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Effect of organic mineral mixtures, probiotics, enzymes, emulsifier and liver supplements on serum protein and serum biochemical profile of broilers

SS Chauhan, RK Sharma, DV Singh, SK Shukla and Jyoti Palod

Abstract
A feeding trial was conducted to evaluate the effects of organic mineral mixture, probiotics, enzymes, emulsifier and liver supplements on serum protein profile and serum biochemical profile. For this purpose, a total of 396 day-old commercial broiler chicks (Cobb) were used and randomly allocated into 11 groups with three replicates of 12 chicks each. The results revealed that at 42nd day, all the supplemented groups were significantly (P<0.05) higher in total protein than T_0 group. All the supplemented groups were significantly (P<0.05) higher in albumin than T_0 group. All the supplemented groups were significantly (P<0.05) higher in globulin than T_0 group. All the supplemented groups were significantly (P<0.05) higher in albumin/ globulin ratio than T_0 group. Glucose values in T_4, T_9 and T_{10} groups were significantly (P<0.05) lower than T_0 (control) group. Total cholesterol values in T_2, T_3, T_4, T_5, T_9 and T_{10} groups showed significant (P<0.05) decrease compared to the T_0 (control). HDL-cholesterol values in T_3, T_4, T_5, T_9 and T_{10} showed significant (P<0.05) increase compared to the T_0 (control). All the supplemented groups showed significant (P<0.05) decrease in LDL- cholestertol values compared to the T_0 (control) except T_6, T_7, and T_8. All the supplemented groups showed significant (P<0.05) decrease in triglycerides values compared to the T_0 (control) except T_6 and T_7. All the supplemented groups showed significant (P<0.05) decrease in AST values compared to the T_0 (control) except T_1, T_6, T_7 and T_8. All the supplemented groups showed significant (P<0.05) decrease in ALT values compared to the T_0 (control) except T_4 and T_9.

Keywords: Organic mineral mixtures, serum protein, serum biochemical

Introduction
Poultry is one of the fastest growing segments of the agricultural sector in India. Globally, India ranked 3rd after China and USA with a production of 88.1 billion eggs (2016 – 2017) and 6th after USA, China, Brazil, Mexico and Indonesia with a production of 3.46 million tons of chicken meat (2016- 2017). The poultry sector in totality accounts for 0.60 per cent of the national GDP (2017–2018). The annual egg and broiler production of India is 70 billion eggs and 3.8 million tons respectively, with per capita consumption of 68 eggs and 2.5 kg chicken meat against the ICMR recommendations of 180 eggs and 11 kg poultry meat (CARI VISION 2050) [3]. Poultry meat has significant role in Indian diet valued at US $ 6.6 billion. Favoured by socio- economic conditions like rising purchasing power and changing food habits of the people this sector is driven by ever increasing domestic demand. Poultry meat is an excellent source of high quality protein, vitamins, and minerals and is not subjected to cultural and religious restrictions. Rising input cost in poultry production has necessitated the need to look for feed supplements which can enhance the nutrient utilization efficiency of feeds thereby improving performance of poultry and resultant increase in profitability. In this context use of organic minerals, probiotics, enzymes, emulsifiers and liver supplements seems promising. Use of organic minerals in poultry diets has been shown to have multiple beneficial effects including higher absorption and increased antibody levels as they may provide alternative pathways for absorption, by decreasing mineral excretion. Similarly, use of probiotics and feed enzymes have been reported to regulate gut integrity, reduce digestive disorders, improve nutrient absorption/ feed efficiency, increases production, check the mortality and lowering of feed cost. Poultry produces emulsifiers in the form of bile, however, at times it is insufficient in view of added fats and oils. Also, as the digestive tract in young birds is not completely developed, fat absorption from the feed matrix is hampered. Hence, addition of emulsifier into the diet can overcome this problem by reducing the size of the fat globules forming small micelles and increasing the total surface available for enzymatic digestion.

The addition of synthetic emulsifier to broiler diets is a recent practice as compared to other dietary supplements. Polyherbal liver stimulants possess hepato – protective, hepatogenic, immunomodulatory and antioxidant properties, which tone up liver resulting in increased utilization of feed and better performance. Keeping the above facts in view, an experiment was conducted to determine the effect of supplementation of organic mineral mixture, probiotics, enzymes, emulsifier and liver stimulants on serum protein and serum biochemical profile of broilers.

Materials and Methods

A total number of 396 day old commercial broiler chicks (Cobb) were procured for undertaking the experiment. All the chicks were individually weighed and randomly allotted to elevin different groups each with three replicates of 12 chicks. The groups were designated as T_0; basal diet, T_1; chicks fed basal diet along with organic mineral mixture 1 (Organomin forte) @ 0.5 g per kg feed, T_2; basal diet along with organic mineral mixture 2 (Vannamin) @ 0.5 g per kg feed,T_3; basal diet along with probiotics (Microguard) @ 0.1g per kg feed,T_4; basal diet along with enzymes + probiotics (Brozyme - XPR) @ 0.5 g per kg feed, T_5; basal diet along with emulsifier (Lipigon) @ 0.5 g per kg feed, T_6; basal diet with 3% less energy, T_7; basal diet with 3% less energy along with liver supplement 1(Superliv premix) @ 0.5 g/kg feed, T_8; basal diet with 3%2 less energy along with liver supplement 2 (X- liv Pro) @ 0.5 g/kg feed, T_9; basal diet along with enzymes with probiotics (Brozyme - XPR) and liver supplement 1(Superliv premix) @ 0.5 g/kg feed, and T_{10}; basal diet along with enzyme with probiotics (Brozyme - XPR), liver supplement 1(Superliv premix) and emulsifier (Lipigon) @ 0.5 g/kg feed. Average body weight of chicks was similar for all the treatment groups. The broiler chicks were housed in deep litter system under standard management practices. Blood samples were collected from six experimental birds of each group i.e. two broiler chicks from each replicate on 42nd day of experimental feeding. Total protein concentration in serum was estimated by Biuret method with the help of Span Diagnostic Kit at 540 nm wavelength (Johnson *et al.*, 1999) [6]. Albumin concentration in serum was estimated by bromocresol green, end point assay method with the help of Span Diagnostic Kit at 630 nm wavelength (Johnson *et al.*, 1999) [6]. Serum globulin was estimated by Beckman coulter model no – Au 480 fully automatic biochemistry analyser. The albumin-globulin ratio was calculated by dividing albumin by globulin. Glucose was done spectrophotometrically by enzymatic GOD-POD method with the help of Span Diagnostic Kit at 505 nm wavelength (Sacks, 1998). Total cholesterol was estimated spectrophotometrically using Span Diagnostic Kit with enzymatic CHOD-PAP method at 505 nm wavelength (Tietz, 1998) [10, 15]. HDL cholesterol concentration was estimated spectrophotometrically at 560 nm by phosphotungstate method (Tietz, 1998) [10, 15]. LDL cholesterol was estimated by Beckman coulter model no–Au480 fully automatic biochemistry analyser. Triglycerides was estimated at 505 nm spectrophotometrically using Span Diagnostic Kit by GPO – PAP, end point assay (Stein and Mayer, 1995) [14]. AST was measured by Kinetic method using Labkit diagnostic kit at 330 nm wavelength (Tietz, 1998) [10, 15]. ALT was estimated using ecoline diagnostic kit based on reference method of International Federation of Clinical Chemistry (IFCC) at 340 nm wavelengths (Bergmeyer *et al.*, 1986) [2]. The experimental data obtained were analyzed statistically using completely randomized design (CRD) as per the methods given by (Snedecor and Cochran, 1994) [12]. The significant mean differences between the treatments were determined by using Duncan's Multiple Range Test (DMRT) as given by Kramer (1957) [7].

Results and Discussion

Serum protein profile (42nd day)

The values of the serum protein profile of broiler supplemented with organic mineral mixture, probiotics, enzymes, emulsifier and liver supplements at different concentrations are furnished in Table 1.

Serum total protein

All the supplemented groups were significantly (P<0.05) higher in total protein than T_0 group however, there were no significant differences in the total protein values between T1 and T2, T3 and T5, T6 and T7, T9 and T10 groups of broilers. Maximum and significantly (P<0.05) higher total protein compared to the control was found in broilers of group T_{10} (4.44 ± 0.02 g/ dl) which was statistically similar to the total protein of group T_9. Minimum and significantly lower total protein was observed in the broilers of control group T_0 ((3.53 ± 0.07).

Findings of the present investigation are in accordance with the reports of Shareef and Al-Dabbagh (2009) [11] who found significant increase in serum total protein levels of broilers supplemented with probiotics.

Albumin

All the supplemented groups were significantly (P<0.05) higher in albumin than T_0 group. Maximum and significantly (P<0.05) higher albumin compared to the control was found in broilers of group T_{10} (2.85 ± 0.02 g/ dl). Minimum and significantly lower albumin was observed in the broilers of group T_0 (1.29 ± 0.03 g/ dl).

The results of the present experiment are in accordance with the findings Shareef and Al-Dabbagh (2009) [11] who found significant increase in serum albumin levels of broilers supplemented with probiotics.

Table 1: Effect of feed supplementations on serum protein profile of broilers (42nd day)

Treatments	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio
T_0	3.53^{a} ± 0.07	1.29^{a} ± 0.03	1.51^{a} ± 0.00	0.85^{a} ± 0.00
T_1	4.00^{b} ± 0.00	2.28^{b} ± 0.13	2.04^{b} ± 0.02	1.11^{b} ± 0.00
T_2	4.10^{b} ± 0.04	2.52^{c} ± 0.03	2.18^{c} ± 0.05	1.15^{c} ± 0.00
T_3	4.22^{c} ± 0.00	2.69^{d} ± 0.00	2.35^{d} ± 0.03	1.14^{c} ± 0.00
T_4	4.31^{ce} ± 0.03	2.74^{e} ± 0.02	2.43^{e} ± 0.00	1.12^{b} ± 0.00
T_5	4.18^{c} ± 0.00	2.62^{f} ± 0.02	2.27^{f} ± 0.01	1.15^{c} ± 0.01
T_6	3.83^{d} ± 0.00	1.69^{g} ± 0.06	1.76^{g} ± 0.06	0.96^{d} ± 0.00
T_7	3.74^{d} ± 0.04	1.45^{h} ± 0.06	1.58^{h} ± 0.02	0.91^{e} ± 0.01

T_8	$3.93^{db} \pm 0.04$	$1.90^{i} \pm 0.05$	$1.93^{i} \pm 0.04$	$0.98^{d} \pm 0.00$
T_9	$4.38^{e} \pm 0.00$	$2.79^{j} \pm 0.00$	$2.47^{j} \pm 0.01$	$1.12^{b} \pm 0.00$
T_{10}	$4.44^{e} \pm 0.02$	$2.85^{k} \pm 0.02$	$2.55^{k} \pm 0.02$	$1.11^{b} \pm 0.01$

Means bearing different superscripts in a column differ significantly (P<0.05).

Globulin

All the supplemented groups were significantly (P<0.05) higher in globulin than T_0 group. The concentration of globulin was maximum (2.55 ± 0.02 g/ dl) in the T_{10} group and minimum in T_0 group (1.51 ± 0.00, g/ dl) of broilers.

Similar to the findings of present experiment, Hassen and Chauhan (2003) [5] found significant increase in serum globulin levels of broilers supplemented with enzymes.

Albumin-globulin ratio (A/ G ratio)

All the supplemented groups were significantly (P<0.05) higher in albumin - globulin ratio than T_0 group. There were no significant differences in the serum albumin-globulin ratio among T_1, T_4, T_9 and T_{10}, T_2 and T_3, T_6 and T_8 treatment groups. The albumin-globulin ratio was maximum in group T_2 (1.15 ± 0.00) and T_5 (1.15 ± 0.01) and minimum in T_0 (0.85 ± 0.00) group of broilers.

The results of the present experiment were in accordance with the findings of Stef *et al.* (2016) [13] who found significant increase in the serum albumin-globulin ratio of broilers supplemented with probiotics compared to the control.

Serum biochemical parameters (42nd day)

The values of serum biochemical profile of broiler supplementation with organic mineral mixtures, probiotics, enzymes, emulsifier and liver supplements are furnished in Table - 2

Glucose

Glucose values in T_4, T_9 and T_{10}, groups were significantly (P<0.05) lower than control group, however, there were no significant differences in the glucose values between T_1,T_2, T_3, T_5, T_7, and T_8 groups of broilers. The glucose was maximum in group T_0 (205.58 ± 0.05 mg/ dl) and minimum in T_{10} (199.47 ± 0.21 mg/ dl) group. Similar results of significant reduction also support the results in which the dietary supplementation of probiotics reduced serum glucose levels in broilers (Shareef and Al-Dabbagh, 2009 [11]. Reduction in the serum glucose levels of supplemented groups may be due to enhanced insulin secretion.

Total cholesterol

Total cholesterol values in T_2, T_3, T_4, T_5, T_9 and T_{10} groups were significantly (P<0.05) lower than T_0 (control) group. Maximum cholesterol level (122.62 ± 0.04 mg/ dl) was found in broilers of group T_0 (control) and minimum in group T_{10} (117.09 ± 0.08 mg/ dl). The levels of cholesterol in T_1, T_6, T_7 and T_8, T_3, T_4, T_5 and T_9 groups of broilers did not show any significant difference between them. Similar to the findings of the present studies, Chuka (2014) [4] found significant decrease in total cholesterol of broilers supplemented with enzymes and probiotics. The reduction in serum cholesterol levels due to supplementation might be due to the inhibition of the of activity of hydroxymethyl glutamate Co A reductase, the rate limiting enzyme in cholesterol synthesis and deconjugation of bile salts in the intestine, thereby preventing them from acting as precursors in cholesterol synthesis.

HDL-cholesterol

HDL- cholesterol values in T_3, T_4, T_5, T_9 and T_{10} groups were significantly (P<0.05) higher than T_0 (control) group. Maximum (65.04 ± 0.12 mg/ dl) HDL-cholesterol level was noted in broilers of T_{10} group. Minimum HDL-cholesterol (62.26 ± 0.12 mg/ dl) level was recorded in broilers of control (T_0) group which was significantly (P<0.05) lower than other treatment groups. HDL-cholesterol concentration between broilers of T_1 and T_2, T_3 T_4, T_5, T_9 and T_{10}, T_6 T_7 and T_8 groups were statistically similar. Findings of the present investigation with respect to probiotic supplementation are not in accordance with the findings of Panda *et al.* (2000) [8] who found significant decrease in HDL-cholesterol of broilers supplemented with probiotics.

LDL-cholesterol

LDL- cholesterol values in T_1, T_2, T_3, T_4, T_5, T_9 and T_{10} groups were significantly (P<0.05) lower than T_0 (control) group. Maximum (50.67 ± 0.05 mg/dl) level of LDL-cholesterol was observed in broilers of control (T_0) group, whereas minimum (46.53 ± 0.14 mg/ dl) level of LDL-cholesterol was found in broilers of T_{10} group. LDL-cholesterol levels between the broilers of T_3, T_4, T_9 and T_{10}, T_7 and T_8, T_2 and T_5 groups were also statistically similar. Abdel-Fattah *et al.* (2008) [1] found significant decrease in LDL-cholesterol of broilers supplemented with probiotics.

Triglycerides

Triglycerides values in T_1, T_2, T_3, T_4, T_5, T_8 T_9 and T_{10} groups were significantly (P<0.05) lower than T_0 (control) group. Maximum (52.45 ± 0.06 mg/ dl) triglyceride concentration was found in the broilers of control (T_0) group whereas, in the T_{10} group broilers the concentration of triglyceride was minimum (45.69 ± 0.19 mg/ dl). However, the levels of triglyceride in T_1 T_2, and T_5, T_3, and T_5 groups of broilers did not show any significant difference between them.

Table 2: Effect of feed supplementation on Serum biochemical profile of broilers (42nd day)

Treatments	Glucose (mg/dl)	Total cholesterol (mg/dl)	HDL-cholesterol* (mg/dl)	LDL- Cholesterol* (mg/dl)	Triglycerides (mg/dl)	AST* U/L	ALT* U/L
T_0	$205.58^{a} \pm 0.05$	$122.62^{a} \pm 0.04$	$62.26^{a} \pm 0.12$	$50.67^{a} \pm 0.05$	$52.45^{a} \pm 0.06$	$172.17^{a} \pm 0.26$	$33.37^{a} \pm 0.21$
T_1	$203.47^{ab} \pm 0.05$	$120.55^{ab} \pm 0.05$	$63.59^{a} \pm 0.08$	$48.87^{b} \pm 0.23$	$49.75^{b} \pm 0.05$	$169.23^{ab} \pm 0.32$	$30.33^{b} \pm 0.31$
T_2	$202.84^{ab} \pm 0.26$	$119.86^{b} \pm 0.31$	$63.81^{a} \pm 0.02$	$48.36^{bc} \pm 0.07$	$49.07^{b} \pm 0.32$	$168.67^{b} \pm 0.06$	$31.00^{b} \pm 0.05$
T_3	$201.65^{ab} \pm 0.30$	$118.85^{bc} \pm 0.33$	$64.29^{b} \pm 0.03$	$47.23^{c} \pm 0.05$	$47.57^{c} \pm 0.34$	$167.80^{b} \pm 0.31$	$31.93^{c} \pm 0.05$
T_4	$200.66^{b} \pm 0.29$	$118.09^{bc} \pm 0.04$	$64.59^{b} \pm 0.11$	$46.99^{c} \pm 0.20$	$47.01^{c} \pm 0.07$	$167.11^{b} \pm 0.07$	$32.43^{a} \pm 0.22$
T_5	$202.36^{ba} \pm 0.07$	$119.20^{bc} \pm 0.07$	$64.04^{b} \pm 0.10$	$47.70^{bc} \pm 0.32$	$48.36^{b} \pm 0.07$	$168.47^{b} \pm 0.05$	$31.47^{c} \pm 0.20$
T_6	$205.08^{a} \pm 0.22$	$122.12^{ab} \pm 0.22$	$62.71^{ab} \pm 0.14$	$50.43^{a} \pm 0.08$	$51.61^{ad} \pm 0.36$	$171.36^{a} \pm 0.07$	$28.62^{d} \pm 0.05$

T_7	$204.57^{ab} \pm 0.06$	$121.65^{ab} \pm 0.05$	$63.00^{ab} \pm 0.03$	$49.90^{ab} \pm 0.20$	$51.01^{ad} \pm 0.06$	$170.61^{a} \pm 0.33$	$29.21^{d} \pm 0.26$
T_8	$204.02^{ab} \pm 0.25$	$121.08^{ab} \pm 0.25$	$63.25^{ab} \pm 0.10$	$49.44^{ab} \pm 0.06$	$50.26^{d} \pm 0.31$	$169.97^{a} \pm 0.05$	$29.72^{e} \pm 0.05$
T_9	$200.02^{b} \pm 0.08$	$118.06^{bc} \pm 0.43$	$64.80^{b} \pm 0.02$	$46.72^{c} \pm 0.05$	$46.44^{ce} \pm 0.25$	$166.88^{b} \pm 0.05$	$32.91^{a} \pm 0.06$
T_{10}	$199.47^{b} \pm 0.21$	$117.09^{c} \pm 0.08$	$65.04^{b} \pm 0.12$	$46.53^{c} \pm 0.14$	$45.69^{e} \pm 0.19$	$166.17^{b} \pm 0.32$	$28.06^{f} \pm 0.25$

Means bearing different superscripts in a column differ significantly ($P<0.05$)

Similar to the findings of the present studies, Shareef and Al-Dabbagh (2009) [11] reported significant decrease in triglyceride levels of broilers supplemented with probiotic.

Serum aspertate aminotransaminase (AST)

Serum aspertate aminotransaminase (AST) values in T_2, T_3, T_4, T_5, T_9 and T_{10} groups were significantly ($P<0.05$) lower than T_0 (control) group. Maximum (172.17 ± 0.26 U/L) AST concentration was found in the broilers of control (T_0) group whereas, in the T_{10} group broilers the concentration of AST was minimum (166.17 ± 0.32 U/L). However, the AST concentrations in broilers of T_2, T_3, T_4 T_5 T_9 and T_{10}, T_6 T_7 and T_8 groups were statistically similar. Similar to the findings of the present studies, Rahman *et al.* (2013) found significant decrease in AST of broilers supplemented with enzymes and probiotics

Serum alanine aminotransaminase (ALT)

Serum alanine aminotransaminase (ALT) values in T_1, T_2, T_3, T_5, T_6, T_7, T_8 and T_{10} groups were significantly ($P<0.05$) lower than T_0 (control) group. ALT concentration in broilers of T_0 group (33.37± 0.21 U/ L) was significantly (%<0.05) higher than the supplemented groups. Minimum (28.06 ± 0.25 U/ L) concentration of ALT was noted in the broilers of T_{10} group. However, the ALT concentrations in broilers of T_3 and T_5, T_6 and T_7, T_1 and T_2 groups were statistically similar.

Rahman *et al.* (2013) found significant decrease in ALT of broilers supplemented with enzymes and probiotics.

Conclusion

Thus, it may be concluded that better protein retention and mobilization may be induced by the feed supplementations. These supplementations has been found beneficial in birds indicating that it is possible to produce lean meat using these supplements and fetch more income.

Reference

1. Abdel-Fattah SA, El-Sanhoury MH, El-Mednay NM, Abdel-Azeem F. Thyroid activity, some blood constituents, organs morphology and performance of broiler chicks fed supplemental organic acids. Int. J of poult. Sci. 2008; 7(3):215-222.
2. Bergmeyer HU, Horder M, Rej R. Approved recommendation on IFCC methods for the measurement of catalytic concentration of enzymes. J. of Clin. Chem. and Clin. Biochem. 1986; 24:497-520.
3. CARI VISION. 2050. Central Avian Research Institute, Izatnagar, Bareilly, India.
4. Chuka, E. Comparative study of the effects of probiotic and commercial enzyme on growth rate, haematology and serum biochemistry of broiler chicken. Food Proc. and Technol. 2014; 5:9.
5. Hassen BH, Chauhan SS. Phytase supplementation on biochemical blood parameters of maize-based-diets-fed broilers. Agri. Topica. 2003; 18:2-3.
6. Johnson AM, Rohlfs EM, Silverman LM. Proteins. In: Burtis C. A. and Ashwood E. R. eds. Tietz Textbook of Clinical Chemistry. 3rd ed. W. B. Saunders Company, Philadelphia. 1999, 477-540.
7. Kramer CY. Extension of multiple range tests to group correlated adjusted means. Biometrics. 1957; 13:13-17.
8. Panda AK, Reddy MR, Rama Rao SV, Raju MVLN, Paraharajj NK. Growth, carcass characteristics, immunocomponence and response to *Escherchia coli* of broiler fed diets with various level of probiotic. Archive fur geflugelkunde. 2000; 64:152-156.
9. Rahman MS, Mustari A, Salauddin M, Rahman MM. Effects of probiotics and enzymes on growth performance and haematobiochemical parameters in broilers. J. Bangladesh Agril. Univ. 2013; 11(1):111-118.
10. Sacks DB. Carbohydrate. In: Tietz Textbook of Clinical Chemistry. 3rd ed. W. B. Saunders Company, USA, 1998.
11. Shareef AM, Al-Dabbagh ASA. Effect of probiotic (*Saccharomyces cerevisiae*) on performance of broiler chicks. Iraqi J Vet. Sci. 2009; 23:23-29.
12. Snedecor GW, Cochran WB. Statistical Methods. 8th ed. The Iowa State University Press, Ames, IOWA, USA, 1994.
13. Stef, L, Julean C, Cean A, Mot D, Stef DS, Simiz E, et al. Evaluation of the Administration effect of Probiotic against Campylobacter Jejuni on the immune system of broiler chickens. Animal Science and Biotechnologies, 2016, 49(1).
14. Stein A, Mayer GL. Clin. Chem. 1995; 41:1421-1426.
15. Tietz NW. Fundamentals of Clinical Biochemistry. 3rd ed. W. B. Saunders Company USA, 1998.