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## Performance of Broilers Fed with Different Levels of Methionine Hydroxy Analogue and DL-Methionine

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### ABSTRACT

One-day-old male Ross chicks were used in an experiment designed to compare two methionine sources, DL-methionine and methionine hydroxy analogue free acid (MHA-FA), and four different levels: 0.41; 0.47; 0.53; 0.59% (starter diet); 0.35; 0.41; 0.47; 0.53% (grower diet); and 0.30; 0.36; 0.42; 0.48% (finisher diet). One thousand two hundred and eighty chicks were housed in 32 experimental floor-pens (40 birds each) and fed 8 experimental diets based on corn and soybean meal for 47 days. The effects of methionine sources and levels were evaluated by performance data, carcass and cut yields, feather yield and abdominal fat content. Data were analyzed as a completely randomized design in a 2x4 factorial arrangement (2 sources and 4 levels), with 8 treatments and 4 repetitions. Analysis of variance was performed using PROC GLM of SAS<sup>®</sup>. Data indicated DL-methionine to be more effective in promoting growth than MHA-FA, and weight gain increased numerically in response to increasing levels of methionine in all phases.

### INTRODUCTION

Maximum carcass yield is an important objective in the modern poultry industry, which demands an optimum protein supply. Supplementation should avoid the excess of nutrients in the formulated diets, and, consequently, the unnecessary expenses with nutrition. Nevertheless, low-cost diet formulation promoted a decrease in the crude protein (CP) content of diets, resulting in a higher fat concentration in the carcass (Garlich, 1985).

Methionine is an important amino acid in protein synthesis in broilers. Synthetic sources are available for over four decades: DL-methionine and DL-2-hydroxy-4-[methyl] butanoic acid (DL-HMB), also known as methionine hydroxy analogue (MHA), is bought as a calcium salt (MHA-Ca) or as a free acid (MHA-FA). Whereas L-methionine and D-methionine are actively absorbed (transported against a concentration gradient), MHAs are passively absorbed, by diffusion, from a more concentrated medium to a less concentrated medium. As a result, lower intestinal absorption efficiency is expected for the hydroxy analogues in comparison to DL-Met, as reported by Esteve-Garcia & Austic (1987). Broilers under stressing conditions absorb less MHA-FA than DL-methionine (Rostagno & Barbosa, 1995). Baker & Boebel (1980) concluded that the bioavailability of MHA-FA was lower than that of DL-methionine. They also reported that the efficacy of the purified form of MHA-Ca, MHA-FA and a polymer preparation of free-acid methionine (MHAP-FA) were 87, 78 and 67% if compared to DL-Met, when used as the only source of sulphur amino acids. Thomas *et al.* (1991) reported that MHA-FA showed 72 and 73 %



of bioefficacy for weight gain and feed conversion when compared to DL-Met, respectively. However, Romoser *et al.* (1976) and Waldroup *et al.* (1981) used practical and semi-purified diets and reported no differences in the performance of broilers that had been supplemented with levels of MHA-FA, MHA-Ca, DL-Met or L-Met at the same equimolar basis.

Diets with protein contents of 16 to 20% resulted in smaller abdominal fat deposit during the finisher period, with increasing sulphur amino acid supplementation (Mendonça & Jensen, 1989).

This study was conducted with the objective of comparing the performance of broilers given different levels of DL-methionine and methionine hydroxy analogue free acid, based on carcass yield, abdominal fat and feather yield.

## MATERIAL AND METHODS

The experiment was conducted in the poultry rearing facility of the Faculdade de Medicina Veterinária e Zootecnia from USP, Campus de Pirassununga, SP, during 47 days. Male Ross chicks (1,280) were housed in 32 pens of 4.28m<sup>2</sup> (40 birds/pen) in a completely randomized design according to a 2x4 factorial (2 sources and 4 methionine levels), with 8 treatments and 4 repetitions. Experimental diets (Table 1) were formulated in order to support the nutrient requirements of the birds, except for methionine, which was supplemented by the two sources (DL-MET and MHA-FA). The supplementation was equimolar, following the recommendations of the manufacturer. Four levels were used: 0.41, 0.47, 0.53 and 0.59 % in the starter diet (1 to 21 days); 0.35, 0.41, 0.47 and 0.53 % in the grower diet (22 to 42 days), and 0.30, 0.36, 0.42 and 0.48 % in the finisher diet (43 to 47 days).

The following performance variables were determined: weight gain (WG), feed intake (FI) and feed conversion (FC), for each phase and for the total period (1 to 47 days). At the end of the experimental period, 4 birds per repetition were randomly chosen and killed, totaling 144 birds. Carcass yield (CY) was obtained by weighing the birds before and after fasting for 8 hours, bleeding, weighing after bleeding, scalding, plucking, weighing after plucking, evisceration, viscera and abdominal fat weighing, eviscerated carcass weighing. Carcasses were chilled in a counter flow chiller and stored for 24 hours in a cold chamber. After the excess of water had been eliminated, carcasses and cuts were weighed. Abdominal fat, including the fat surrounding the gizzard, was determined according to Cabel *et al.*

(1987). The yields of the carcass, cuts and viscera were determined based on the methodology adapted by Souza *et al.* (1994).

Data were analyzed using the PROC GLM of the Statistical Analysis System (SAS, 1985), and means were compared by Tukey's test at the 5% probability level. Data referring to the different supplementation levels used for each variable were submitted to orthogonal contrast analysis, and deviation equations were determined when the contrast analysis was significant ( $p < 0.05$ ). The interaction between the source x level was analyzed and further analysis was performed when interaction was significant ( $p < 0.05$ ), in order to study

**Table 1** – Composition and nutritional levels of the experimental diets.

| Ingredient                       | Starter | Grower | Finisher |
|----------------------------------|---------|--------|----------|
| Corn                             | 52.26   | 57.11  | 63.70    |
| Soybean Meal                     | 40.13   | 34.00  | 28.00    |
| Soya oil                         | 3.52    | 4.90   | 4.50     |
| Salt                             | 0.35    | 0.35   | 0.35     |
| Limestone                        | 1.24    | 1.60   | 1.60     |
| Dicalcium phosphate              | 1.60    | 1.14   | 0.95     |
| Inert + methionine <sup>1</sup>  | 0.50    | 0.50   | 0.50     |
| Vitamin/Mineral mix <sup>2</sup> | 0.40    | 0.40   | 0.40     |
| Nutrient Content (Calculated)    |         |        |          |
| ME, kcal/kg                      | 2,950   | 3,100  | 3,150    |
| Crude Protein, %                 | 22.5    | 20.0   | 18.0     |
| Methionine %                     | 0.35    | 0.32   | 0.30     |
| Methionine + Cystine, %          | 0.71    | 0.65   | 0.60     |
| Calcium, %                       | 0.95    | 0.95   | 0.90     |
| Available P, %                   | 0.45    | 0.35   | 0.30     |

1 - Supplementation with four different levels of DL-methionine and MHA-FA.

2 - Starter vitamin and mineral premix, levels/kg: vit. A, 2,250,000 IU; vit. D<sub>3</sub>, 450,000 IU; vit. E, 5,250 mg; vit. K, 360 mg; vit. B<sub>2</sub>, 1,130 mg; vit B<sub>12</sub>, 3,750 mcg; niacine, 7,465 mg; choline, 62,250 mg, calcium pantothenate, 2,580 mg, iron, 14,000 mg, copper, 12,500 mg, manganese 14,500 mg; cobalt, 21 mg; iodine, 150 mg; zinc, 12,500 mg; selenium, 52 mg; monensin and nicarbazin, 250,000 mg; growth promoter (avilamicin), 2,500 mg; antioxidant (BHT), 1,280 mg; Grower and finisher vitamin mineral premix, levels/kg: vit. A, 1,875,000 IU; vit. D<sub>3</sub>, 375,000 IU; vit. E, 3,250 mg; vit. K, 270 mg; vit. B<sub>2</sub>, 900 mg; vit B<sub>12</sub>, 2,500 mcg; niacine, 6,270 mg; choline, 48,800 mg, calcium pantothenate, 1,990 mg; iron, 14,000 mg, copper, 1,750 mg; manganese, 14,500 mg; cobalt, 21 mg; iodine, 150 mg; zinc, 12,500 mg; selenium, 43 mg; salinomycin, 250,000 mg; growth promoter (avilamicin), 2,500 mg; antioxidant



changes of each source within each level and vice-versa.

## RESULTS AND DISCUSSION

In the starter phase (1 – 21 days, Table 2), DL-MET was more effective in promoting bird growth when compared to MHA-FA ( $p < 0.05$ ), similar to the result reported by Van Weerden *et al.* (1983). Further analysis of the orthogonal contrasts showed a linearity of the data ( $p < 0.05$ ), producing the following equation: Initial WG =  $0.76 + 0.10X$ , where X is the methionine level. There was an increase of 2.72% in weight gain as a response to the highest methionine level (0.53%) when compared to the lowest level (0.35%). The increasing weight gain with increasing methionine levels in the

diet have also been reported by Schutte & Van Weerden (1981), Twining & Hochstetler (1982) and Elkin & Hester (1983). Nevertheless, Garlich (1985) found differences for weight gain at 21 days of age that were attributed to methionine levels, but not due to the source that was used. Feed intake was not affected by levels or sources in the initial phase. Furthermore, no significant effects were seen for sources, levels and source x level interaction on feed conversion, differently from the results reported by Van Weerden *et al.* (1983), who observed better feed conversion of DL-MET compared to MHA-FA. Garlich (1985) found that feed conversion was better when methionine was supplemented, but no differences were seen due to methionine source.

During the grower phase (22 to 42 days), weight

**Table 2** – Weight gain (kg/bird), feed intake (kg/bird) and feed conversion in broilers at the different phases for the four methionine dietary levels.

| Levels (%)     | Weight Gain <sup>1</sup> |                    |       | Feed intake |       |       | Feed conversion    |                    |       |
|----------------|--------------------------|--------------------|-------|-------------|-------|-------|--------------------|--------------------|-------|
|                | DL-MET                   | MHA                | Mean  | DL-MET      | MHA   | Mean  | DL-MET             | MHA                | Mean  |
| Starter Phase  |                          |                    |       |             |       |       |                    |                    |       |
| 0.41           | 0.808                    | 0.790              | 0.799 | 1.120       | 1.158 | 1.171 | 1.380              | 1.407              | 1.394 |
| 0.47           | 0.813                    | 0.800              | 0.806 | 1.183       | 1.135 | 1.158 | 1.455              | 1.418              | 1.437 |
| 0.53           | 0.823                    | 0.803              | 0.813 | 1.163       | 1.133 | 1.148 | 1.413              | 1.428              | 1.421 |
| 0.59           | 0.830                    | 0.805              | 0.818 | 1.170       | 1.145 | 1.133 | 1.410              | 1.410              | 1.410 |
| Mean           | 0.818 <sup>a</sup>       | 0.799 <sup>b</sup> | 0.809 | 1.159       | 1.145 | 1.152 | 1.419              | 1.416              | 1.418 |
| CV%            | 2.530                    | 1.470              | 2.370 | 3.053       | 2.450 | 2.793 | 2.352              | 2.500              | 2.437 |
| Grower Phase   |                          |                    |       |             |       |       |                    |                    |       |
| 0.35           | 1.548                    | 1.568              | 1.558 | 3.048       | 3.415 | 3.232 | 1.975              | 2.148              | 2.062 |
| 0.41           | 1.578                    | 1.573              | 1.576 | 3.225       | 3.280 | 3.253 | 2.048              | 2.060              | 2.054 |
| 0.47           | 1.610                    | 1.583              | 1.597 | 3.128       | 3.150 | 3.139 | 1.940              | 1.990              | 1.965 |
| 0.53           | 1.620                    | 1.615              | 1.618 | 3.290       | 3.255 | 3.273 | 2.035              | 2.013              | 2.024 |
| Mean           | 1.589                    | 1.585              | 2.510 | 3.173       | 3.275 | 3.224 | 1.955 <sup>a</sup> | 2.053 <sup>b</sup> | 2.026 |
| CV%            | 2.698                    | 2.384              | 1.586 | 4.440       | 3.620 | 4.289 | 3.581              | 4.059              | 4.000 |
| Finisher Phase |                          |                    |       |             |       |       |                    |                    |       |
| 0.30           | 0.266                    | 0.268              | 0.267 | 0.710       | 0.727 | 0.719 | 2.618              | 2.733              | 2.676 |
| 0.36           | 0.273                    | 0.280              | 0.277 | 0.710       | 0.748 | 0.729 | 2.635              | 2.670              | 2.653 |
| 0.42           | 0.290                    | 0.283              | 0.287 | 0.760       | 0.730 | 0.745 | 2.598              | 2.563              | 2.581 |
| 0.48           | 0.300                    | 0.320              | 0.310 | 0.720       | 0.780 | 0.750 | 2.558              | 2.343              | 2.451 |
| Mean           | 0.282                    | 0.288              | 0.285 | 0.725       | 0.746 | 0.736 | 2.602              | 2.577              | 2.589 |
| CV%            | 17.46                    | 12.86              | 15.16 | 4.78        | 5.13  | 5.10  | 14.20              | 13.27              | 13.54 |

1- Means followed by different letters in each line and within each parameter are different ( $p < 0.05$ ) independent of the methionine source.



**Table 3** – Weight gain (kg/bird), feed intake (kg/bird) and feed conversion of broilers reared from 1 to 47 days of age.

| Levels (%) | Weight gain <sup>1</sup> |       |       | Feed intake |       |       | Feed conversion    |                    |       |
|------------|--------------------------|-------|-------|-------------|-------|-------|--------------------|--------------------|-------|
|            | DL-MET                   | MHA   | Mean  | DL-MET      | MHA   | Mean  | DL-MET             | MHA                | Mean  |
| 0.41       | 2.630                    | 2.643 | 2.637 | 5.218       | 5.398 | 5.308 | 1.985              | 2.018              | 2.002 |
| 0.47       | 2.680                    | 2.653 | 2.667 | 5.310       | 5.258 | 5.284 | 2.008              | 1.980              | 1.994 |
| 0.53       | 2.720                    | 2.698 | 2.709 | 5.500       | 5.340 | 5.420 | 2.018              | 2.010              | 2.014 |
| 0.59       | 2.725                    | 2.748 | 2.737 | 5.195       | 5.628 | 5.412 | 1.913              | 2.040              | 1.977 |
| Mean       | 2.689                    | 2.686 | 2.690 | 5.306       | 5.406 | 5.356 | 1.981 <sup>a</sup> | 2.012 <sup>b</sup> | 1.997 |
| CV%        | 2.840                    | 3.440 | 3.110 | 4.058       | 3.433 | 3.812 | 3.285              | 3.112              | 3.536 |

1- Means followed by different letters in each line and within each parameter are different ( $p < 0.05$ ) independent of the methionine source.

gain increased ( $p < 0.05$ ) due to increasing methionine levels (Table 2). Analysis of the linear orthogonal contrast was significant ( $p < 0.01$ ), and the corresponding equation was: Growing WG =  $1.44 + 0.34X$ , where X is the methionine level supplemented on the diet. A weight gain increase of 4.65% was seen in the highest methionine level (0.53) in comparison to the lowest level (0.35). Garlich (1985) reported a weight gain 12.4% higher compared to the weight gain of broilers fed the basal diet, that was deficient in methionine. The same was observed by Huyghebaert (1993), who compared DL-MET and MHA-FA, and reported that the increase in methionine levels promoted an increase of 14% in weight gain when compared to the methionine-deficient basal diet. A similar result was described by Rostagno & Barbosa (1995), who observed an increase in 13% of weight gain for birds fed a basal diet without methionine. Source or level did not affect feed intake during the growing period, and no interaction was seen between the two factors. Nevertheless, Garlich (1985) reported that feed intake differed significantly due to methionine supplementation level. Feed conversion in the growing phase was significantly different ( $p < 0.05$ ) among the different sources, so that the birds fed with DL-MET had a better feed conversion than the birds supplemented with MHA-FA. No effect of level was seen for feed conversion. On the other hand, Huyghebaert (1993) reported that feed conversion was 11% better with the increase in the percentage of methionine in the diet, and Schutte & Pack (1995) found a similar result.

During the finisher phase (43 to 47 days), no significant difference was seen for weight gain due to sources and levels, but there was an increasing trend with higher methionine levels, for the two studied sources. Identical results were reported by Schutte & Pack (1995), however Visentini *et al.* (1996) studied

substitution levels of DL-MET by MHA-FA and concluded that the total substitution (100%) resulted in smaller weight gain. Feed intake during this phase was not affected by source, but orthogonal contrast analyzed within levels showed a significant linear contrast ( $p < 0.05$ ), according to the equation: Final FI =  $0.66 + 0.18X$ , where X is methionine level for this period. This result is in accordance to those reported by Schutte & Pack (1995), who reported that the increase in methionine levels increased feed intake. There was no effect of different methionine levels or sources on feed conversion in the final phase.

No effect of source, level or interaction between source x level was seen for weight gain and feed intake during the total period (Table 3). Feed conversion was significantly different ( $p < 0.05$ ) between sources, so that broilers fed with DL-MET showed better feed conversion than the birds supplemented with MHA-FA.

No significant differences were seen for sources, treatments and interaction between the factors for carcass yield, thigh yield, leg yield, breast yield and abdominal fat (Table 4). Nevertheless, Jensen *et al.* (1989) observed that the abdominal fat in males and females slaughtered at 42 days of age decreased with the increase in sulphur amino acids in the diet. Hickling *et al.* (1990) suggested that the response of breast meat production to methionine + lysine levels might result in economic benefits that would obviously depend on the cost of the supplemented amino acid and the price of broiler meat. Since they reported an increase of 15 to 20 g in breast meat per bird with an adequate supplementation of methionine and lysine, these data support the hypothesis that higher levels of essential amino acids in the diet, above those required for maximizing weight gain, increase meat production.

The interaction between source and level was significant for feather yield, but no significant contrast



**Table 4** – Carcass yield (%), abdominal fat (%), legs (%), thighs (%) and breast (%) at 47 days of age in broilers submitted to different treatments and sources of methionine.

|                            | Sources    |         |        |        |
|----------------------------|------------|---------|--------|--------|
|                            | Levels (%) | DL-MET  | MHA-FA | Mean   |
| Carcass yield <sup>1</sup> | 0.41       | 78.693  | 78.765 | 78.729 |
|                            | 0.47       | 78.493  | 79.098 | 78.796 |
|                            | 0.53       | 79.095  | 78.610 | 78.853 |
|                            | 0.59       | 79.597  | 78.568 | 79.083 |
|                            | Mean       | 78.970  | 78.760 | 78.865 |
|                            | CV %       | 0.898   | 0.800  | 0.847  |
| Abdominal Fat <sup>2</sup> | 0.41       | 2.075   | 2.540  | 2.308  |
|                            | 0.47       | 2.235   | 2.258  | 2.247  |
|                            | 0.53       | 1.988   | 2.610  | 2.299  |
|                            | 0.59       | 2.473   | 2.510  | 2.492  |
|                            | Mean       | 2.193   | 2.472  | 2.335  |
|                            | CV %       | 24.0739 | 17.268 | 21.178 |
| Leg <sup>3</sup>           | 0.41       | 13.335  | 13.055 | 13.195 |
|                            | 0.47       | 13.288  | 13.175 | 13.232 |
|                            | 0.53       | 13.040  | 13.288 | 13.164 |
|                            | 0.59       | 13.070  | 13.000 | 13.035 |
|                            | Mean       | 13.183  | 13.130 | 13.156 |
|                            | CV %       | 1.650   | 2.169  | 1.90   |
| Thigh <sup>3</sup>         | 0.41       | 15.730  | 15.578 | 15.654 |
|                            | 0.47       | 15.328  | 15.510 | 15.419 |
|                            | 0.53       | 15.128  | 15.618 | 15.373 |
|                            | 0.59       | 15.575  | 15.468 | 15.522 |
|                            | Mean       | 15.440  | 15.544 | 15.492 |
|                            | CV %       | 2.319   | 2.597  | 2.447  |
| Breast <sup>3</sup>        | 0.41       | 25.303  | 26.305 | 25.804 |
|                            | 0.47       | 26.003  | 26.228 | 26.116 |
|                            | 0.53       | 26.155  | 25.698 | 25.927 |
|                            | 0.59       | 25.500  | 26.428 | 25.964 |
|                            | Mean       | 25.740  | 26.165 | 25.952 |
|                            | CV, %      | 2.169   | 3.212  | 2.830  |

1- Percentage of live weight.

2 - Percentage of hot eviscerated carcass.

3 - Percentage of cold eviscerated carcass.

was seen for DL-MET and MHA-FA (Table 5). Bastiani *et al.* (1994) reported better feathering at 35 days of age for the birds fed higher methionine levels in the diet.

**Table 5** – Feather yield (%) of 47-day-old broilers submitted to different sources and levels of methionine.

|                            | Sources    |        |        |       |
|----------------------------|------------|--------|--------|-------|
|                            | Levels (%) | DL-MET | MHA-FA | Mean  |
| Feather Yield <sup>1</sup> | 0.41       | 7.415  | 6.993  | 7.204 |
|                            | 0.47       | 7.180  | 6.495  | 6.802 |
|                            | 0.53       | 6.655  | 7.573  | 7.114 |
|                            | 0.59       | 7.430  | 7.403  | 7.417 |
|                            | Mean       | 7.170  | 7.116  | 7.143 |
|                            | CV %       | 8.296  | 9.133  | 8.588 |

1- Percentage of live weight.

## CONCLUSIONS

DL-methionine was more effective in promoting a better live performance of the birds when compared to methionine hydroxy analogue, and higher methionine levels increased weight gain, but feed intake was not affected by methionine level or sources, except in the final phase. Feed conversion was affected by methionine source and dietary level, since it was better for broilers fed DL-methionine than methionine hydroxy analogue and with higher levels.

Feather yield was higher in the birds receiving the highest methionine levels when compared to the broilers fed with lower methionine levels. Nevertheless, sources and/or levels of methionine did not affect carcass yield, thigh yield, leg yield, breast and abdominal fat.

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