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Studies on Comparative Efficacy of Herbal Amino Acid (Methiorep) Supplement with Synthetic Dl Methionine on Broiler Growth Performance and Carcass Quality Traits

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Abstract- An experiment was conducted on commercial Ven cob broiler chickens to investigate the effect of supplementing herbal methionine (methiorep) that replaced synthetic DL methionine (DLM) in the diets of broilers. One hundred and twenty day-old, commercial broiler chicks (Ven Cobb) were randomly divided into two experimental groups, each of which comprised four replicates. Each replicate consisted of 15 birds. Groups were group I - Diet with synthetic DL-methionine at 2 kg ton^{-1} of feed and group II - Diet with Methiorep (herbal-methionine) at 1 kg ton-1 of feed & DL-methionine at 1 kg ton^{-1} of feed. The experiment lasted for 42 days. Diets were iso nitrogenous in control and treatment groups and based on corn soy-bean meal. The experiment was designed to investigate comparative efficacy of herbal methiorep and synthetic DL methionine on growth performance, nutrient utilization, immune response and carcass quality traits of broilers. The replacement of 0.1% DLM by 0.1% Methiorep significantly improved live weight gain (P>0.05) and feed conversion ratio (P>0.05). The nitrogen, calcium and phosphorus retention was significantly higher in group II as compared to group I. Dressing % among groups did not differ significantly suggesting that combination of 0.1% herbal methiorep & 0.1% DLM could efficiently replaced 0.2% synthetic DL methionine. Combination of Methiorep & DLM supplemented group was found to elicit humoral as well as cell mediated immune response at par to the group supplemented DLM alone. Group I has also been observed to exert a superior hypocholesterolemic effect than 0.2% synthetic methionine. Methiorep & DLM supplemented group was found to produce higher serum globulins and serum proteins than DLM alone. Considering growth performance, economics and other attributes it could be recommended to replace 0.1% synthetic methionine by 0.1% herbal Methiorep in the diet of broiler chickens.

I. INTRODUCTION

There is great renewed interest in developing natural alternative supplements to maintain animal performance and well being. More synthetic methionine in the diet is metabolized into highly toxic compounds like methyl propionate, thereby, adversely altering the performance of birds (Bender, 1975). Herbal methionine as a source of active methionine is claimed to be effective in its optimum activity for proper protein accretion of birds so that they can reach better growth and performance potential. In this perspective a study was conducted to evaluate the effects of herbal methionine (Methiorep), supplied by M/S Ayurvet Limited, Baddi, India in commercial broiler chicken in comparison with synthetic methionine.

II. MATERIALS AND METHODS

One hundred and eighty day-old, commercial broiler chicks (Ven Cobb) were randomly divided into three groups, each of which comprised four replicates. Each replicate consisted of 15 birds. Groups were group I - Diet with synthetic DL-methionine at 2 kg ton^{-1} of feed and group II - Diet with Methiorep (herbal-methionine) at 1 kg ton^{-1} of feed & DL-methionine at 1 kg ton^{-1} of feed. The diets were isonitrogenic in control and treatment groups and formulated according to BIS (1992). The diet was based on corn soy-bean meal. Birds were raised under deep liter system and experiment was continued for a period of 42 days. The research was carried out at the Department of Animal Nutrition, College of Veterinary Science and Animal Husbandry, Durg, India.

Management

Both broiler starter and broiler finisher rations were offered to the birds in mash form. Starter mash was fed from second day of experiment to 21st day of experiment. After that finisher ration was provided up to the end of experiment. Birds were maintained on a 24 h constant light schedule. Feed and water were offered for consumption *ad libitum*. The brooding temperature was maintained close to the requirement. Vaccination of birds was done time to time.

Body Weight, feed consumption, feed conversion ratio and Energy and protein utilization

The weekly and cumulative feed consumption, body weights and FCR were recorded in each replicate. To evaluate the energy and protein utilization efficiency of birds, a 5 day metabolic trial was conducted starting from 38th day of experiment. The energy value of feed and excreta was determined by bomb calorimeter and protein value by standard method of AOAC (1995).

Meat quality

At 42 days of age, 4 birds from each replicate were sacrificed as per standard procedure. Then the carcasses were defeathered, eviscerated and evaluated for dressing, bleeding, thigh muscle, breast muscle and abdominal fat percentage. Water holding capacity of meat samples was determined (Offer and Knight, 1988). The pH of the finely minced meat was also determined (Gillespie, 1960) by using digital pH meter (Systronics, model 335).

Blood collection

Collection of blood was done at 42nd day from 10 birds of each replicate. Approximately 5 mL of blood was collected.

Total protein, albumin, globulin, AST and ALT

Total plasma protein was determined with the help of UV visible Spectrophotometer (Keller, 1991). Plasma albumin was determined with by auto analyzer, Micro lab 200 (Tietz, 1994). The globulin fraction in the plasma samples was calculated by subtractions of plasma albumin from total plasma protein. The plasma AST and ALT were measured as per the method suggested by Schlebusch *et al*. (1974).

Immune response

Humoral immune response was assessed by Haemagglutination inhibition test against Newcastle disease virus (NCDV). Ranikhet vaccination was done on 7^{th} and 28^{th} day. The blood samples were collected on 28th and 42nd day. The serum was separated for the evaluation of HI titer against NCDV. The HA unit of NCDV (R2B) was determined by the standard procedure (Allan, 1974). The cell mediated immune response was evaluated by using 1% 2-4, di nitrochlorobenzene at thoracolumbar region on 26^{th}, 27^{th} and 28^{th} days (sensitizing dose) and 40^{th}, 41^{st} and 42^{nd} days (challenge dose) in all the groups as per the method described by Valsala *et al.* (1981). The thickness of the skin and diameter of spreading lesion at thoracolumbar region, indicating reaction zone, was measured at 24, 48 and 72 hours after challenging with DNCB (photo1).Weight of bursa of fabricius, spleen and thymus were taken at the end of experiment from the sacrificed. birds of each group to calculate the relative organ by the formula: Organ weight factor = (organ weight (g)/ whole body weight (g)) x 1000. The representative samples of bursa of fabricius, spleen and intestine were taken at the end of experiment from the sacrificed birds of each group to study the organization of lymphoid tissue stained by haematoxylin and eosin method (Drury and Wallington, 1967).

Statistical Analysis: The data obtained were subjected to statistical analysis by the software SPSS 10 (SPSS, 1997). Levels of significance were calculated as per the standard method described by Duncan (1995) whenever any effect was found significant.

III. RESULTS AND DISCUSSION

Composition of feed ingredients and diet

The chemical composition of feed ingredients viz., maize, Hypro Soya, deoiled rape seed meal, de-oiled rice bran (DORB), rice polish and meat cum bone meal (MBM) has been given in table 1.

Growth performance, feed consumption and feed conversion ratio

The weekly body weight, weight gain, feed consumption and FCR of broilers have been presented in table 2. The body weight was significantly higher in group II as compared to group I (0.2 % DL methionine). The cumulative feed intake up to 6^{th} week was non-significantly different among two groups. The mean FCR of Methiorep+DLM supplemented group (II) was higher than group I. Narayanswamy and Bhagwat (2010) reported that the chicks in herbal methionine group showed a significant (p<0.01) gain in body weight (increase of 343 g) as compared to the chicks in control group and numerically higher weight gain (increase of 133 g) as compared to the chicks in synthetic methionine group. The results of the present trial are in accordance with the findings of Kalbande *et al.,* (2009) and Narayanswamy and Bhagwat (2010). These studies showed that the herbal source of methionine can replace DL-methionine in the diet of commercial broiler chicks. Chattopadhyay (2003) found that supplementation of herbal methionine at the rate of 15g/kg showed better FCR compared to DL-methionine supplemented birds. Mortality was non significant amongst groups. Chattopadhyay (2003) observed that neither DL-methionine nor herbal methionine supplementation had a significant effect on broiler mortality.

Balance study

The apparent metabolizable energy between group I and IIwas non significant. All the birds of groups I, II were in positive nitrogen, Ca and P balances. The percent N retention in Methiorep + DLM supplemented group (II) was significantly higher than group I. Nitrogen retention per cent was significantly (P<0.05) higher in group II (53.94%) than group I however two groups did not differ significantly. The Calcium balance in group II was also significantly higher as compared to I. The P balance (g/d) was significantly higher in group II as compared to group II and control (I). The per cent P retention was found to be significantly (p<0.05) higher in group II (46.78%) as compared to group I.

Carcass traits

The effect of herbal and synthetic amino acids on carcass traits and visceral organs weight expressed as percent of live weight is presented in **table 3.** Data revealed no significant variation in the defeathered and eviscerated weight amongst the two groups, while the dressed weight was numerically higher in group II than I. However, group I and II did not differ significantly from each other in terms of carcass traits, indicating that 0.1% of herbal methiorep can replace 0.1% of synthetic methionine without affecting the carcass parameters. Cut-up parts yield, expressed as per cent of dressed weight has given in table 3. Significantly lower abdominal fat content was found in group II. Percentage of breast yield and thigh yield were more (P<0.01) when DL-methionine and herbal methionine was added in the diet (Chattopadhyay, 2003).

Muscles biochemical parameters

The effect of inclusion of herbal methiorep and synthetic DL Methionine on the dry matter (DM), protein, total lipid and cholesterol contents of thigh and breast muscles has been summarized in **Table 3**. No significant difference could be observed in DM, protein and total lipid contents of thigh muscles amongst the two groups. The lipid content of abdominal fat was similar in herbal and synthetic methionine supplemented groups. Chattopadhyay (2003) reported that abdominal fat (%) and liver lipid (g/kg) was significantly decreased by the addition of 15g herbal methionine/kg diet.

Cell mediated and Humoral immune response

The cell mediated immune response is presented in **table 4.** The results indicated that group I and II resulted into significantly ($P<0.05$) higher mean skin thickness after DNCB application. The increased skin thickness in group II might be due to increased proliferating rate of T lymphocyte and induced secretion of IL-1. The degree of inflammatory reaction at the site of application, following subsequent challenge indicate significant intense reaction which signifies the better capacity of conversion of T lymphocyte into specific sensitized antibodies in birds. The mean serum HI titer against NCDV at 28 as well as 42 days (Table 4) was significantly higher in group II followed by group I.

Organ weight factor

The relative weights of immune organs *viz.,* thymus, bursa and spleen are presented in table 4. The weight of thymus was significantly improved by the inclusion of herbal (0.1% methiorep) and synthetic amino acids (0.1% DLM) and there was no significant difference between them. However DL methionine did not influence the relative weight of spleen as compared to herbal ones.

Organization of lymphoid tissue

It was very interesting to note that the herbal and synthetic methionine supplementation in the diet influenced the organization of medulla and cortex of the lymphoid follicle of bursa. Nodular arteriole of the white pulp (B cell zone) was more developed and larger as compared to control (photo 1). In group I and II nodular artery and organization of the surrounding white pulp was more distinct as compared to control.

Blood biochemical and Hematological parameters

Treatment group II exhibited hypocholesterolemic effect as compared to control and group I. Serum albumin was significantly ($P<0.05$) higher in group II as compared to group I. There was no significant difference in serum total protein between group I and II. In contrast, group II revealed significantly ($P<0.05$) higher serum protein (5.16g/dl) than group I (table 5). The changes in some of the hematological parameters have been presented in table 5. The mean Hb values in herbal (1% methiorep) and synthetic (1% DL methionine) methionine supplemented groups was significantly higher than control however these two groups did not differ significantly. No significant difference was noticed in heterophil counts in broilers due to herbal or synthetic methionine. No pathological lesions of fatty liver syndrome has been observed amongst groups due to herbal or synthetic methionine.

Economics

The economics of raising broilers chickens was better in groups II than group I. There was gain of INR .0.97 per chick in group III in comparison to group I and INR 7.94 per chick in comparison to group II.

IV. CONCLUSION

The study concludes that 1 kg Methiorep per tonne of feed can efficiently replace 1 kg synthetic DL methionine per tonne of feed to improve commercial broiler flock performance (growth, FCR, and livability parameters).

REFERENCES

[1] Allan, W. H and Gough, R. E. 1974. A standard HI test for NCDV 1. A comparison of macro and micro method. Vet. Rec., 95: 120-125.

[2] AOAC, 1995. Official Methods of Analysis, Association of Officials Analytical Chemist. Vol - I, 16th Ed., AOAC.International, Arlington, USA, pp: 31-65.

[3] Bender, D.A. 1975. Amino acid metabolism. John Wiley and Sons. Ltd., 1st Ed., New York, USA, pp: 112-142.

[4] Bureau of Indian Standard (BIS), 1992. Nutrient Requirements for poultry. In: Animal Feeds and Feeding Stuffs- Determination of Calcium and Magnesium in Mineral Supplements IS, 13574.

[5] Chattopadhyay, R.R. 2003. Possible mechanism of hepatoprotective activity of Azadirachta indica leaf extract: part II, J. Ethnopharmacol., 89: 217-9.

[6] DRURY, R. A. B. and WALLINGTON, E. A. (1967). Carleton's Histological Technique, 4th ed. London: Oxford University Press.

[7] Duncan, D.B. 1955. Multiple range and multiple F-tests. Biometrics, 11: 1-42.

[8] Gillespie, E.L. 1960. Methods of Analysis. The Science of Meat and Meat Product. Freeman, W.H. and Company, London, pp: 234.

[9] Kalbande, V.H., Ravikanth K., Maini S. and Rekhe D.S. 2009. Methionine supplementation options in poultry. International Journal of Poultry Science 8(6): 588- 591.

[10] Keller, H. 1991. Klinisch-Chemische Labordianostikfun Die Praxis. 2nd Edn., George Thieme Veriag, Stuttgart, pp: 263.

[11] Narayanswamy, H.D. and Bhagwat, V.G. 2010. Evaluating the efficacy of methionine supplementation options in commercial broiler chickens. Poultry Line 3: 5-7.

[12] Offer, G. and Knight, P. 1984. The Structural Basis of Water Holding in Meat. In: Development in Meat Science,

[13] Lawrie, R. (Ed.). Elsevier Applied Science, London, pp: 63-173.

[14] Schlebusch, H., Rick, W., Lang, H. and Knedel, M. 1974. Dtsch. Med. Wschr., 99: 765-765.

[15] SPSS Base Applications Guide 7.5,©, 1997. Statistical Package for Social Sciences, Chicago, USA.

[16] Tietz, N.W. 1994. Textbook of Clinical Chemistry. 2nd Edn., W.B. Saunders and Company, Philadelphia, pp: 703.

[17] Valsala, K.B., Rajan, A. and Krishnan Nayar, M. 1981. Evaluation of cutaneous hypersensitivity to 2, 4, DNCB in ducks. Kerala J. Vet. Sci., 12 (2): 332-336.

Table 1. The ingredient composition of diets (%)

Group	Starter ration		Finisher Ration	
	I	II	I	II
Feed ingredients	**%**	**%**	**%**	**%**
Maize	50.78	50.78	56.85	56.85
Hypro Soya	32.04	32.04	22.44	22.44
MBM	0	0	2	2
Rape Seed DOC	3	3	3	3
DORB	8.46	8.46	7.45	7.45
Rice Polish	0	0	3.56	3.56
LSP	1.62	1.62	1.46	1.46
DCP	2.11	2.11	1.69	1.69
Salt	0.35	0.35	0.33	0.33
DLM	**0.1**	0.2	**0.1**	0
Soda Bicarb	0.06	0.06	0.04	0.04
Choline	0	0	0	0
Crude soy oil	1	1	1	1
Premix	0.48	0.48	0.28	0.28
Methiorep	-	**0.1**	-	**0.1**
Total	**100**	**100**	**100**	**100**

*Premix: trace mineral premix (mg/kg diet); Mn 25; Fe 30; Zn 27.5; Cu 5.62; Se 0.08; Co 0.07; I 0.62; vitamin premix (per kg diet): vit. A 3900 IU; vit. K 1mg; vit. E 30 mg; vit. D_3 1312.5 IU; vit. B_1 0.9 mg; vit. B_2 3mg; vit. B_{12} 0.005mg; niacin 14.3 mg; pantothanic acid 2 mg; folic acid 1.25 mg; Ca pentothenate 3.75 mg; salinomycin (Coxistac 12%) 30 ppm; bacitracin methylene di salicyclate (BMD110) 55ppm. [1]retinol [2]α tocopherol acetate, [3]Sodium selenite, [4]Zinc-methionine, [5]chromium picolinate

Table 2: Effect of herbal and synthetic amino acids onbody weight gain, FCR & Nutrient balance

Particulars	Group I	Group II
Total feed consumption (g)	4046.74^{ab}±33.41	3998.10^{a} ±53.52
Total body weight gain (g)	2180.28^{ab} ±20.65	2071.14^{a} ±30.26
FCR	1.86^{ab} ±0.03	1.93^{a} ±0.03
N intake (g/day)	4.05^{a}±0.27	3.70^{ab} ±0.18
N balance (g/day)	1.95^{a}±0.28	2.05^{ab} ±0.15
AME (Kcal/d)	414.92^{a}±8.54	425.96^{ab} ±9.24
Ca intake (g/day)	1.54^{ab}±0.10	1.42^{a}±0.07
Ca balance (g/day)	0.47^{a}±0.11	0.48^{a}±0.13
P intake (g/day)	0.94^{a}±0.01	0.99^{a}±0.05
P balance (g/day)	0.35^{a}±0.06	0.49^{ab} ±0.05

Superscripts are read row wise for comparison of mean. Means in the same row with different superscript a, b, are significantly different ($p < 0.05$).

Table 3: Effect of Herbal and synthetic amino acids on carcass traits (% of live wt.) & muscle biochemical parameters at 6th week

Particulars	Groups	
	I	II
Defeathered wt.	88.95^{a} ± 0.59	88.24^{a} ± 0.32
Eviscerated wt.	68.85^{a} ± 0.42	68.39^{a} ± 0.45

Dressed wt.	62.69^{a}± 0.59	61.48^{a}± 0.45
Giblet	4.86^{a} ± 0.29	4.72^{a} ± 0.11
Liver	1.84^{a} ± 0.09	2.11^{b} ± 0.03
Abdominal Fat	1.93^{a} ± 0.50	1.59^{a} ± 0.21
Dressed wt.	62.69^{a}± 0.59	61.48^{a}± 0.45
Breast	27.20^{b}± 0.15	27.15^{b}± 0.44
Thigh	29.87^{ab}± 0.71	31.16^{b}± 0.54
Wing	16.87^{a}± 0.96	19.05^{ab}± 0.47
Back & neck	25.18^{bc}± 0.57	21.81^{a}± 0.37
Dressed wt.	62.69^{a}± 0.59	61.48^{a}± 0.45
Chemical composition		
Thigh muscle		
Dry matter (%)	23.60 ± 0.20	23.26 ± 0.16
Total lipid (%)	2.10 ± 0.04	2.02 ± 0.05
Cholesterol (mg/100g)	75.08 ± 1.65	74.21 ± 1.82
Protein	22.00 ± 0.16	21.48 ± 0.12
Breast muscle		
Dry matter (%)	25.42 ± 0.12	25.80 ± 0.18
Total lipid (%)	1.40 ± 0.06	1.33 ± 0.05
Cholesterol (mg/100g)	59.76 ± 2.05	58.84 ± 1.85
Protein	23.82 ± 0.15	24.40 ± 0.16
Abdominal fat	59.76 ± 2.05	58.84 ± 1.85
Total Lipid	87.92± 1.12	86.70 ± 0.95

Superscripts are read row wise for comparison of mean. Means in the same row with different superscript a, b, c are significantly different ($p < 0.05$).

Table 4: Effect of Herbal and synthetic amino acids on humoral immune response

Particulars	**Day**	**Group**	
		I	**II**
Mean serum HI titer (log 2)	28 d	4.10^{ab} ±0.08	3.88^{a} ± 0.13
	42 d	4.49^{ab} ±0.08	4.18^{a} ± 0.07

Superscripts are read row wise for comparison of mean. Means in the row with different superscript a, b, c are significantly different ($p < 0.05$)

Table 5: The effect of Herbal and synthetic amino acids on certain blood biochemical constituents

Particulars	Groups	
	I	II
Cholesterol (mg/dl)	159.23^{b}±1.04	151.67^{a}±1.88
Albumin (mg/dl)	1.61^{a}±0.02	1.58^{a}±0.01
Serum globulin (g/dl)	2.41^{a}±0.01	3.37^{b}±0.02
Serum total protein (g/dl)	4.24^{a}±0.02	5.16^{b}±0.03
SGPT (U/l)	8.40^{a} ± 1.47	8.50^{a} ± 0.86
SGOT (U/l)	241^{a}±0.59	281^{a}±0.52
Serum triglycerides (mg/dl)	177^{a}±0.22	159^{a}±0.22

Superscripts are read row wise for comparison of mean. Different superscript differ significantly ($p < 0.05$).

Photo 1 Skin hypersensitivity test showing erythema, induration challenge with DNCB at 24 hrs post infection

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