249 ¹	206	241 B
B + Level 2	278 ¹	259 ¹	210	249 AB
B + Level 3	280 ¹	261 ¹	221	254 A
Averages	275 a	256 b	213 c	
CV (%)	5.83			

¹Means differ ($P \leq 0.05$) from the control treatment by Dunnett's test.

Means followed by different letters (lowercase in row and uppercase in column) differ ($P < 0.05$) by Tukey's test.

CV: coefficient of variation; DLM: DL-methionine, HMBA: DL-2-hydroxy-4-(methyl) butanoic acid; PHI: poly-herbal ingredient.

Taken together, all parameters analyzed in the present study show that the average bioavailability of DL-HMBA in relation to the DLM is 42% on a product basis.

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Resumen

C.P. Sangali, L.D.G. Bruno, R.V. Nunes, A.R. de Oliveira Neto, P.C. Pozza, J.R. Henz, F.C.N. Giacobbo, and E. Berwanger. 2015. Biodisponibilidad de las diferentes fuentes de metionina para broilers del 1 a los 21 días de edad. Cien. Inv. Agr. 42(1): 35-43. El objetivo de este trabajo fue evaluar la biodisponibilidad de DL-2-hidroxi-4- (metil) butanoico (DL-HMB) y de un poli ingrediente herbal (PIE) en relación con DL-metionina (DLM) en pollos de engorde. 1100 pollos Cobb 500, machos y hembras, fueron alimentados con una dieta basal sin suplementación de metionina industrial, o una dieta suplementada con tres niveles de DL-HMB (0,170; 0,340; 0,511%), o tres niveles de DLM (0,111; 0,221; 0,332%) en una cantidad equivalente al 65% del nivel de DL-HMB o tres niveles de PIE (0,111; 0,221; 0,332%), en una cantidad equivalente a los niveles de DLM, de 1 a 21 días de edad. El análisis de regresión lineal simultánea (slope ratio) reveló biodisponibilidad relativa de 39% de DL-HMB, en comparación con el DLM para aumentar el peso y de 44% para la conversión alimenticia. Sin embargo, los datos de rendimiento de las aves suplementadas con PIE no se ajustaron

significativamente a los modelos de regresión simultáneos, por consiguiente, no es posible determinar la biodisponibilidad relativa del PIE a la DLM. Consideran todos los parámetros estudiados, la biodisponibilidad relativa de DL-HMB fue del 42% en comparación con el DLM en la base del producto.

Palabras clave: Aminoácidos azufrados, DL-2-hidroxi-4- (metil) butanoico, DL-metionina, Poli ingrediente herbal.

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