



## Methionine Sources do not Affect Performance and Carcass Yield of Broilers Fed Vegetable Diets and Submitted to Cyclic Heat Stress

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### ABSTRACT

The supplementation of vegetal diets with L-methionine (100% molar), methionine hydroxyl analogue (HMB) (88% molar) or DL-methionine (99% molar) was compared as to the performance of broilers allocated in cages and submitted to cyclic heat stress (CHS). The trial was carried out from 21 to 42 days of age. Two levels of synthetic methionine were supplemented for each methionine source (0.1 or 0.3%), and the control treatment was not supplemented with synthetic methionine (negative control). Statistical analyses included the negative control treatment or were performed in a 3 x 2 factorial design (sources x levels). Addition of synthetic methionine to the basal level containing 0.63% of total sulphur amino acids significantly improved feed conversion (FC) independent of the source. On the other hand, improvements in weight gain (WG) and body weight (BW) were more consistent comparing the negative control to HMB-supplemented treatments. Factorial analysis showed better FC for L-Met compared to DL-Met, whereas HMB showed intermediate results. The supplementation level of 0.3% methionine showed better FC than 0.1%. Methionine levels or sources had no effects on carcass, yields of cuts or feathering results. Therefore, results of DL-Met and HMB added to vegetal-based diets in comparable molar terms promoted similar performance in broilers under CHS conditions.

### INTRODUCTION

The demand for poultry fed exclusively vegetal diets is a reality due to the BSE (bovine spongiform encephalomyelitis) crisis in European countries. The formulation of diets in this new scenario requires attention to issues such as amino acid availability (especially if the vegetable protein source is other than soybean meal), levels of non-starch polysaccharides and type of fat deposited in the carcass of birds fed vegetal feeds (Petri, 2002). The use of synthetic methionine supplementation in poultry feeds is a common practice in cereal and vegetable protein-based diets. The available methionine sources for animal industry are L-methionine (L-Met), DL-methionine (DLM) and HMB (2-hydroxy-4-methyl-thio-butanoic acid). The first is commonly used in experiments and is considered as the reference standard, since only the L isomer of methionine is deposited in the muscles or incorporated into enzymes by animals. The L isomer of methionine is present in natural sources of methionine, such as corn, soybeans, wheat, etc. D-methionine (D-Met) must be converted into the L form in order to be nutritionally active, and the conversion efficiency has been reported as 90% (Baker, 1994). Noll *et al.* (1984) also found that the bioavailability of L-Met is higher than that of D-Met (131±10%). When compared to the hydroxy analogue, the latter is similar (96±7%). Saunderson (1985) evaluated the incorporation of methionine sources



in the tissues using Carbon 14 and reported lower incorporation levels of DLM and HMB as compared to L-Met.

Experimental evidences suggest differences in the bioavailability of HMB and DLM, when these sources are included in diets based on crystalline amino acids (Boebel & Baker, 1982; Van Weerden *et al.*, 1982, 1983). In contrast, when diets based on intact proteins are used, the differences between these two sources are small or do not exist (Rohmer & Abel, 1999; Elkin & Hester, 1983). In some studies HMB has outperformed DLM (Ribeiro *et al.*, 2001; Vasquez-Anon *et al.*, 2004) and, in others, DLM has outperformed HMB (Schuttle & De Jong, 1996; Balnave & Oliva, 1990). Some results question the validity of using DLM as a standard in the slope ratio analysis when evaluating methionine sources (Vasquez-Anon *et al.*, 2004) and Gonzalez-Esquerria *et al.* (2004) suggested that an appropriate model for testing bioefficacy should include methionine intake, and not methionine levels, as the independent variable.

It has been suggested that under heat stress conditions (HS) birds fed HMB presented better growth rates, feed efficiency, absorption rate and lower mortality (Swick & Pierson, 1988; Swick *et al.*, 1990). In vitro experiments have shown that HS influenced D-Met absorption as compared to L-Met absorption (Knight *et al.*, 1994). This fact would be disadvantageous to DLM-fed birds in comparison to HMB-fed birds, when they enter into the thermoneutral period. There were no differences in performance between different methionine sources in a period of 5 days of thermoneutralty after the birds had experienced HS (Ribeiro *et al.*, 2001). However, these authors did observe an effect of methionine level on leg yield.

Shutte & Pack (1995) and Albino *et al.* (1999) questioned the sulphur amino acid (SAA) levels recommended by the NRC (1994) for broilers. On the other hand, Whitaker *et al.* (2002) concluded that there was no improvement in performance or carcass with the addition of methionine above the levels proposed by the NRC.

This study aimed to compare the effect of methionine levels and sources (L-Met, DLM, and HMB) added to vegetal diets (corn-soybean meal) on performance and carcass yield of broilers submitted to cyclic heat stress.

## MATERIAL AND METHODS

One experiment was carried out in order to compare the effects of three synthetic methionine sources (L-Met, DLM, or HMB) and two supplementation levels of sulphur amino acids (Met+ Cys) (0.73 or 0.93%) on the performance and carcass weight of male broilers submitted to cyclic heat stress (CHS) from 21 to 42 days of age. It was assumed that L-Met, DLM or HMB sources contained 100, 99 or 88% of methionine on the same molar basis. Methionine levels added to each treatment were based on the levels commonly used in the Brazilian broiler industry for grower diets (Lima, 1996). In addition to the treatments that contained synthetic methionine sources, a basal diet with no inclusion of synthetic methionine was used (negative control).

Four hundred and forty-eight Ross 308 male broilers were raised in batteries in an environmentally controlled room, with 24 hours of light, and standard temperature management during the starter phase. In this phase, all birds were fed a single corn and soybean meal-based diet, with 3,050 kcal ME/kg and 22% CP, using HMB as the supplementary source of methionine (Table 1). At 21 days of age, broilers were individually weighed and assigned to three different blocks, according to weight range (light, average, or heavy weight). A total number of 112 birds were culled. The remaining birds were distributed into 56 cages (measuring 0.5 m<sup>2</sup>) divided into 7 treatments, with 8 replicates per treatment and 6 birds per cage.

The diets of the experimental phase (22 to 42 days) were prepared from a common basal diet containing 3,150 kcal ME/kg, 19.5% CP, and the first and second limiting amino acids formulated to achieve 110% of the requirements suggested by NRC (1994) (Table 1). A proximate analysis (AOAC, 1990) was made before formulation, as well as amino acid analyses of the ingredients. Treatment diets were submitted to mixing test, determination of supplemental methionine or HMB, determination of calcium and phosphorus levels, and proximate analysis. At 42 days of age, two birds per cage with body weight as close as possible to the average weight of the parcel (Guidoni, 1994) were slaughtered to determine carcass, breast, leg, thigh and feather weights. The weight of feathers was calculated by the difference of bird weight before and after defeathering.

Cyclic heat stress (CHS) started when birds were 24 days old, as follows: 12 hours at 24°C, 3 hours of increasing temperature up to 32°C, 6 hours at 32°C,



and 3 hours of decreasing temperature down to 24°C. Birds were exposed to an adaptation period (22 to 24 days of age) with gradual temperature increase to prevent mortality during CHS. After 35 days of age, maximum temperature was 30- 31°C, because of the high relative humidity of the environment (85%). Temperature was monitored every 30 minutes using a DeltaTRAK ICON Data Logger thermometer. The following parameters were evaluated weekly throughout the experimental period: feed intake (FI), weight gain (WG), and feed conversion (FC). pH values were assessed in the duodenum, jejunum and ileum contents. Intestinal contents were homogenized, diluted in deionized water at 5.65 pH and measured by a digital pHmeter (Digimed DM 20).

The experiment was carried out as a randomized block design with 7 treatments allocated in 3 blocks. Statistical analysis were performed using the software SAS (1986). Collected data were submitted to analysis of variance and means were compared by the Tukey's test ( $p < 0.05$ ). Data were also statistically analyzed without considering the negative control treatment, according to a 3 x 2 factorial design – 3 sources of methionine and 2 levels of methionine within the 3 blocks of initial body weight ranges.

## RESULTS AND DISCUSSION

The results presented in Table 2 indicate that the calculated levels of Met were similar to the analyzed levels in the feed. The basal diet presented slightly higher values of SAA than calculated based on the results of amino acids analyses of raw material (0.66% vs 0.63%), but this could be attributable to analytical variation.

Performance results are shown in Table 3, including data of the negative control. There was a numerical improvement in WG, FC and BW at 42 days of age for the majority of the treatments with supplemental methionine, independently of methionine source. Nevertheless, significant differences were noted only among L-Met-0.93 and HMB-0.73 and 0.93%, showing better WG compared to the negative control. DLM treatments and L-Met-0.73 had intermediate WG. BW was higher only in HMB treatments comparing to the negative control. HMB-0.93 showed better FC than DLM-0.73 and the negative control. No significant differences were found for FI ( $p > 0.05$ ), despite the trend of increased consumption in the HMB-fed birds. Baker (1984) suggests that pair-feeding methodologies would be more adequate to compare feed

supplements, since feed intake affected other performance parameters. FC was the most sensitive parameter comparing basal and supplemented diets.

**Table 1** - Ingredients (%) and nutritional levels of the experimental diets.

Diet	1-21 days	21-42 days SAA levels		
		0.73	0.93	Basal diet
Corn	57.13	61.58	61.58	61.58
SBM 48%	36.42	30.94	30.94	30.94
Vegetable Oil	2.27	3.37	3.37	3.37
Dical. Phosphate	1.71	1.50	1.50	1.50
Limestone	1.48	1.62	1.62	1.62
Salt	0.46	0.41	0.41	0.41
HMB <sup>1</sup>	0.26	0.121	0.353	-
DL – Methionine	-	0.108	0.314	-
L – Methionine	-	0.107	0.311	-
Min <sup>2</sup> and Vit <sup>3</sup> premix	0.13	0.13	0.13	0.13
Lysine	0.061	-	-	-
Choline	0.045	0.07	0.07	0.07
Anticoccidial <sup>5</sup>	0.0225	0.0225	0.0225	0.225
Growth promoter <sup>5</sup>	0.012	0.012	0.012	0.012
Kaolin		0.23	-	0.35
ME (kcal/kg) <sup>6</sup>	3050	3156	3156	3156
CP (%) <sup>7</sup>	22.3	20.2	20.2	20.2
Ca (%) <sup>7</sup>	1.0	1.0	1.0	1.0
Total P (%) <sup>7</sup>	0.73	0.70	0.70	0.70
Lys (%) <sup>7</sup>	1.20	1.15	1.15	1.15
Arg (%) <sup>7</sup>	1.49	1.31	1.31	1.31
Met+Cys (%) <sup>7</sup>	0.94			0.63
Met (%) <sup>7</sup>	0.56			0.28

1 - Used alternately with the other sources. 2 - Starter and Finisher phases: 0.18 mg Selenium; 0.38 mg Iodine; 25 mg Iron; 6 mg Copper; 0.30 mg Cobalt; 35 mg Zinc; 72 mg Manganese per kg feed. 3 - Starter: 8,000 IU vit. A; 1,600 IU vit. D3; 30 mg vit. E; 1.5 mg vit K3; 1 mg vit. B1; 6 mg vit. B2; 2 mg vit. B6; 12 mcg vit. B12; 9 mg pantothenic acid; 28 mg niacin; 0.25 mg folic acid; 20 mcg biotin per kg feed. Finisher: 7,000 IU vit. A; 1,400 IU vit. D3; 20 mg vit. E; 1.5 mg vit. K3; 0.6 mg vit. B1; 4 mg vit. B2; 0.6 mg vit. B6; 10 mcg vit. B12; 9 mg pantothenic acid; 23 mg niacin; 0.25 mg folic acid; 20 mcg biotin per kg feed. 5 - Monensin; Flavomycin + olaquinox. 6 - Calculated values. 7 - Analyzed values; for methionine contents see Table 2.

**Table 2** - Analyzed content of methionine and cystine and molar contents of supplemental methionine sources in treatment diets.

Treatment	Synthetic Met (%)	HMB (%)	Total (SAA%)
Negative control	-	-	0.66
DLM	0.73	0.110	0.77
DLM	0.93	0.309	0.97
L-Met	0.73	0.133	0.79
L-Met	0.93	0.316	0.98
HMB	0.73	0.105	0.77
HMB	0.93	0.320	0.98

When the experiment was analyzed as a factorial design (Table 4), no significant interactions were found between sources and levels for FC, WG and BW. In regard to methionine supplementation, FC improved as the level of inclusion increased ( $p < 0.003$ ). Some authors have questioned whether SAA levels



**Table 3** - Feed intake (FI), weight gain (WG) and feed conversion (FC) from 21 to 42 days, and body weight (BW) at 42 days of age of broilers under heat stress and submitted to different methionine sources and levels (analysis including the negative control).

Treatment	FI (g)	WG (g) <sup>1</sup>	FC (g:g) <sup>1</sup>	BW(g) <sup>1</sup>
Negative control	2698	1296 b	2.098 a	2055 b
DLM 0.73	2612	1440 ab	1.962 b	2186 ab
DLM 0.93	2673	1442 ab	1.890 bc	2195 ab
L -Met 0.73	2564	1414 ab	1.883 bc	2175 ab
L -Met 0.93	2565	1497 a	1.850 bc	2252 ab
HMB 0.73	2776	1525 a	1.924 bc	2280 a
HMB 0.93	2720	1520 a	1.801 c	2266 a
CV	7.3	8.9	6.2	6.1
Probability	0.27	0.01	0.001	0.02

1 - Means in the same column with different letters are statistically different [Tukey (p<0.05)].

**Table 4** - Feed intake (FI), weight gain (WG) and feed conversion (FC) from 21 to 42 days, and body weight (BW) at 42 days of age of broilers under heat stress and submitted to different methionine sources and levels (factorial analysis, excluding the negative control).

Treatment	FI (g)	WG (g) <sup>1</sup>	FC (g:g) <sup>1</sup>	BW(g) <sup>1</sup>
<b>Methionine Source</b>				
DLM	2639	1434	1.930b	2195
L-Met	2621	1450	1.826a	2208
HMB	2744	1518	1.864ab	2275
<b>Methionine Level</b>				
0.73	2688	1453	1.926a	2215
0.93	2648	1482	1.821b	2237
<b>Probability</b>				
Source	0.31	0.16	0.04	0.20
Level	0.57	0.44	0.003	0.56

1 - Means in the same column with different letters are statistically different [Tukey (p<0.05)].

recommended by NRC (1994) would be insufficient to maximize broiler performance (Mendonça & Jensen, 1989; Hickling *et al.*, 1990; Shutte & Pack, 1995; Albino *et al.*, 1999). On the other hand, Whitaker *et al.* (2002) evaluated addition of methionine 100 to 140% of the requirement suggested by NRC (1994) and concluded that broiler performance was not affected. Regarding the sources, L-Met showed statistically better FC than DLM. Birds fed HMB showed intermediate FC. Since only L-isomers of methionine are used for protein synthesis, and transport systems for absorption have much lower an affinity for the D-isomer than the L-isomer (Lerner & Taylor, 1967), it was expected a better performance of birds fed the latter. Nevertheless, Waldroup *et al.* (1981) and Garlich (1985) did not observe differences between HMB, DLM and L-Met. On the other hand, based on results of two experiments involving over 11,000 broilers grown to market weight (49 days), Elkin & Hester (1983)

observed that HMB and DLM were both superior to L-Met, but were not different from each other regarding weight gain. FC was not different among the three sources.

The relationship between methionine source and dietary Arg:Lys ratio, which affected the feed intake of heat stressed broilers in previous studies (Balnave *et al.*, 1999) was not observed in the present trial using vegetal diets containing an Arg:Lys ratio of 1.30. Nevertheless, differences of more than 100 g in FI were detected when comparing HMB with DLM or L-Met treatment. Besides, Wiernusz (1994) and Ribeiro *et al.* (2001) did not observe differences in the performance of broilers fed either HMB or DLM and submitted to heat stress. Conversely, Swick & Pierson (1988), Swick *et al.* (1990) and Knight *et al.* (1994) have reported advantages of HMB as compared to DLM birds submitted to HS, particularly for feed efficiency, whereas Balnave & Oliva (1990) found improved feed efficiency under HS conditions for DLM, but not for HMB.

The supposition that vegetable diets can lead to feathering problems, due to the lower cystine level in feed, was not evidenced in this trial. Cannibalism or feather pecking were not observed. Methionine sources did not influence feathering, corroborating findings reported by López-Coello (1994). Results have also shown that the levels of methionine did not influence feathering. It is possible that the experimental conditions to which birds were submitted resulted in lower stress as compared to birds under field conditions. In this trial, even the imposed heat stress was not enough to inhibit feather growth in birds fed the methionine-deficient diet, despite causing a negative impact on performance. Elkin & Hester (1983) reported that birds fed 0.66% SAA in the diet under commercial rearing conditions have eaten feathers that were on the pen floor.

There was no influence of the levels or sources of supplemented methionine on carcass assessments or intestinal pH when the negative control treatment was compared to the other treatments (Table 5). Ribeiro *et al.* (2001) observed that birds supplemented with HMB in thermoneutral environment (25°C) had higher leg weight than those fed with DLM. Mendonça & Jensen (1989), Hickling *et al.* (1990), Gorman & Balnave (1995), Schutte & Pack (1995) and Albino *et al.* (1999) observed that higher methionine levels increased carcass and breast yields and reduced abdominal fat.



**Table 5** - Yields at slaughter (%) in 42 day-old broilers submitted to different methionine sources and levels.

Treatments	Feather	Carcass	Breast	Leg	Thighs	pH <sup>1</sup>
<b>Analysis including negative control treatment</b>						
Negative control	6.2	80.6	22.7	12.8	12.3	6.7
DLM – 0.73	5.6	82.0	23.5	12.8	12.6	6.5
DLM – 0.93	5.8	81.5	23.5	12.7	12.0	6.4
L-Met – 0.73	6.0	80.0	23.1	13.0	11.8	6.5
L-Met – 0.93	5.7	81.4	24.2	13.0	12.5	6.4
HMB – 0.73	6.2	81.8	24.5	12.8	12.1	6.5
HMB – 0.93	5.7	81.1	23.6	12.9	12.1	6.7
CV	15.9	2.0	5.3	4.4	5.8	3.8
Probability	0.82	0.24	0.36	0.89	0.25	0.21
<b>Factorial design analysis</b>						
DLM	5.7	81.7	23.5	12.8	12.3	6.5
Source L-Met	5.8	80.7	23.7	13.0	12.1	6.5
HMB	5.9	81.4	24.0	12.9	12.1	6.6
Level 0.73	5.9	81.2	23.7	12.9	12.2	6.5
0.93	5.7	81.3	23.8	12.8	12.2	6.5
<b>Probability</b>						
Source	0.71	0.21	0.65	0.31	0.77	0.21
Level	0.68	0.83	0.77	0.79	0.88	0.75

1 - Intestinal content pH.

## CONCLUSIONS

- The addition of supplementary methionine to a vegetal diet based on corn and soybean meal improved broiler performance, but had no effect on feed intake.
- Similar molar concentrations of DL-methionine or HMB, used as supplemental sources of methionine in vegetal diets, resulted in similar broiler performance.
- The supplementation of methionine above NRC (1994) recommendations was not advantageous in terms of carcass yield or feathering.

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