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# Methionine Supplementation Options in Poultry

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**Abstract:** Methionine is essential or limiting amino acid for poultry. An experiment was conducted to determine the comparative efficacy of DL-methionine and herbal methionine supplement on growth and performance of broiler chickens. Three hundred commercial broiler (VenCobb) chicks were procured and randomly divided into three groups (n = 100), one control ($T_0$) and two treatments ($T_1$ and $T_2$). All the groups were offered standard maize-soyabean meal based ration. $T_1$ was offered herbal sources of methionine (Methiorep®, supplied by Ayurvet Ltd., India) @1kg/tonne of feed and $T_2$ was administered DL- methionine@ 1 kg/tonne of feed from 0-42 days. A significant improvement in overall growth and performance was observed in birds supplemented with methionine. This study demonstrates that herbal methionine can replace DL-methionine very efficiently when used at the rate 1 g/kg diet of commercial broiler chicken.

**Key words:** DL-methionine, herbal methionine, growth, performance, fatty liver, hypocholesterolemic

## INTRODUCTION

Severe or chronic deficiencies of certain nutrients impair immune response and increases susceptibility to infectious diseases affecting growth and performance. Methionine is usually first limiting amino acid in maize and soyabean meal based diet. DL-methionine supplementation in growing chick is a common practice (Swick *et al*., 1990). Amino acids can exist as D- or L-isomers or mixture of two products. The D-isomers are biologically inactive while L-form is commonly occurring in most of the tissues. However, birds possess the ability to utilize both D and L-forms also called racemic mixture (Lesson and Summers, 2001). Methionine is commonly supplemented as dry DL-methionine (DL-Met; 99%pure) or as liquid DL-methionine hydroxy analog-free acid (MHA-FA, containing 88% of active substance). Methionine is essential for various vital functions in body such as: protein synthesis, regulation of cell division, methyl donor, reduces reactive oxygen species etc. An important aspect of protein and methionine inter-relationship is the ability of both to act as lipotropic agents. The production of lean carcasses has become especially important as producers are changing gradually from selling live birds to selling meat. Methionine may act as a lipotropic agent through its role as an amino acid in balancing protein or through its role as a methyl donor and involvement in choline, betaine, folic acid and vitamin B metabolism (Young *et al*., 1955). Synthetic methionine is metabolized into highly toxic compounds such as methylpropionate, thereby, adversely altering the performance of poultry birds (Bender, 1975). Synthetic methionine is listed among the prohibited synthetic substances and its usage has been questioned in organic farming practices (Anonymous, 1999). The aim of the present experimental study is to evaluate efficacy of herbal formulation Methiorep (supplied by M/S Ayurvet Limited, Baddi, .P., India) and synthetic methionine in improving overall growth, productivity and performance in poultry birds.

## MATERIALS AND METHODS

Three hundred day chicks of VenCobb broiler strain were purchased from *M/S Vaishnavi hatcheries Pvt. Ltd. (Maharashtra, India)*. Chicks were vaccinated via drinking water for Newcastle disease (ND) on 7$^{th}$ and 22$^{nd}$ day and at 14$^{th}$ day for infectious bursal disease. Day old chicks were randomly divided into three groups, one control ($T_0$) and two treatments ($T_1$ and $T_2$), with two replicates each on equal body weight basis, each replication was of 50 chicks. Chicks were offered maize soybean meal based diet (broiler starter and broiler finisher in mash form) as per standards of nutritional requirement recommended by NRC, 1994. Ingredients and chemical composition of basal diet are presented in Table 1. All the groups were subjected to similar managemental and nutritional regimens except the treatments given to them. Control group wasn't subjected to any treatment, $T_1$ was supplemented with herbal source of methionine@1kg/tonne of feed (*Methiorep, supplied by M/S Ayurvet Limited, Baddi, India)* and $T_2$ group was offered with synthetic DL-Methionine@1kg/tonne of feed, from 0-42 days.

The standard techniques of the proximate analysis were used to determine nutrient content of experimental diets (AOAC, 1995). Individual body weight and feed consumption of broilers from all pens were measured on day 0, 21 and 42 day of age. Mortality of each pen was recorded on a daily basis. Six birds were randomly selected from each replicate of each treatment group

Table 1a: Percent ingredient composition of starter and finisher ration

| Feed Ingredients | Starter | Finisher |
|---|---|---|
| Maize | 46.6 | 56.2 |
| Soybean meal | 39.0 | 32.2 |
| Deoiled rice bran | 11.5 | 8.7 |
| Vitamins | 0.2 | 0.2 |
| Mineral Mixture | 0.2 | 0.2 |
| Limestone | 1.0 | 1.0 |
| Dicalciumphosphate | 1.0 | 1.0 |
| Salt | 0.3 | 0.4 |
| Total | 100 | 100 |
| Crude protein (%)estimated | 23.35 | 20.76 |
| Metabolizable energy (kcal/kg) | 2801.2 | 2903.3 |
| Lysine % (calculated) | 1.222 | 1.046 |
| Methionine %(calculated) | 0.5 | 0.5 |
| E/P ratio | 119.65:1 | 139.5:1 |

Table 1b: Percent chemical composition of experimental broiler ration on dry matter basis

| Sr.No. | Nutrients | Percent in starter ration | Percent in finisher ration |
|---|---|---|---|
| 1 | Crude protein | 23.35 | 20.76 |
| 2 | Crude fibre | 3.15 | 3.35 |
| 3 | Ether extract | 4.48 | 5.01 |
| 4 | Total ash | 6.60 | 6.85 |
| 5 | Acid insoluble ash | 1.40 | 1.60 |
| 6 | Nitrogen free extract | 62.42 | 64.03 |
| 7 | Metabolizable energy calculated (Kcal/kg) | 2802.2 | 2903.3 |
| 8 | E/P ratio | 120:1 | 139.5:1 |

and control group for Blood samples collection for estimation of biochemical parameters at 3rd and 5th week of experimental study. Samples (approx. 2 ml/bird) were collected in heparinised vacutainer tube (Becon Dickinson India Pvt. Ltd., New Delhi, India) for biochemical study. Immediately after collection, tubes were placed in an ice bath and transported to the laboratory. Plasma was harvested subsequently by centrifuging the whole blood samples at 3000 rpm for 15 min in centrifuge machine. The heparinized plasma samples were stored at -20°C in Eppendorf tubes and analyzed subsequently. Plasma samples were analyzed for proteins (total proteins, albumin and globulin), enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST). Plasma total protein, albumin, ALT and AST were analyzed in the Automatic Blood Analyzer (*Microlab 200, E-Merck India Ltd., Mumbai*). While serum cholesterol and serum triglyceride were analyzed by CHOD and GPO method using clinical Autoanalyser.

## RESULTS AND DISCUSSION

Proximate analysis of ration offered to experiemental birds revealed that diet was containing 23.35% and 20.76% crude protein with a metabolizable energy content of ration 2802.2 and 2903.3 kcal/kg for starter and finisher, respectively (Table 1a and 1b). At the end of 6th week, average cumulative body weight (g) in $T_0$, $T_1$, $T_2$ were 1694.00, 1981.8 and 1643.8 g, respectively (Table 2). The broiler chicks in $T_1$ had significantly ($p<0.01$) higher final body weight as compared to those in $T_0$ and $T_2$ groups Tsiagbe *et al*., 1987 also reported improvements in body weight gain and food conversion with choline supplementation of a chick diet either alone or in combination with methionine. Increase in weight gain with increasing level of methionine in diet was also reported by (Meirelles *et al*., 2003 and Wang *et al*., 2004). Herbal methionine supplemented birds had significantly higher weekly body weight gain than untreated control and synthetic methionine administered group. *Constituent herbs of polyherbal methionine supplement formulation namely Cicer arientinum, Phaseolus mungo, Mucuna pruriens, Triticum sativum* and many more are scientifically well established for having highly bioavailable methionine and for potential role in promoting growth (Harms *et al*., 1961).

Table 2: Average weekly body weight gain (g) of birds at weekly intervals

| Age in weeks | $T_0$ | $T_1$ | $T_2$ |
|---|---|---|---|
| Initial weight | 46.89 | 46.86 | 46.75 |
| 1st | 63.54 | 94.69 | 67.25 |
| 2nd | 269.91 | 297.53 | 278.27 |
| 3rd | 486.10 | 580.71 | 502.22 |
| 4th | 921.94 | 863.07 | 857.60 |
| 5th | 1238.60 | 1400.8 | 1305.80 |
| 6th | 1694.00$^a$ | 1981.8$^b$ | 1743.80$^a$ |
| Mean gain at the end of 6th week | 282.33$^a$ | 330.01$^b$ | 273.96$^a$ |
| SE ± | 9.8578 | 12.073 | 19.86 |
| CD | 282.32 | | |

The feed consumption of experimental broiler chicks was recorded at weekly interval throughout the experimental period. Total average feed consumption per bird ranged from 3239.28-3513.70 g during experimental period of 6 weeks. Average feed consumption per bird per day (g) was 83.68, 80.87 and 81.23 g for $T_0$, $T_1$, $T_2$ respectively from 0-6 weeks of age, feed consumption in all the groups was non significantly different. The cumulative Feed Conversion Ratio (FCR) was significantly ($p<0.01$) better for $T_2$ (1.80) than $T_0$ (1.84) and $T_1$(1.91), respectively. Addition of methionine over and above the recommended requirement of broilers improves their performance in terms of body weight gain and food conversion efficiency (Ozturkan *et al*., 1993; Simone *et al*., 1995 and Ohta and Ishibashi, 1995). Supplementing herbal or synthetic sources of methionine improved feed to gain ratio in grower and total period of the experiment ($p<0.05$) which is in consist with other research (Simone *et al*., 1995 and Ohta and Ishibashi, 1995). Denner and Bessi (2003) also reported efficacy of liquid DL-methionine hydroxy analogue free acid (DL-MHA-FA) compared to DL methionine in improving growth and performance in laying hens. Similar observations were reported by many scientists in broiler and layers supplemented methionine alongwith maize-soyabean, maize-barley-soyabean, sorghum-wheat-soyabean meal based diet

Table 3: Biochemical parameters in control and treatment groups after 3rd and 5th week

| | Alkaline Phosphatase (ALP) ( IU/L) | | Aspartate amino transferase(AST) ( IU/L) | | Serum triglycerides mg/dL | | Serum Cholesterol mg/dL | |
|---|---|---|---|---|---|---|---|---|
| Groups | 3rd week | 5th week | 3rd week | 5th week | 3rd week | 5th week | 3rd week | 5th week |
| Control group | 258.09$^{a}$±15.93 | 183.87$^{a}$±11.78 | 32.38±3.31 | 13.94±9.78 | 199.59$^{a}$±15.77 | 181.85$^{a}$±14.73 | 156.78$^{a}$±11.09 | 141.14$^{a}$±7.73 |
| Group $T_1$ | 184.81$^{b}$±12.59 | 197.78$^{ab}$± 9.22 | 21.82±3.11 | 17.43±4.84 | 114.85$^{b}$±11.13 | 145.52$^{b}$±10.67 | 101.54$^{b}$±4.65 | 106.95$^{a}$±6.73 |
| Group $T_2$ | 189.60$^{b}$±6.62 | 255.31$^{c}$±10.69 | 17.29±2.57 | 16.59±4.42 | 139.06$^{b}$±9.96 | 182.95$^{b}$±30.89 | 125.88$^{b}$±4.59 | 113.29$^{a}$±5.46 |

*Means bearing different superscript in the row differ significantly (p<0.05)

(Bertram *et al*., 1991; Brennan, 1998; Lemme *et al*., 2002).

Previous works revealed that supplementation of methionine in diet improved performance in terms of FCR but feed intake was similar between methionine supplemented and unsupplemented groups. Similarly in the present investigation, supplementation of DL methionine improved FCR compared to control whereas supplementation of herbal methionine@1kg/tonne of feed showed better FCR than DL methionine supplemented birds. The survivability of birds during the experiemental period did not differ significantly among treatments. It indicates that dietary supplements have no detrimental effect on survivability. (Rajurker *et al*., 2009), also reported herbal methionine supplement (Methiorep supplied by Ayurvet Ltd Baddi, India) to be totally safe and having no adverse effect at the highest limit dose of 5 g/kg body weight in male Wistar rats, when tested for acute toxicity by OECD 423 guidelines.

Effect of supplementing DL methionine and herbal methionine on serum biochemicals is depicted in Table 3. Liver marker enzymes serum Alkaline Phosphatase (ALP) and Aspartate Amino Transferase (AST) were observed to be significantly higher in control ($T_0$) than treatment groups ($T_1$ and $T_2$).A moderate increase in AST and ALP activity is seen in soft injury, slight elevations in liver marker enzymes may be associated with glucocorticoid excess, due to stress or fatty liver syndrome which increases the enzyme activity. Values of enzymes recorded after 3rd and 5th week in DL-methionine supplemented and herbal methionine treated group were significantly lower than untreated control but in normal range. Deficiency of methionine in diet here was no increase in liver enzymes in treated groups during complete experiemental period, it can be inferred that supplementation of herbal or DL methionine did not cause any liver dystrophy or other vital organ abnormalities where from these enzymes are secreted, rather exhibited hepatoprotective action. Normalization of values of liver marker enzymes by herbal methionine supplementation in group $T_1$ is attributed to the activity of constituent herbs of Methiorep namely *Azadirachta indica, Boerhaavia diffusa, Trigonella foenumgraecum* and many other (Chattopadhyay, 2003; Rawat *et al*., 1997; Kaviarasan and Anuradha, 2007).

Table 3 dipicts significantly higher values Serum cholesterol and serum triglycerides. Serum triglyceride value as recorded after 3rd and 5th week in Methiorep supplemented group ($T_1$) (114.85 ±11.13 and 145.52 ±10.67mg/dl) was significantly lower than T2 (139.06 ±9.96 and 182.95±30.89mg/dl) followed by Control group (156.78±11.09 and 141.14±7.73 mg/dl). The findings of current experiment can be well correlated to those reported by (Roth and Milstein, 1957) that dietary methionine insufficiency may induce fatty liver thereby increasing total serum cholesterol and triglycerides. Fowler, 1996 reported about important role of methionine in lipid metabolism. Methionine deficiency in ration may lead to fatty liver syndrome in poultry as studied by many authors (Coles, 1986; Riddell, 1997). Since Its, non-polar sulphur containing amino acid, precursor of lecithin and potent lipotropic and chelating agent and diet inadequate in this essential amino acid may lead to fatty liver and other metabolic disturbances (Keshavarz, 2003). Methionine plays an important role in cysteine, carnitine and taurine synthesis by trans-sulfuration pathway. However supplementation of methionine has been observed to normalize the values of serum biochemicals. Among the two supplements of methionine, herbal formulation proved better than DL methionine in lowering total serum cholesterol and this may be attributed to the hypocholesterolemic activity of certain constituent herbs of Methiorep namely, *Allium Sativum, Allium cepa, Phaseolus mungo Mucuna pruriens and* many more (Augusti, 1974; Iauk *et al*., 1989 and Thomas *et al*., 1983).

The data from the feeding trial indicate that herbal methionine supplement (Methiorep) can replace DL-methionine very effectively in the diet of commercial broiler birds when used at the rate of 10 g/kg diet as the performance of broiler birds supplemented with DL-methionine can be well compared with birds supplemented with herbal methionine. However, further trials are warranted to consolidate the present findings and cost effectiveness of using herbal methionine.

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