

# IMPACT OF HERBAL METHIONINE SUPPLEMENTATION ON BROILER GROWTH PERFORMANCE, SERUM BIOCHEMICAL INDICES, AND RELATIVE ORGAN WEIGHTS

DEMIREL, R.<sup>1\*</sup> – ALKAN, C.<sup>2</sup>

<sup>1</sup>*Department of Animal Science, Faculty of Agriculture, Dicle University, Diyarbakır, Türkiye*

<sup>2</sup>*Ministry of Agriculture and Forestry, Mardin, Türkiye  
(e-mail: calkan47@hotmail.com; ORCID: 0000-0001-8870-3533)*

*\*Corresponding author*

*e-mail: ramazand@dicle.edu.tr; phone: +90-535-820-9816; ORCID:0000-0003-0816-4125*

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**Abstract.** Methionine is essential for the daily physiological activities of animals, as well as maintaining health and ensuring sustainable production levels. The amino acid methionine plays a critical role in facilitating various vital functions in animals, including cell growth and serving as a methyl donor in biological processes. In the study, a total of 312 one-day-old male Ross 308 broiler chicks were randomly allocated into three groups for a 6-week feeding period. The experimental treatments included commonly used synthetic DL-methionine (1 g/kg of feed) and two proposed doses of herbal extract-derived methionine (1.0 and 1.25 g/kg of feed) as alternative methionine sources. At the end of the study, no statistically significant differences were observed among the groups in terms of live weight gain, feed intake, feed conversion ratio, or the relative weights of the gizzard, carcass, and abdominal fat. Similarly, no significant differences were found in blood serum glucose, total protein, creatinine, triglyceride, cholesterol and calcium concentrations. However, statistically significant differences were observed among the groups concerning the relative weights of the intestine, spleen, and liver, as well as blood serum uric acid, albumin, and phosphorus levels. In conclusion, herbal extract-derived methionine can be confidently used as a natural methionine source in place of synthetic DL-methionine without negatively impacting animal health and performance.

**Keywords:** *broiler chickens, feeding efficiency, relative organ weights, serum biochemical parameters, synthetic methionine*

## Introduction

Proteins are fundamental structural components of human and animal bodies as well as having high nutritional value. Additionally, they play a crucial role in all biological functions of animals, including growth, wound healing, hemoglobin synthesis, antibody production, animal fiber formation, keratinous structure development, and the production of insulin. Chicken meat is becoming increasingly popular due to its short production time, low cost, and high nutritional value. Therefore, the poultry industry will focus more on animal welfare and health in the future (Korver, 2023). Although there are over 300 different amino acids found in nature, only 20 of them are incorporated into protein structures. Sulfur-containing essential amino acids, such as methionine and cystine, are particularly deficient in plant-based feedstuffs. Exogenous amino acids must be supplied daily to ensure healthy and well-balanced nutrition for both humans and animals. Sulfur-containing amino acids play a crucial role in enhancing an animal's resistance to infectious diseases (Kaplan and Yıldız, 2012).

Sulfur-containing amino acids are powerful antioxidants that protect cells from free radical damage. Insufficient intake of sulfur-containing amino acids can lead to antioxidant imbalance as well as various health and productivity issues (Anonymous, 2009).

The methionine content of commonly used feedstuffs is limited, except for animal by-products. Methionine is the most limiting amino acid in commonly used corn-soybean-based poultry diets. In conventional poultry diets, feedstuffs supply only 75% of the total methionine requirements of the animals. Approximately 25% of the methionine requirements of the birds should be met by synthetically produced DL-methionine sources, which are 99.5% pure and highly effective (Fanatico, 2010).

In organic poultry and aquaculture production, the use of synthetically produced DL-methionine is being increasingly restricted or completely banned. According to organic poultry production standards, the recommended levels of synthetic methionine have been reduced to 2 pounds per ton for laying and broiler chickens, 3 pounds per ton for turkeys and other poultry (Bazadeh and Ahmadi, 2022). To address this issue, the protein content in poultry diets should be increased, despite the associated rise in feed costs and environmental concerns. Meeting the methionine requirements of traditionally reared poultry, especially fast-growing commercial lines, is quite difficult. Methionine plays a vital role in various life functions and cell growth as a methyl donor (Demirel and Şentürk Demirel, 2017). Compared to growth, broilers require higher amounts of methionine and choline to enhance their immune system, and there are synergistic relationships between these two nutrients (Swain and Johri, 1010).

Insufficient methionine intake leads to a reduction in the relative weight of *bursa of Fabricius* (immune system organ in poultry), lymphocyte counts, and the lymphocyte proliferation index (Wu et al., 2013). Diets deficient in methionine can lead to decreased feed intake, feed utilization rate, and productivity. Additionally, methionine deficiency can result in uniformity disorders, reduced breast meat yield, increased fattening and cannibalism, consequently leading to higher production costs (Corzo et al., 2006). Similar adverse effects were observed on live weight gain, feed intake, and feed conversion ratio in turkeys (Gonzalez-Esquerra et al., 2007).

Partridge et al. (1985) reported that absorption of amino acids varies depending on the source, with synthetic forms being more effective than plant-derived methionine (Silva et al., 2010). Dematte Filho et al. (2015) reported that the use of plant-extract-derived methionine resulted in lower live weight and live weight gain compared to synthetic methionine sources in male broilers. In a similar study, live weight gain and feed conversion ratio were found to be lower compared to those achieved with synthetic methionine sources in broilers. However, no significant differences were observed between the groups in terms of relative digestive and immune organ weights. Consequently, plant-derived methionine sources are insufficient for young, growing broilers (Yuan et al., 2012).

Daljeet et al. (2013) compared two plant-extract derived methionine sources (HM-6 and HM-7 - (M/s Indian Herbs Research and Supply Co. Ltd. Saharnpur, India) with synthetic DL-methionine in broilers, and as a result, higher live weight gain was achieved with the synthetic methionine source. Feed conversion and energy utilization were found to be slightly better. Carcass weight and meat characteristics were not affected by the methionine sources. Additionally, synthetic DL-methionine was 100% effective for growth, whereas HM-6 and HM-7 were 91.9% and 92.3% effective, respectively. Consequently, both plant-derived methionine sources were found to be insufficient for fast-growing birds.

Methiorep and Meth-o-Tasr® are two commercially produced plant-extract-derived methionine sources in India (Kalbande et al., 2009). *Allium sativum*, *A. Cepa*, *Phaseolus mungo*, and *Mucuna pruriens* are potential plant-extract-derived methionine sources for

Methiorep production. Methiorep at a dosage of 10 kg<sup>-1</sup> was found to be as effective as DL-methionine in broiler diets when the basal diet contains sufficient levels of methionine (Waskar et al., 2010). Supplementation of Methiorep and Meth-o-Tasr® to basal diets deficient in methionine content was found to be ineffective in both broiler and laying hens (Salome et al., 2010; Yuan et al., 2012; Igbasan et al., 2012; Igbasan and Olugosi, 2013).

Animal protein sources contain a maximum %2 methionine, whereas synthetic methionine contains %99. Therefore, it is challenging to obtain sufficient levels of methionine, especially in organic poultry production (Jakob, 2013). Synthetic methionine is permitted in conventional poultry feeds at a rate of 2270 g per ton, while in organic production permission, the limit is 908 g for hens and turkeys. For other poultry species, a limit of 1362 g is allowed (Fanatico, 2017). Replacing synthetic methionine with Methiorep® at substitution rates of 0%, 25%, 50%, 75%, and 100% did not affect the growth performance, blood profiles, or carcass parameters of broilers (Makinde et al., 2017).

Given the essential role of methionine in poultry nutrition and the growing restrictions on the use of synthetic methionine in organic production systems, there is an urgent need to explore sustainable and natural alternatives. Plant-derived methionine sources, such as Methiorep® and Meth-o-Tasr®, have shown promise; however, their effectiveness remains inconsistent, particularly in meeting the nutritional demands of fast-growing broilers. The aim of the study was to evaluate the effects of two proposed doses (1.0 and 1.25 kg<sup>-1</sup> of feed) of herbal extract methionine source, as a substitute for synthetically produced DL-methionine (1 kg<sup>-1</sup> of feed) on feeding performance, serum biochemistry, and relative organ weights of broilers.

## Materials and methods

### *Animal material*

In the study, a total of 312 day-old male Ross 308 broiler chicks were used as animal material. Following vaccination against Newcastle Disease Virus, the chicks were randomly allocated into three groups based on their live weight. The live weights between individuals were quite close, so, birds were weighed and randomly allocated to groups. Differences in initial live weights of chick groups were controlled and found to be statistically insignificant. The chicks were housed in electrically heated compartments, with floors coated in wood shavings, for a period of 42 days. The trial was conducted using a completely randomized design, consisting three experimental groups. During the six-week feeding period, the birds were individually weighed at weekly intervals. Feed and water were provided ad libitum throughout the experiment. Feed conversion ratio (FCR) values were calculated accordingly. The experimental area was illuminated using white fluorescent lamps, maintaining a 23-h light/1-h dark cycle. During the first week, the ambient temperature was maintained at 33°C, and in subsequent weeks, it was reduced by approximately 3°C per week until it stabilized at 22°C.

### *Feed material*

In this study, two experimental diets were formulated. The broiler starter (1-22 days) and the grower (22-42 days) were produced at a private feed mill. The diets were formulated in accordance with the NRC (1994) recommendations. DL-methionine, with a purity of 99.55%, was procured from a private supplier. Additionally, plant-extracted methionine (PEM), commercially known as Igumeth, was obtained from a private

company (Prodimix) based in Spain. The composition of diets is presented in *Table 1*. The chemical analysis of the feeds was conducted at the Feed and Animal Nutrition Laboratory of the Faculty of Agriculture, Dicle University.

### **Relative organ weights**

At the conclusion of the trial, 10 birds from each group were slaughtered. The carcass weight and the weights of several internal organs, including the liver, gizzard, spleen, intestine and abdominal fat, were measured. The relative organ weights were calculated using the following formula:

$$\text{Relative Organ Weight} = (\text{Organ Weight} / \text{Carcass Weight}) \times 100$$

**Table 1.** Nutritional composition of feeds and feedstuffs (%)

| <b>Feedstuffs</b>           | <b>Starter (1-22 days) %</b> | <b>Finisher (23-42 days) %</b> |
|-----------------------------|------------------------------|--------------------------------|
| Corn                        | 54.0                         | 56.6                           |
| Soybean meal (44% CP)       | 26.0                         | 19.0                           |
| Full-fat soybean meal       | 16.0                         | 17.0                           |
| Sunflower meal              | -                            | 4.0                            |
| DCP <sup>a</sup>            | 2.20                         | 1.60                           |
| Lime stone                  | 0.90                         | 1.0                            |
| NaCl                        | 0.30                         | 0.35                           |
| Vitamin premix <sup>b</sup> | 0.15                         | 0.10                           |
| Mineral premix <sup>c</sup> | 0.15                         | 0.15                           |
| L-lysine                    | 0.20                         | 0.10                           |
| DL-methionine               | 0.10                         | 0.10                           |
| <b>Chemical composition</b> |                              |                                |
| Crude protein               | 22.0                         | 20.0                           |
| ME (kcal/kg)                | 3010                         | 3260                           |
| Calcium                     | 1.0                          | 0.96                           |
| Available P                 | 0.49                         | 0.40                           |
| L-lysine                    | 1.36                         | 1.05                           |
| Methionine + cystine        | 0.80                         | 0.78                           |

<sup>a</sup>Content: 24% Ca, 17.5% P

<sup>b</sup>Provides per kg of diet: 8000 IU vitamin A; 1200 IU vitamin D3; 10 IU vitamin E; 2 mg vitamin K3; 2 mg vitamin B1, 5 mg vitamin B2; 0.2 mg pyridoxine; 0.03 mg vitamin B12; 10 mg vitamin B5; 50 mg niacin; 0.1 mg biotin; and 0.5 mg folic acid

<sup>c</sup>Provides per kg of diet: 80 mg Fe; 40 mg Zn; 60 mg Mn; 0.8 mg I; 8 mg Cu; 0.2 mg Se; and 0.4 mg Co

### **Bood serum analysis**

At the time of slaughter, blood samples were collected from ten birds per groups via brachial vein (3 mL). After centrifugation at 4000 rpm for 10 min, the serum was separated and transferred to Eppendorf tubes, then stored at -80°C for biochemical analyses. Biochemical analyses (glucose, uric acid, total protein, albumin, creatinine, triglycerides, cholesterol, phosphorus and calcium) were performed using an Architect

C16000 autoanalyzer (Abbott Laboratories, USA) at the Department of Biochemistry, Dicle University.

### **Statistical analysis**

The statistical evaluations were performed using the SPSS Statistics v. 16.0 Statistical Package (SPSS Inc., Chigaco, IL, USA). An analysis of variance (ANOVA) was conducted to evaluate mean differences among treatment groups. Tukey's multiple range test was used to distinguish the statistical difference among the mean value of each experimental group for each tested parameter.

### **Ethical approval**

This study was conducted with the approval of the Local Ethics Committee for Animal Experiments of Dicle University (DUHADEK: 2017/07).

### **Results and discussion**

In this study, the effects of synthetic methionine and two recommended doses of plant extract methionine (PEM) sources on feeding performance, relative organ weights, and serum biochemical parameters were evaluated. During the trial, no mortality was observed in any of the groups.

### **Live weight gain (LWG)**

All birds were weighed at the beginning of the trial, and subsequent weighings were conducted individually at weekly intervals. The weekly LWG values of the birds are presented in *Table 2*. No statistically significant differences were observed among the methionine groups for LWG at 14, 28, 35, and 42 days of age ( $P > 0.05$ ). However, the difference between the LWG values at the end of the first and third weeks of age was found to be significant ( $P < 0.05$ ).

**Table 2.** Effect of dietary methionine supplementation on weekly live weight gain in broilers (g/bird)

| Groups | Treatments           | Live weight gain (g/bird) |                    |                     |                    |                    |                    |
|--------|----------------------|---------------------------|--------------------|---------------------|--------------------|--------------------|--------------------|
|        |                      | 1 <sup>st</sup> wk        | 2 <sup>nd</sup> wk | 3 <sup>rd</sup> wk  | 4 <sup>th</sup> wk | 5 <sup>th</sup> wk | 6 <sup>th</sup> wk |
| 1      | DL-Meth. (1.0 g/kg)  | 99.8 <sup>a</sup>         | 253.2              | 516.5 <sup>b</sup>  | 980.1              | 1550.6             | 2142.0             |
| 2      | PEM (1.0 g/kg feed)  | 98.9 <sup>a</sup>         | 247.7              | 536.3 <sup>ab</sup> | 1000.4             | 1590.4             | 2161.0             |
| 3      | PEM (1.25 g/kg feed) | 94.1 <sup>b</sup>         | 251.9              | 558.2 <sup>a</sup>  | 1028.3             | 1618.5             | 2162.5             |
|        | SEM                  | 0.760                     | 2.90               | 6.29                | 9.06               | 13.02              | 14.60              |
|        | P                    | **                        | NS                 | *                   | NS                 | NS                 | NS                 |

<sup>a,b</sup>Within each column, means superscripted with the same letter are not significantly different ( $P > 0.05$ ). SEM: Standard error of the mean; NS: Non-significant, \* $P < 0.05$ , \*\* $P < 0.01$ . wk: week

Similar to our findings, Kalbande et al. (2009) reported that the dietary inclusion of Methiorep as a herbal methionine source (10 g/kg) was as effective as DL-methionine for growth performance in broilers. Dhumal et al. (2017) stated that supplemental methiorep levels did not affect LWG of the birds. Similarly, Ahmed and Abbas (2015) and Bhutyal et al. (2022) reported that plant-based methionine, used as a substitute for DL methionine,

did not negatively affect the fattening performance of broiler chickens. Makinde et al. (2017) also stated that Methiorep is as effective as synthetic methionine. Giannenas et al. (2022) stated that herbal extract methionine sources are effective in improving the performance of broiler chickens. However, our results do not align with the findings of Yuan et al. (2012), Daljeet et al. (2013) and, Dematte Filho et al. (2015), who reported that herbal methionine decreased live weight gain compared to synthetically produced DL-methionine. Itoe et al. (2010) highlighted that herbal methionine was inferior in LWG compared to birds fed on DL-methionine.

### **Feed intake (FI)**

Feed intake values were determined at weekly intervals. Weekly and cumulative FI values of the birds are presented in *Table 3*. No statistically significant differences were observed among the methionine groups for cumulative FI ( $P > 0.05$ ).

Similar to our findings, Igbasan et al. (2012) reported that dietary inclusion of herbal methionine source (Meth-o-Tas<sup>®</sup>) (0.5-1.0 g/kg) did not affect FI compared to DL-methionine in laying hens. Makinde et al. (2017) also stated that Methiorep is as effective as synthetic methionine for FI. On the contrary, Dhupal et al. (2017) reported that supplementary methiorep levels increased FI values.

**Table 3.** Effect of dietary methionine supplementation on weekly feed intake in broilers (g/bird)

| Groups | Treatments               | Feed intake (g/bird) |                     |                      |                    |                    |                    |
|--------|--------------------------|----------------------|---------------------|----------------------|--------------------|--------------------|--------------------|
|        |                          | 1 <sup>st</sup> wk   | 2 <sup>nd</sup> wk  | 3 <sup>rd</sup> wk   | 4 <sup>th</sup> wk | 5 <sup>th</sup> wk | 6 <sup>th</sup> wk |
| 1      | DL-methionine (1.0 g/kg) | 176                  | 635.2 <sup>b</sup>  | 1215.4 <sup>a</sup>  | 1960.2             | 2947.1             | 4014.7             |
| 2      | PEM (1.0 g/kg feed)      | 163.1 <sup>ab</sup>  | 591.7 <sup>ab</sup> | 1155.7 <sup>ab</sup> | 1909.9             | 2922.8             | 4010.2             |
| 3      | PEM (1.25 g/kg feed)     | 154.1 <sup>b</sup>   | 564.8 <sup>a</sup>  | 1119.4 <sup>b</sup>  | 1876.9             | 2894.5             | 4005.2             |
|        | SEM                      | 3.76                 | 9.22                | 13.30                | 17.09              | 20.79              | 23.80              |
|        | P                        | *                    | **                  | *                    | NS                 | NS                 | NS                 |

<sup>a,b</sup>Within each column, means superscripted with the same letter are not significantly different ( $P > 0.05$ ). SEM: Standard error of the mean. NS: Non-significant, \* $P < 0.05$ , \*\* $P < 0.01$ . wk: week

### **Feed conversion ratio (FCR)**

No statistically significant differences were observed among the methionine groups for FCR ( $P > 0.05$ ) (*Table 4*).

**Table 4.** Effect of dietary methionine supplementation on weekly feed conversion ratio in broilers

| Groups | Treatments               | Feed conversion ratio |                    |                    |                    |                    |                    |
|--------|--------------------------|-----------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
|        |                          | 1 <sup>st</sup> wk    | 2 <sup>nd</sup> wk | 3 <sup>rd</sup> wk | 4 <sup>th</sup> wk | 5 <sup>th</sup> wk | 6 <sup>th</sup> wk |
| 1      | DL-Methionine (1.0 g/kg) | 1.25                  | 1.45               | 1.67               | 1.85               | 2.00               | 2.11               |
| 2      | PEM (1.0 g/kg feed)      | 1.24                  | 1.44               | 1.66               | 1.84               | 1.99               | 2.09               |
| 3      | PEM (1.25 g/kg feed)     | 1.22                  | 1.42               | 1.64               | 1.82               | 1.97               | 2.08               |
|        | SEM                      | 0.009                 | 0.009              | 0.009              | 0.009              | 0.009              | 0.009              |
|        | P                        | NS                    | NS                 | NS                 | NS                 | NS                 | NS                 |

<sup>a,b</sup>Within each column, means superscripted with the same letter are not significantly different ( $P > 0.05$ ). SEM: Standard error of the mean. NS: Non-significant, \* $P < 0.05$ , \*\* $P < 0.01$ . wk: week

Our FCR results are consistent with the findings of Dhumal et al. (2017), while it is not align with the findings of Itoe et al. (2010) and Igbasan et al. (2012), who reported that herbal methionine caused poorer FCR. However, Chattopadhyay et al. (2006) stated that chickens fed 15 g/kg herbal methionine showed significantly better FCR compared to those fed 10 g/kg DL-methionine. Makinde et al. (2017) also reported that Methiorep is as effective as synthetic methionine for improving FCR.

### **Relative organ weights (ROW)**

Relative organ weights are presented in *Table 5*. No statistically significant differences were observed among the methionine groups for the relative weights of carcass, abdominal fat, and gizzard ( $P > 0.05$ ). However, significant differences were observed among the groups for relative weights of the liver, spleen and, intestine ( $P < 0.05$ ).

**Table 5.** Effect of dietary methionine supplementation on relative organ weights in broilers

| Groups | Treatment                | Carcass weight (g) | Relative organ weight (%) |               |                    |         |                    |
|--------|--------------------------|--------------------|---------------------------|---------------|--------------------|---------|--------------------|
|        |                          |                    | Liver                     | Abdominal fat | Spleen             | Gizzard | Intestine          |
| 1      | DL-Methionine (1.0 g/kg) | 1489.2             | 2.50 <sup>a</sup>         | 0.68          | 0.13 <sup>ab</sup> | 2.52    | 6.38 <sup>a</sup>  |
| 2      | PEM (1.0 g/kg)           | 1541.2             | 2.02 <sup>b</sup>         | 0.80          | 0.09 <sup>b</sup>  | 2.50    | 5.75 <sup>ab</sup> |
| 3      | PEM (1.25 g/kg)          | 1620.7             | 2.24 <sup>ab</sup>        | 0.86          | 0.14 <sup>a</sup>  | 2.62    | 5.56 <sup>b</sup>  |
|        | SEM                      | 37.766             | 0.079                     | 0.051         | 0.008              | 0.054   | 0.12               |
|        | P                        | NS.                | *                         | NS            | *                  | NS      | *                  |

<sup>a,b</sup>Within each column, means superscripted with the same letter are not significantly different ( $P > 0.05$ ). SEM: Standard error of the mean. NS: Non-significant, \* $P < 0.05$ , \*\* $P < 0.01$ .

The highest relative weights of the liver (2.50%), spleen (0.13%), and intestine (6.38%) were obtained from the DL-methionine group. The results obtained from our study are not similar to the results of Kanagaraju and Rathnapraba (2019), who reported that the relative weights of the gizzard, heart and liver were higher as a result of the use of herbal methionine. The results in the present study are in same line with findings of Helder and Roy (2007) they reported non-significant influence on abdominal fat pad. These results are in agreement with the findings of Yuan et al. (2012), who compared synthetic and herbal extract methionine and found that synthetic methionine is superior to herbal extract methionine. In addition, our results for liver weights are align with the findings of Igbasan et al. (2012), who reported herbal methionine increased liver weight but reduced abdominal fat. Makinde et al. (2017) also stated that Methiorep is as effective as synthetic methionine for ROW.

### **Serum biochemical parameters**

Serum glucose, total protein, creatinine, triglycerides, cholesterol, and calcium concentrations were not affected by methionine sources and doses ( $P > 0.05$ ) (*Table 6*).

Serum albumin and uric acid values in group I (DL-methionine) were significantly higher than in both of PEM groups ( $P < 0.05$ ). The highest values of uric acid and albumin were measured in group I (DL-methionine) at 3.30 mg/dL, and 0.57 g/dL, respectively. These results are consistent with the findings of Igbasan et al. (2012), who reported that lower plasma biochemical parameters were observed with herbal methionine sources.

However, our results do not align with the findings of Daljeet et al. (2013), who pointed out that herbal methionine increased liver lipid content. The highest phosphorus values were obtained from the higher dose PEM group as 6.55 mg/dL. On the other hand, blood phosphorus levels in the higher dose PEM group were significantly higher than those in the synthetic methionine group ( $P < 0.05$ ).

When the basal diet contains sufficient methionine, PEM sources are found to be more effective than synthetically produced methionine (Waskar et al. 2010). However, if the basal diet does not contain sufficient methionine, PEM sources will be inadequate (Salome et al. 2010; Yuan et al. 2012; Igbasan et al. 2012; Igbasan and Olugosi, 2013). Makinde et al. (2017) also stated that Methiorep did not affect the blood serum profiles of broilers.

**Table 6.** Effect of dietary methionine supplementation on serum biochemical parameters in broilers

| Parameters            | Treatments               |                     |                      | SEM   | P  |
|-----------------------|--------------------------|---------------------|----------------------|-------|----|
|                       | DL-Methionine (1.0 g/kg) | PEM (1.0 g/kg feed) | PEM (1.25 g/kg feed) |       |    |
| Glucose (mg/dL)       | 224.2                    | 205.4               | 207.6                | 4.88  | NS |
| Uric acid (mg/dL)     | 3.30 <sup>a</sup>        | 2.57 <sup>b</sup>   | 2.75 <sup>b</sup>    | 0.13  | *  |
| Tot. protein (g/dL)   | 3.10                     | 2.88                | 3.09                 | 0.12  | NS |
| Albumin (g/dL)        | 0.57 <sup>a</sup>        | 0.50 <sup>b</sup>   | 0.47 <sup>b</sup>    | 0.01  | ** |
| Creatinine (mg/dL)    | 0.28                     | 0.25                | 0.28                 | 0.001 | NS |
| Triglycerides (mg/dL) | 40.6                     | 35.9                | 37.9                 | 1.34  | NS |
| Cholesterol (mg/dL)   | 101.0                    | 106.7               | 110.4                | 4.08  | NS |
| P (mg/dL)             | 5.61 <sup>b</sup>        | 5.97 <sup>ab</sup>  | 6.55 <sup>a</sup>    | 0.17  | *  |
| Ca (mg/dL)            | 8.21                     | 7.90                | 8.55                 | 0.22  | NS |

<sup>a,b</sup>Within each column, means superscripted with the same letter are not significantly different ( $P > 0.05$ ). SEM: Standard error of the mean. NS: Non-significant, \* $P < 0.05$ , \*\* $P < 0.01$

## Conclusion

Compared to the synthetic methionine group, the proposed higher dose of PEM (1.25 g/kg) resulted in higher live weight gain values only during the first and third weeks of the trial. Feed intake values were statistically significant at 7, 14 and 21 days; however, no statistically significant differences were observed at 28, 35, and 42 days of trial. No significant differences were found among the methionine groups for feed conversion ratios. Statistically significant differences were observed in some relative organ weights (liver, spleen, intestine) expressed as a percentage of body weight, while no significant differences were found for abdominal fat, gizzard, and carcass weights among the groups. Serum glucose, total protein, creatinine, triglycerides, cholesterol, and calcium concentrations were not affected by treatments; however, albumin, uric acid, and phosphorus levels were significantly influenced.

The absence of significant differences in live weight gain and feed conversion ratios between the synthetic methionine group and the two proposed PEM doses (1.0 and 1.25 g/kg) suggests that PEM can effectively meet methionine requirements, particularly in organic poultry production systems where synthetic methionine use is restricted. Furthermore, these results indicate that plant extract methionine sources at these levels can be safely utilized without compromising the health and performance of broilers.

Future studies should focus on evaluating long-term effects and economic feasibility to further validate the potential of PEM as a sustainable alternative to synthetic methionine.

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