



ISSN (E): 2277- 7695

ISSN (P): 2349-8242

NAAS Rating: 5.03

TPI 2018; 7(10): 13-17

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www.thepharmajournal.com

Received: 25-08-2018

Accepted: 26-09-2018

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## Substitution of herbal methionine with dl-methionine in poultry rations enhances the growth, performance and contributes towards the antioxidant defence potential in broilers

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### Abstract

To determine the differential influence of supplementing the broiler diet with two different sources of methionine namely, the herbal methionine (HM) and DL-methionine (DLM) and examine the influence on growth, performance and the effect on the antioxidant effects in 120 days-old broiler chicks were purchased and randomly assigned into 3 treatment groups (G1, G2 & G3) with 40 chicks in each group, having 4 replicates of 10 birds in each replicate. The diets included Corn soybean meal-based, control diet supplemented with DLM or HM. G1 as a control supplemented with DLM (3.4 and 2.3 kg/ton, respectively in starter and finisher ration) feed only, G2 was given feed supplemented with HM @ 6.5 and 5.2 kg/ton of feed, respectively in starter and finisher ration and G3 was provided commercial feed supplemented with combination of DLM and HM @ 1.7 + 3.2 and 1.15 + 2.60 kg/ton each, respectively in starter and finisher ration. The DLM-Methionine fed group showed significantly increased growth performance in live body weight and weekly body weight gain, feed consumption and feed conversion ratio as well as antioxidant parameters like TABARS, SOD, CAT and GPx significantly higher in group 1 as compared to group 2 and 3. Therefore, based on these findings, it can be suggested that herbal methionine can be effectively substituted for synthetic methionine in the poultry broiler diets up to the extent of 50%.

**Keywords:** Herbal Methionine, DLM-Methionine, Growth performance, Antioxidant parameters.

### 1. Introduction

Poultry is one of the fastest growing segment among the component of veterinary and agricultural sector in India. Today india occupied 3<sup>rd</sup> position in egg production (which was 5<sup>th</sup> in 2000) and 5<sup>th</sup> position in poultry meat production (which was in 13<sup>th</sup> in 2000) in world. The average growth rate of poultry industry in terms of broiler sector is 10% over last decade. the contribution of poultry sector to the country GDP is 411.5 billion during 2010-2011. therefore about 66.7 % of the total output only from poultry meat sector. the poultry meat production has increased from 0.069 million tone in 1961 to 2.337 million tone in 2010-2011 (Prabhakaran, 2012) [18]. Poultry farming in terms of broiler production is the best way of supplying good quality animal protein for human consumption. Broiler are the quickest and most economic and most efficient converter of plant material into food of high biological value (Bootwalla, 2005) [5]. Poultry meat contribute around 20% of total meat production in country. The per capita availability of poultry meat is 2.15 kg/annum which is very less as against 11 kg meat/annum recommendation by national institute of nutrition prabhakaran, 2012) [18]. Hence there is tremendous scope for further development in poultry meat sector. The economics of production is very important criteria for broiler (poultry) farming. Feed is the major constituent in total cost of production in broiler farming. Feed constituents near about 60-70% total cost of broiler production. Hence to increase the profit margin, one has to reduce feed cost. Using feed additives mainly methionine in broiler feed is one of the way to reduce feed cost in poultry production. Methionine is first limiting essential amino acid which is not synthesized by bird so has to be supplemented from outside sources like feed. The methionine holds a number of vital functions like protein synthesis, glutathione precursor formation, regulation of cell division as well as most importantly it is a methyl donor. Conventional poultry diets are typically corn and soybean meal based containing low in lysine (grain) and methionine (soybeans).

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The requirement of the methionine in feed as excessible protein which result in excretion of excess nitrogen which is not environmentally friendly. Methionine is one of the few amino acids that contain sulfur, and sulfur is a major constituent of feathers. If bird diets are deficient in any single amino acid, it will most likely be methionine. An adequate level of methionine is required in the diet and a deficiency results in reduced growth and feather development. A methionine deficient bird will tend to eat feathers in an attempt to satisfy a craving for this amino acid. A bird may even pull them from its own body. The use of herbal and synthetic methionine in poultry diets makes it possible to feed lower levels of dietary protein that still meet the daily methionine requirement.

Methiorep is a scientifically developed combination of herbs that contains herbal ingredients rich in methionine in free form and as conjugated form. Methiorep also contains SAME and phosphatidyl choline required for conversion of homocysteine to methionine. This cycle ensures that more methionine is available for protein accretion and other functions of birds, which in turn results in maximum performance. Methiorep contain various herbs including *Allium cepa*, *Allium sativum*, *Cicer arietinum*, *Glycine max* (full fat soya), *Mucuna pruriens*, *Phaseolus mungo*, *Triticum sativum* and *Trigonella foenum-graecum*. The DL-methionine supplementation in growing chicken feeds is a common practice especially in cereal and vegetable protein based ration. Amino acids can be exist as D or L isomers is commonly occurring in most of the tissues however birds has ability to utilizes both D and L-forms which is called as racemic mixture. Al though the D-form is not biologically active, poultry have the ability to utilize both D- and L-forms. Methionine supplementation is typically in the form of dry DL-methionine which is 99% pure, or as liquid D, L-methionine hydroxy analog-free acid which is the equivalent of 88% methionine after the conversion of the analog to the biologically active form. (Srijit Tripathi 2016) [20]. Balanced nutrition plays crucial role in the health and productivity of commercial poultry. Several novel approaches are being incorporated into the poultry diet on continuous basis to better the nutritional quality to accomplish enhanced production and profitability. However, In spite of advances made on the nutritional aspects, a lot of several aspects of the poultry nutrition still remain unsolved and continue to challenge to researchers. Substituting herbal methionine in poultry rations with DL-methinine is one such effort to reduce the costs along with enhancement of nutritional quality of poultry diet (Kanduri A.B *et al.*, 2014) [12].

## 2. Materials and Methods

Methiorep® is a polyherbal combination containing herbs like *Allium cepa*, *Allium sativum*, *Cicer arietinum*, *Glycine max* (full fat soya), *Mucuna pruriens*, *Phaseolus mungo*, *Triticum sativum* and *Trigonella foenum-graecum*. And pure form of synthetic methionine were obtained from Ayurved Limited, Solan, and Himachal Pradesh. Broiler chicks (Vencobb) of 120 day old were procured from local hatchery in Bidar. The Chicks were acclimatized for one week and then randomly divided into 3 groups consisting of 40 chicks in each group, having 4 replicates of 10 birds in each group. The trial was conducted for a period of 42 days. Vaccination was done as per routine farm practice. The diets included Corn soybean meal-based, control diet supplemented with DLM or HM. G1 as a control supplemented with DLM (3.4 and 2.3 kg/ton,

respectively in starter and finisher ration) feed only, G2 was given feed supplemented with HM @ 6.5 and 5.2 kg/ton of feed, respectively in starter and finisher ration and G3 was provided commercial feed supplemented with combination of DLM and HM @ 1.7 + 3.2 and 1.15 + 2.60 kg/ton each, respectively in starter and finisher ration. Performance parameters like average body weight, weekly body weight gain, feed consumption and feed conversion ratio (FCR) were recorded at weekly intervals in all groups. TBARS, A oxidative stress marker and Antioxidant enzymes viz., Superoxide dismutase, Catalase and glutathione peroxidase were evaluated in liver tissue collected at the end of the experiment.

### 2.1 Anti-oxidant defense profile in Liver

The birds were sacrificed at the end of week six and liver tissues were rapidly excised into ice cold normal saline and then blotted dry and stored at -20 °C for further analysis. Liver tissues were homogenized with ice cold 0.1 mol/L Tris HCl buffer of pH 7.4 to make 10% homogenate w/v (1 g of liver crushed in 10 ml of ice cold 0.1 mol/l Tris HCl buffer of pH 7.4). This homogenate was centrifuged at 3000 rpm for 10 min, the supernatant was collected and used for assay of super oxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and thiobarbituric acid reacting substances (TBARS) in the liver tissue homogenate.

#### 2.1.1 Superoxide dismutase (SOD) estimation

8 to 0.5 ml of tissue homogenate, 0.25 ml of ethanol and 0.15 ml of chloroform was added and mechanically shaken for 15 minutes and centrifuged at 13,000 rpm for 15 minutes at 4 °C. The supernatant was separated and used for the test. Assay mixture consists of 2 ml of 0.1 M Tris-HCl, 0.5 ml of homogenate, 1.5 ml of distilled water and 0.5 ml of pyrogallol. SOD value was taken for 3 min at 420 nm wave length. The enzyme activity was expressed in terms of unit per minute per g of protein. One unit of enzyme corresponds to the amount of enzyme that inhibits pyrogallol auto-oxidation reaction by 50 percent (Markuland S and Markuland G, 1974).

#### 2.1.2 Thiobarbituric acid reacting substances (TBARS)

1 gm of tissue sample with 10 ml of 0.2 M Tris HCl buffer (pH 7.2) was taken in a tissue homogenizer to get a 10% homogenate. 500 µl of supernatant from the homogenate, 1 ml of 10% trichloroacetic acid and 1 ml of 0.67% thiobarbituric acid were taken in a tightly stoppered tube. The tube was heated to boiling temperature for 45 min. After cooling the tube, the contents were centrifuged. The supernatant was read at 532 nm against blank. The concentration of test samples was obtained using molar extinction coefficient of MDA and enzyme activity was expressed in terms of n moles of MDA/mg of tissue. (Balasubramanian *et al.*, 1988) [3].

#### 2.1.3 Glutathione peroxidase (GPx)

2 ml of 0.1 M PBS was added to 100 µl of 1:20 diluted RBC lysate. To this solution, 100 µl each of reduced glutathione and H<sub>2</sub>O<sub>2</sub> buffer was added. The reaction mixture was incubated for 5 min at room temperature and then 100µl NADPH was added. The absorbance was measured in a spectrophotometer at 320 nm for 5 min at every 60 sec interval. The enzyme activity was calculated using extinction coefficient of 3,781 and expressed as U/ml. (Moron *et al.*, 1979) [16].

### 2.1.4 Catalase

To assay mixture containing 0.4 ml of 0.2 M H<sub>2</sub>O<sub>2</sub> and 0.5 ml of 0.01 M phosphate buffer (pH 7), 0.1 ml of homogenate was added and mixed well. Into this, 2 ml of dichromate acetic acid solution was blown exactly after 60 sec. Then kept in boiling water bath for 10 min. The absorbance was read at 570 nm against reagent blank containing 0.4 ml of 0.2 M H<sub>2</sub>O<sub>2</sub> and 0.5 ml of 0.01 M phosphate buffer (pH 7). The enzyme activity was expressed as µg of H<sub>2</sub>O<sub>2</sub> decomposed /mg protein/min (Asru, 1972).

### 3. Statistical Analysis

The data was subjected to statistical analysis by applying one way ANOVA using statistical package for social sciences (SPSS) version 15. Differences between means were tested using Duncan's multiple comparison test and significance was set at P<0.05.

### 4. Results

#### 4.1 Growth Parameters

The result of effect of herbal methionine and DL-methionine supplementation shown in fig 1,2,3,4.

**Table 1:** Live body weight (g) of different groups of broiler chicks

Groups	0Wk	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
G1	39.70±1.27	171.40± 4.66	362.00±5.00	63.50±5.94	1255.50±12.67	1712.50±17.86	2353±11.56
G2	38.70±2.03	138.40 ±5.18	268.40±2.82	537.20±11.56	1060.06±10.26	1350.70±12.59	1916.10±24.35
G3	38.50±0.98	155.10 3.51	312.00±11.08	594.70±8.83	1173.70±23.98	1542.70±18.05	2136.80±18.21

**Table 2:** Weekly body weight gain (g) per bird of broiler chicks

Groups	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
G1	131.70±4.45 <sup>A</sup>	190.60±6.06 <sup>A</sup>	301.50±7.14 <sup>A</sup>	592.00±11.14 <sup>A</sup>	457.00±22.76 <sup>A</sup>	640.50 ±17.74 <sup>A</sup>
G2	99.70±6.34 <sup>C</sup>	130.00±6.06 <sup>C</sup>	268.80±12.11 <sup>C</sup>	522.86±16.50 <sup>B</sup>	290.64±11.54 <sup>C</sup>	565.40±31.04 <sup>B</sup>
G3	116.60±4.16 <sup>B</sup>	156.90±12.58 <sup>B</sup>	282.70±9.60 <sup>AB</sup>	579.00±22.29 <sup>A</sup>	369.00±31.44 <sup>B</sup>	594.10±21.24 <sup>AB</sup>

**Table 3:** Weekly feed consumption (g) per bird in different groups

Groups	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
G1	147.70 <sup>B</sup>	281.65 <sup>C</sup>	430.61 <sup>C</sup>	729.95 <sup>C</sup>	1062.31 <sup>B</sup>	1394.30 <sup>C</sup>
G2	172.9 <sup>A</sup>	336.99 <sup>A</sup>	476.51 <sup>A</sup>	835.86 <sup>A</sup>	1116.35 <sup>A</sup>	1566.30 <sup>A</sup>
G3	170.29 <sup>A</sup>	310.73 <sup>B</sup>	454.41 <sup>B</sup>	47.26 <sup>B</sup>	1102.24 <sup>A</sup>	1472.80 <sup>B</sup>

**Table 4:** Feed conversion ratio (FCR) per bird of broiler chicks.

Groups	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
G1	1.13 <sup>C</sup>	1.49 <sup>B</sup>	1.43 <sup>C</sup>	1.24 <sup>B</sup>	2.39 <sup>B</sup>	2.19 <sup>B</sup>
G2	1.79 <sup>A</sup>	2.65 <sup>A</sup>	1.80 <sup>A</sup>	1.62 <sup>A</sup>	3.91 <sup>A</sup>	2.85 <sup>A</sup>
G3	1.48 <sup>B</sup>	2.19 <sup>A</sup>	1.63 <sup>B</sup>	1.31 <sup>B</sup>	3.29 <sup>A</sup>	2.51 <sup>AB</sup>

**Fig.5.** Markers of Oxidative stress and antioxidant defenses in liver of different groups of broilers.

Groups	TBARS (n moles of MDA released/mg protein)	Superoxide dismutase (U/mg protein)	Catalase (U/mg protein)	Glutathione peroxidase (U/mg protein)
G1	2.85 ± 0.07 <sup>C</sup>	1.09 ± 0.00 <sup>A</sup>	0.16 ± 0.00 <sup>A</sup>	4.51 ± 0.13 <sup>A</sup>
G2	3.67 ± 0.03 <sup>A</sup>	0.85 ± 0.01 <sup>C</sup>	0.06 ± 0.00 <sup>B</sup>	3.40 ± 0.21 <sup>B</sup>
G3	3.16 ± 0.06 <sup>B</sup>	0.90 ± 0.01 <sup>B</sup>	0.15 ± 0.01 <sup>A</sup>	4.35 ± 0.25 <sup>A</sup>

The growth performance of broiler bird analyzed in term of live body weight and weight gain significantly ( $p<0.05$ ) higher in group 1 followed by group 3 than group 2 last day of experiment observed (Fig.1 and 2 respectively). Feed consumption and FCR were significantly lower in group 1 and 3 as compared to group 2 at last day of experiment (Fig.3 and 4 respectively).

#### 4.2 Antioxidant

The antioxidant parameter measured at the 6<sup>th</sup> week of experiment showed TBARS concentration in DL-Met group 1 was significantly ( $p<0.05$ ) lower as compared to groups 2 and 3. The activity of SOD (U/mg protein) in DL-Met group 1 was significantly ( $p<0.05$ ) higher as compared to groups 2 and 3. The activity of catalase (U/mg of protein) and glutathione peroxidase (U/mg of protein) in DL-Met group 1 was significantly ( $p<0.05$ ) higher as compared to H-Met group 2. There was no significant difference in these values when group 3 was compared to group 1 (Fig.5).

### 5. Discussion

Oxidative stress marker enzymes like Thiobarbituric acid reactive substances (TBARS) are less and antioxidant enzyme like Superoxide dismutase, catalase and glutathione showed significantly increased level in both liver tissues of synthetic methionine treated group 1 compared to group 3 followed group 2. This can be attributed to the fact that, in addition to acting as an essential nutrient, herbal methionine can combat the free radical-induced damage when used in combination with DL-Met than using it alone. Methionine induces reduction of lipid peroxidation, offers protection against membrane damage and restores changes in the glutathione system (Selvam and Ravichandran, 1991) [19]. Methionine residues can act as powerful endogenous antioxidants in proteins and the free radical scavenging activities of methionine can only partially be explained by the chelating function of its sulfur moiety. The growth parameters showed significantly increased in synthetic methionine (DLM) treated group 1 followed by (DLM+HM)

Group 3 and then HM group 2. The growth performance of broiler bird in term of live body weight and weight gain significantly ( $p < 0.05$ ) higher in group 1 followed by group 3 than group 2 in last day of experiment observed (Fig.1 and 2 respectively). Feed consumption and FCR were significantly lower in group 1 and 3 as compared to group 2 at last day of experiment (Fig.3 and 4 respectively). Bhagora *et al.*, (2013)<sup>[4]</sup>. Demonstrated that significant improvement in overall growth and performance was observed in birds supplemented with 50% herbal methionine and 50% DL-methionine which is more economical as compared to 100% DL-methionine, 100% herbal methionine and 125% herbal methionine in commercial broilers. The result of present study corroborate with other studies those reported by Ibrahim and Hamid I, 2014 where in 100% DLM group and 100% HM fed group gave similar performance as well as the broiler group fed with 50% DLM and 50% HM gave best performance. The results in the present study are also in corroboration with those reported by Halder and Roy (2007)<sup>[8]</sup>, Schutte and Pack (1995)<sup>[21]</sup> that addition of herbal source of methionine with feed improved performance in terms of body weight gain and feed efficiency in broilers. Herbs namely *Cicer arietinum*, *Phaseolus mungo*, *Mucuna pruriens* are rich source of proteins and essential amino acid. The supplementation of herbal methionine as well as synthetic methionine could influence the growth, performance and antioxidant profiles and has good impact on the survivability of the birds. Waskar *et al.*, (2009)<sup>[23]</sup>, Chattopadhyay *et al.*, (2006)<sup>[6]</sup>. And Halder and Roy (2007)<sup>[8]</sup>. Were reported a 1:1 replacement for synthetic methionine with herbal methionine in broiler rations.

## 6. Conclusions

The present study, concluded that supplementation of herbal and synthetic methionine (50% + 50%) in group 3 resulted in significant improvement on growth performance and antioxidant as compared to herbal methionine (H-Met) alone in group 2. The results of group 3 were comparable to synthetic methionine alone used group 1. The increase in demand for poultry meat has given rise to the use of synthetic compounds in feed and the high cost of such compounds like synthetic methionine increases the cost of finished feeds (Anonymous, 1999)<sup>[1]</sup>. Therefore, instead of using 100% H-methionine as a replacement to DL-Methionine in the poultry broiler diets, a combination of 50% synthetic methionine + 50% H-Met could be a better alternative for 100% DL-met. However, more number of trials are warranted to consolidate the present findings and cost effectiveness of using H-Met and to arrive at the ideal ratio of incorporation of herbal methionine in the poultry diets for improving the farmer economy as well as enhancing the growth performance.

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