ISSN 1682-8356
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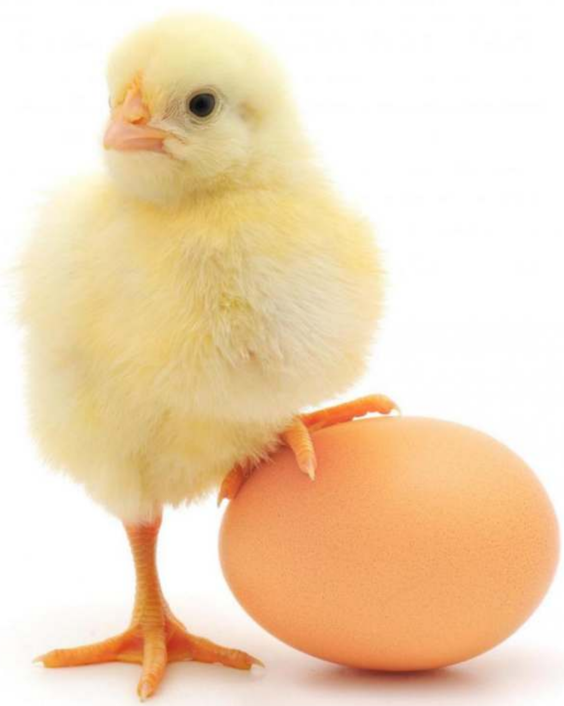

INTERNATIONAL JOURNAL OF POULTRY SCIENCE

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International Journal of Poultry Science 5 (11): 1034-1039, 2006
ISSN 1682-8356

Comparative Efficacy of DL-Methionine and Herbal Methionine on Performance of Broiler Chicken

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Abstract: An experiment was conducted to determine the comparative efficacy of DL-methionine and herbal methionine on performance and carcass characteristics of broiler chickens. Two thousand and four hundred d-old commercial broiler (VenCobb) chicks were purchased and randomly divided into four dietary treatment groups of 600 birds each. Each treatment group was further subdivided into three replicates of 200 broilers per replicate. The treatments groups were control; control plus 10g DL-methionine/kg diet; control plus 10g herbal methionine (Herbomethion®, supplied by Indian Research and Supply Co. Ltd.) /kg diet and control plus 15g herbal methionine/kg diet. There were significant effects of dietary treatments on body weight, body weight gain, feed intake and feed conversion ratio at 0 to 41 day. The body weight and body weight gain of the broilers fed the 15g herbal methionine/kg diet were heavier than other treatments. Feed conversion ratio of broiler fed 15g herbal methionine/kg diet was significantly better than that of broilers fed on 10g herbal methionine or DL-methionine/kg diet. Neither DL-methionine nor herbal methionine supplementation had a significant effect on broiler mortality. Plasma protein and enzyme concentration was unaffected by the dietary treatments. Abdominal fat (%) and liver lipid (g/kg) was significantly decreased by the addition of 15g herbal methionine/kg diet. This study demonstrates that herbal methionine can replace DL-methionine very effectively when used at the rate 15g/kg diet of commercial broiler chicken.

Key words: DL-methionine, herbal methionine, performance, Carcass characteristics, Broiler chicken

Introduction

Amino acids can exist as D- or L-isomers or mixture of two products. All amino acids occurring in animal tissues however are L-isomers, since D-isomers have no biological function. The exception to this is methionine, where the bird is able to use D- or L-forms or as occurs most frequently, a DL- 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). An important aspect of protein and methionine interrelationship 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_{12} metabolism (Young *et al.*, 1955; March and Biely, 1956; Chen *et al.*, 1993). Methionine serves as an integral portion of body protein, is a precursor for cystine and an important source of dietary sulfur. Sulfur-adenosyl methionine is a potent donor of methyl groups, which contributes to the synthesis of many important substances including epinephrine, choline and creatine (Bender, 1975). Fancher and Jensen (1989) set methionine in the first position among amino acids limiting chicken growth. The increase in demand for cheap meat has given rise to the use of synthetic compounds in feed. Recently the safety of such practices has been questioned and their use is becoming restricted in many regions of the world. Therefore, there is great renewed interest in developing natural alternative supplements to maintain animal performance and well being. Methionine is required at higher level when bird is predisposed to fast growth along with high production performance. Herbal methionine as a source of ‘active methionine’ is claimed to be effective in its optimum activity for proper protein accretion and other functions in poultry birds so that they can reach better growth and performance potential. Under these circumstances, this study was designed to determine the comparative efficacy of DL-methionine and herbal methionine on performance of broiler chicken.

Materials and Methods

Birds and experimental design: A farm trial on DL-methionine and herbal methionine supplementation in commercial broiler chicken was conducted in an organized farm near university. Two thousand four hundred d-old commercial broilers (VenCobb) were purchased from nearby hatcheries and were randomly divided into four dietary treatment groups of 600 broilers each. Each treatment group was further subdivided into

Table 1: Ingredients and chemical composition of starter and finisher diet

Name	Starter	Finisher
Ingredients (as air dried basis), kg/1000 kg		
Maize	554.6	615.0
Rice polish	40.0	50.5
Soybean	335.0	250.4
Fish meal	50.0	50.0
Calcite	4.2	5.5
Di-Calcium Phosphate	11.0	11.0
Soybean oil	-	12.0
Salt	0.2	0.95
Trace mineral mixture†	1.0	1.0
Vitamin premix‡	0.3	0.45
Choline chloride	0.8	0.8
Maduramycin	0.5	0.5
Sodium bicarbonate	1.0	1.0
Zinc oxide	0.2	0.2
Manganese sulfate	0.2	0.2
Natuzyme¶	0.5	0.5
Lincomycin (1%)	0.5	0.4
Chemical composition		
Dry matter, %[a]	89.23	89.76
Crude Protein, %[a]	22.87	20.00
Metabolizable Energy, Kcal/kg[c]	2850	2960
Calcium, %[a]	0.92	0.90
Available Phosphorous, %[c]	0.46	0.45
Methionine, %[c]	0.42	0.29
Lysine, %[c]	1.20	1.06

† Composition of each Kg trace mineral mixture : Cu, 15g.; Co, 02g.; Fe, 60g.; Zn, 80g.; Mn, 80g.; I, 02g.; Se, 0.3g.; Mo, 0.1g. ‡ Each Kg contains : Vitamin A- 80 MIU; Vitamin D_3- 12 MIU; Vitamin E- 70g; Vitamin K_3 -8g; Vitamin B_1- 6.4g; Vitamin B_2- 40g; Vitamin B_6-12.8g; Vitamin B_{12}-160mg; Nicotinic acid- 80g; Vitamin B_5- 115g; Folic acid-4g; Biotin-24mg. ¶ Each Kg contains: Cellulase-6,000,000 U; Xylanase-10,000,000 U; α glucanase-700,000 U; α amylase-700,000 U; Pectinase-70,000 U; Protease-3,000,000 U; Phytase-400,000 U. [a]Assayed value [c]Calculated on the basis of standard values applicable under Indian condition (Singh and Panda, 1996).

three replicates of 200 birds. Commercial brooding and management procedures were followed, and all broilers were fed a typical commercial broiler starter diet for the first 3 weeks of the experiment followed by finisher diet up to 6 weeks. The treatment groups were control diet without methionine; control plus 10g DL-methionine/kg diet, control plus 10g herbal methionine (Herbomethion®, supplied by Indian Research and Supply Co. Ltd.) /kg diet and control plus 15g herbal methionine/kg diet.

Housing and management: The broilers were housed in floor pens and rice husk served as litter material. The house was cleaned thoroughly with formaldehyde and potassium permanganate solution three days prior to arrival of birds. The d old chicks were offered electrolyte solution upon arrival. Birds were maintained on a 24 hours constant light schedule. The brooding temperature was maintained close to their requirement, first by heating device for 3 days following arrival of chicks. Then no additional heating was required as the summer room temperature was found appropriate up to 3 weeks and finally by turning cooler fan during day time for the last 3 weeks of rearing period. The birds were vaccinated against Ranikhet disease and Infectious Bursal Disease on d 7, 14 and 21 and provided antibiotic for the first 5 days as per recommendation.

Details of the feeding regimens: The chicks were offered maize soybean meal based diet (broiler starter and broiler finisher in mash form). These diets were formulated to meet or exceed the BIS (1992) nutritional requirement of broiler chicken. The diets were fortified with mineral and vitamin premix as per the standard stipulated by the Bureau of Indian Standard for broiler chickens (1992). Ingredients and chemical composition of basal diet were presented in Table 1. Total amount of feed offered during 24 hours to a replicate under a specific treatment groups was divided into 3 equal proportions. The amount and timing of feed was adjusted in such a way that the birds consume the whole of the diet offered at any one time. As a result hardly any residue can be obtained from the replicate after a days feeding. The standard techniques of the proximate analysis were used to determine nutrient content of experimental diets (AOAC, 1995).

Record keeping: Individual body weight and feed consumption of broilers from all pens were measured at the 0, 21 and 42 day of age. Mortality of each pen was recorded on a daily basis. Feed conversion ratio was adjusted according to the feed consumption of the dead broilers. Body weight was recorded before offering feed. Body weight gain was obtained by calculation.

Collection, processing and analysis of blood samples: Two birds from each replicate were slaughtered on d 21 and 42 by severing the carotid artery and jugular vein and blood samples were collected for analyses of plasma proteins (total proteins, albumin and globulin), plasma enzymes (aspertate aminotransferase and alanine aminotransferase). Blood samples (approx. 10 ml) collected in heparinized vacuitainer 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), aspertate aminotransferase (AST)}. Plasma total protein, albumin, ALT and AST were analyzed in the Automatic Blood Analyzer (Microlab 200, E-Merck India Ltd., Mumbai,

Table 2: Performance of broiler chicken fed DL-methionine and herbal methionine

Attributes	Age, day	C	T_1	T_2	T_3	SEM	P
Body weight, g	0	39.18	39.22	39.77	39.42	0.52	0.214
	21	486.8^c	535.9^{ab}	499.5^{bc}	553.5^a	3.94	0.004
	42	1811.3^c	1851.5^b	1809.4^c	1924.0^a	6.51	0.003
Body weight gain, g	0-21	447.6^c	496.7^{ab}	459.7^{bc}	514.1^a	4.93	0.004
	0-42	1772.1^c	1812.3^b	1769.6^c	1884.6^a	6.86	0.003
Feed intake, g	0-21	885.7^a	854.1^b	811.5^c	823.2^c	4.46	0.008
	0-42	3505.4^a	3243.8^b	3231.8^b	3283.7^b	35.32	0.006
FCR, g/g	0-21	1.85^d	1.72^b	1.77^c	1.60^a	0.01	0.009
	0-42	1.98^c	1.79^{ab}	1.83^b	1.74^a	0.01	0.006
Survivability, %	0-42	99.2	99.5	99.8	99.3	0.01	0.315

C-Control without methionine; T_1-Control plus 10g DL-methionine/kg diet; T_2-Control plus 10g herbal methionine/kg diet; T_3-Control plus 15g herbal methionine/kg diet; $^{a-d}$Means with in row with no common superscripts differ significantly (P<0.05).

India) using commercial kit (Transasia Bio-Medical Ltd., Ringanwada, Daman, India).

Carcass dissection and abdominal fat collection: Another 10 birds were identified at 43 day from each replicate with weights closest to the mean body weight of pen to study dressing percentage, cut up parts and abdominal fat content. The birds were starved for 12 hrs (indeed drinking water was supplied ad-libitum) and were sacrificed as per standard procedure of evaluation of carcass characteristics including the yield of breast and thigh. After removal of viscera, the abdominal fat pad (from the proventriculus surrounding the gizzard down to the cloaca) was removed and weighed (Cahaner *et al.* 1986) which is indicative of abdominal fat content. After removal of liver it was packed into a zipper and transferred to the laboratory into an ice box. After collection, livers were dried in hot air oven at 50°C for 4 days and then grounded and were kept in desiccators for analysis of protein and fat content.

Statistical analysis: The data were analyzed using the General Linear Models procedure of SPSS (1997). Significant differences between treatment means were separated using the Duncan's multiple range test.

Results and Discussion

Performance: The effects of DL-methionine and herbal methionine supplementation on the performance of broiler are shown in Table 2. Fifteen g herbal methionine/kg diet increased (P<0.01) body weight at d 21 and 42, and body weight gain at day 0-21 and 0-42 compared to others though it is comparable with the 10g DL-methionine supplemented birds at d 21 and 0-21 day. Body weight gain of birds supplemented with 10g herbal methionine/kg of feed was similar with the performance of control birds. Responses to DL-methionine in maize-soyabean, maize-barley-soybean and sorghum-wheat-soybean meal have been observed by earlier workers (Bertram *et al.*, 1991; Brennan, 1998; Lemme *et al.*, 2002; Danner and Bessei, 2002). In earlier experiment (Bertram *et al.*, 1991) with the highest level of DL-methionine supplementation (0.15%), the growth was improved by 8.3% than the unsupplemented control group. 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%) (Meirelles *et al.*, 2003). The increasing weight gain with increasing methionine levels in the diet was also reported by Schutte and Van Weerden (1981), Twining and Hochstetler (1982) and Elkin and Hester (1983). Nevertheless, Garlich (1985) found differences for weight gain at 21 days of age that were attributed to methionine levels. Wang *et al.*, (2004) also reported significant response of birds to methionine supplementation in terms of weight gain in d 21 and 42. In the present investigation methionine supplementation has also given better result. Herbal methionine supplemented birds performed better than DL-methionine at 15g/ke dietary level, which could be explained by its nature and availability of herbal methionine to the birds. Feed intake of control birds at the end of d 21 and 42 were higher (P<0.01) than methionine (DL-or herbal) supplemented groups. At the end of d 42, feed intake of methionine supplemented birds were similar; which indicate herbal methionine at the rate of 15g/kg did not improve the feed intake compared to DL-methionine supplementation (10g/kg of diet).

Supplementation of DL-methionine or herbal methionine improved (P<0.01) FCR compared to control. Better FCR was found in herbal methionine supplemented birds (15g/kg diet). FCR was similar between the DL-methionine and 15g herbal methionine supplemented birds. Feed efficiency improved by about 3.7% in DL-methionine supplemented group on 38 days of age in earlier study (Bertram *et al.,* 1991). Wang *et al.*, (2004) reported that supplementation of methionine did not response feed to gain in broiler chicken. Garlich (1985) found that feed conversion was better when methionine was supplemented in the diet. Huyghebaert (1993) reported that increase in methionine level promoted an increase of 14 % in weight gain compared to methionine deficient basal diet. A similar result was described by Rostagno and Barbosa (1995) who observed that the increase in methionine levels promoted an increase of

Table 3: Plasma proteins and enzyme concentration of broiler chicken fed DL-methionine and herbal methionine

Attributes	Age, day	C	T_1	T_2	T_3	SEM	P
Total protein, g/dl	21	3.21	3.47	3.35	3.53	0.36	0.719
	42	3.72	4.35	4.06	4.76	0.58	0.061
Albumin, g/dl	21	1.52	1.62	1.55	1.63	0.26	0.845
	42	1.73	2.06	1.91	2.18	0.48	0.088
Globulin, g/dl	21	1.69	1.85	1.80	1.90	0.14	0.089
	42	1.99	2.29	2.15	2.58	0.39	0.082
Albumin : Globulin	21	0.89	0.86	0.86	0.85	0.03	0.432
	42	0.86	0.90	0.88	0.84	0.02	0.238
AST, U/L	21	113.67	115.33	121.67	106.33	13.73	0.604
	42	143.00	146.33	155.00	149.00	14.17	0.941
ALT, U/L	21	18.00	18.67	18.00	18.67	2.56	0.995
	42	16.33	15.33	17.67	17.33	1.62	0.740

C-Control without methionine; T_1-Control plus 10g DL-methionine/kg diet; T_2-Control plus 10g herbal methionine/kg diet; T_3-Control plus 15g herbal methionine/kg diet; $^{a-d}$Means with in row with no common superscripts differ significantly (P<0.05).

13% in 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-methionine had a better feed conversion than the birds supplemented with MHA-FA. 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 and Pack (1995) found a similar result. During the finisher phase (43-47 days), no significant difference was seen for weight gain but there was an increasing trend with higher methionine levels, for the two studied sources (Meirelles *et al.,* 2003). Previous works, revealed that supplementation of methionine in the 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 at the rate of 15g/kg showed better FCR compared to DL-methionine supplemented birds. The survivability of birds during the experimental period did not differ significantly between the treatments. The pathophysiological changes observed under post mortem examination of dead birds did not attribute to the dietary treatments. It indicates that dietary supplementation had no detrimental effect on survivability.

Plasma proteins and enzymes: Effect of supplemental DL-methionine and herbal methionine on plasma proteins and enzymes are presented in Table 3. Plasma concentration of total protein and enzyme at both 21 and 42 day was similar (P>0.05) between the treatments. Total protein, albumin and globulin concentration in plasma were in the normal range throughout the experiment (Prabhakaran *et al.,* 1996). The concentration of AST and ALT was in normal range throughout the experimental period (Marjanovic *et al.,* 1975). As there was no increase in AST and ALT during the experimental period; so it can be inferred that supplementation of DL-methionine (10g/kg) or herbal methionine (up to 15g/kg) did not cause any liver dystrophy or other vital organ abnormalities where from these enzymes are secreted. This indicates that all the birds were apparently in healthy condition throughout the experimental period.

Carcass characteristics: Effects of DL-methionine and herbal methionine on carcass characteristics in commercial broiler chicken are presented in table 4. A significant difference (P<0.05) exists between the treatments for dressing percentage. Methionine supplementation at the rate of 15g/kg showed highest dressing percentage compared to others. Bleeding percentage was not affected by dietary treatments. Abdominal fat content of birds reduced (P<0.01) when DL-methionine or herbal methionine was supplemented in the diet compared to control. Lowest abdominal fat content was found in herbal methionine supplemented (15g/kg diet) birds (1.76%) which significantly (P<0.01) varied from others. Percentage of breast yield and thigh yield were more (P<0.01) when DL-methionine and herbal methionine was added in the diet.

Ojano-Dirain and Waldroup (2002) observed significant improvement (P<0.05) in dressing percentage and breast meat yield between the broilers fed NRC methionine level and those fed higher levels. Recent research has suggested that levels of lysine and methionine in excess of NRC (1994) recommendation may result in enhanced performance, especially in regard to breast meat yield (Hickling *et al*., 1990; Moran and Bilgili, 1990; Schutte and Pack, 1995). Earlier studies reported that DL-methionine supplementation decreased fat deposition (Huyghebaert, 1993; Jeroch and Pack, 1995; Schutte and Pack, 1995; Virtanen and Rosi, 1995). Jensen *et al.* (1989) observed that the

Table 4: Carcass characteristics, liver protein and lipid of broiler chicken fed DL-methionine and herbal methionine

Attributes	C	T_1	T_2	T_3	SEM	P
Dressing %	71.43^{ab}	71.90^{ab}	70.90^{b}	72.73^{a}	0.37	0.044
Bleeding %	3.96	5.41	6.95	4.94	0.66	0.068
Abdominal fat %	1.84^{c}	1.81^{b}	1.82^{bc}	1.76^{a}	0.01	0.000
Breast yield %	17.22^{c}	17.69^{b}	17.54^{bc}	18.53^{a}	0.09	0.000
Thigh yield %	10.74^{c}	11.48^{b}	11.47^{b}	12.27^{a}	0.07	0.000
Liver protein, g/kg	167.90^{c}	181.85^{b}	180.56^{b}	187.58^{a}	0.82	0.000
Liver lipid, g/kg	52.34^{d}	41.72^{b}	43.42^{c}	37.22^{a}	0.32	0.000

C-Control without methionine; T_1-Control plus 10g DL-methionine/kg diet; T_2-Control plus 10g herbal methionine/kg diet; T_3-Control plus 15g herbal methionine/kg diet; $^{a-d}$Means with in row with no common superscripts differ significantly (P<0.05).

abdominal fat in males and females slaughtered at d 42 of age decreased with the increase in sulfur 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. Methionine in the system act as a methyl donor and donates methyl group to dimethylethanolamine to form trimethylethanolamine which be used directly for the synthesis of lecithin (Saunderson and McKinley, 1990). Lecithin facilitates the transport of fat through the body. If methionine is deficient in a diet, methionine supplement is used for protein synthesis. Carnitine is of greatest importance for beta-oxidation of long-chain fatty acids. Thus by this way methionine supplementation decreases the abdominal fat content and increases the body weight. From the present findings it is found that supplemental methionine either DL- or herbal increases the proportion off breast and thigh meat, which may be preferred by the Indian consumers.

Liver protein and lipid: Effect of DL-methionine and herbal methionine on liver protein and lipid are presented in Table 4. It has been found that supplemental DL-methionine or herbal methionine significantly (P<0.01) increased liver protein and decreased (P<0.01) liver lipid. Reduction of lipid content of liver was more by DL-methionine (10g/kg) than herbal methionine (10g/kg). Lowest liver lipid content was found in 15g/kg herbal methionine supplemented birds. The reduction of lipid from the liver can be explained by the lipotropic activity of methionine, which is described under carcass characteristics.

The data from the feeding trial indicate that herbal methionine can replace DL-methionine very effectively in the diet of commercial broiler birds when used at the rate of 15g/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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