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Evaluation of mineral mixture, Probiotics, enzymes and acidifiers effect on serum biochemistry of broiler chickens

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ABSTRACT

The present study was conducted to evaluate the effects of dietary supplementation with organic mineral mixtures, probiotics, enzymes, and acidifiers on the serum biochemical parameters of broiler chickens. A total of 150 day-old straight-run VenCobb broiler chicks were randomly assigned to five treatment groups (T0–T4) with three replicates of 10 chicks each. The groups included a control (T0) receiving only a basal diet, while T1 to T4 were supplemented with organic mineral mixture (0.50 g/kg), probiotics (1.0 g/kg), enzymes (0.50 g/kg), and acidifiers (1.0 g/kg), respectively. The birds were reared under uniform management conditions for 42 days. At the end of the trial, serum samples were analyzed for glucose, total cholesterol, triglycerides, urea, creatinine, and BUN/creatinine ratio using commercial diagnostic kits. The results revealed a significant reduction ($p \le 0.05$) in serum glucose, cholesterol, and triglyceride levels in all supplemented groups compared to the control. The enzyme-supplemented group (T3) exhibited the most pronounced reduction in lipid parameters. Urea and creatinine concentrations were significantly lower in the supplemented groups, with the lowest creatinine observed in T3, indicating improved protein metabolism and renal function. The BUN/creatinine ratio was also significantly reduced in all treatment groups, reflecting enhanced nitrogen utilization. These findings suggest that supplementation with organic minerals, probiotics, enzymes, and acidifiers positively modulate the serum biochemical profile of broiler chickens, indicating improved metabolic efficiency and physiological health.

1. Introduction

Poultry production has emerged as one of the fastest-growing sectors in animal agriculture, largely due to its high efficiency in converting feed into animal protein (Erdaw and Beyene, 2022). However, the increasing demand for safe, sustainable, and antibiotic-free poultry products has prompted researchers and industry stakeholders to explore alternative strategies to improve growth performance, gut health, and overall physiological status of broiler chickens

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(Bist et al., 2024). Nutritional modulation through dietary supplementation has become an effective tool to enhance productivity while maintaining health and welfare (Celi et al., 2017). Among the various additives used, mineral mixtures, probiotics, enzymes, and acidifiers have gained considerable attention due to their multifaceted benefits on metabolism, immunity, and nutrient utilization (Mondal et al., 2024). Minerals are essential micronutrients required for numerous physiological functions, including bone development, enzymatic activities, and maintenance of acid-base balance (Akram et al., 2020). Imbalances or deficiencies in mineral intake can lead to poor performance and increased susceptibility to disease (Kiani et al., 2022). Mineral mixtures in poultry diets aim to ensure adequate supply of macro- and micro-minerals, optimizing metabolic functions reflected through various serum biochemical indicators such as calcium, phosphorus, and liver enzyme activities (Jamali et al., 2022). Probiotics, defined as live microorganisms that confer health benefits when administered in adequate amounts, play a crucial role in maintaining gut microbial balance (Sanders et al., 2018). In poultry, probiotics such as Lactobacillus, Bifidobacterium, and Bacillus species have been widely used to improve gut integrity, reduce pathogenic load, and enhance nutrient absorption (Krysiak et al., 2021). These effects can influence systemic metabolism, reflected in serum biochemical parameters including total protein, albumin, glucose, and lipid profile (Khabirov et al., 2022). Enzymes, particularly exogenous feed enzymes like phytase, protease, and xylanase, are incorporated into broiler diets to improve digestibility of nutrients by breaking down anti-nutritional factors present in feed ingredients (Sureshkumar et al., 2023). Improved nutrient assimilation not only enhances growth performance but also influences the biochemical profile by optimizing metabolic energy and protein utilization (Musigwa et al., 2021). Acidifiers, including organic acids like citric acid, fumaric acid, and formic acid, have been used to lower gastrointestinal pH, thereby inhibiting the proliferation of harmful bacteria and promoting beneficial microbial flora (Sardar et al., 2020). The acidified environment enhances the activity of digestive enzymes and improves mineral solubility, which in turn can affect systemic biochemical markers such as blood urea nitrogen, liver enzymes, and electrolytes (Dima et al., 2024). Despite the growing use of these additives, there is limited comparative information available on their individual and combined effects on serum biochemistry in broiler chickens (Żbikowski et al., 2020). Serum biochemical parameters serve as valuable indicators of nutritional status, metabolic function, and overall health, thus providing insights into the physiological impact of dietary interventions (Picó et al., 2019; Abd El-Hack et al., 2025). Understanding how different feed additives influence these parameters can help optimize nutritional strategies for better productivity and welfare in poultry. Therefore, the present study was undertaken to evaluate the effects of dietary supplementation with mineral mixture, probiotics, enzymes, and acidifiers on serum biochemical parameters in broiler chickens. The findings from this study aim to provide scientific evidence on the efficacy of these additives in promoting physiological health and improving metabolic efficiency in broilers under standard commercial rearing conditions.

2. Materials and methods

2.1. Ethical committee clearance

The experiment was conducted between March 2021 to April 2021 at Instructional Poultry farm, college of veterinary and Animal Science, Pantnagar. The experimental work was approved by the Institutional Animal Ethics Committee, Vide No. "IAEC/CVASc/LPM/438 dated February 25, 2020 and conducted according to guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

2.2. Experimental animals

A total of 150 day-old straight-run VenCobb broiler chicks, comprising both males and females, were procured for the experiment. Each chick was individually weighed, and then randomly assigned to five treatment groups, with each group consisting of three replicates of 10 chicks. The broiler chicks were reared under a deep litter system with adequate lighting and ventilation throughout the study period. The ambient temperature of the poultry house was carefully regulated to ensure optimal conditions. A 4–5 cm thick layer of rice husk was used as bedding material on the floor. All chicks were vaccinated according to the recommended vaccination schedule. Uniform housing and management practices were maintained across all treatment groups. Fresh drinking water and feed specific to each treatment group were provided ad libitum for the duration of the experiment.

2.3. Experimental design

The study was conducted using a completely randomized design (CRD), incorporating the supplementation of organic mineral mixture, probiotics, enzymes, and acidifiers. A total of 150 day-old broiler chicks were utilized and randomly distributed into five treatment groups, each comprising three replicates with 10 chicks per replicate. The first treatment group (T0) served as the control, receiving only the basal diet without any supplementation. In treatment group T1, a blend of organic mineral mixture (Vannamin®) was included in the feed at a rate of 0.50 g/kg. Treatment T2 involved the addition of probiotics (Biofav GTH®) at 1.0 g/kg of feed, while treatment T3 received a cocktail of enzymes (Intelzyme®) at 0.50 g/kg. In treatment T4, an acidifier (Zaracid DS®) was supplemented at 1.0 g/kg of feed.

2.4. Samples collection

The supplements were fed from 1 to 42nd day. At the end of feeding trial on 42nd day, 6 birds from each treatment were randomly selected for collection of blood. Serum was separated from the blood for analysis of biochemical parameters.

2.5. Serum biomarkers profile

Serum separated from the collected blood samples was utilized for the analysis of serum enzymes. Commercial diagnostic kits from Erba Diagnostics Mannheim Ltd., Baddi, Himachal Pradesh were employed to estimate serum Glucose, Total cholesterol, Triglycerides, Urea, Creatinine, and BUN/Creatinine ratio following the instructions provided in the kit protocol.

2.6. Statistical analysis

The experimental data from the study were statistically analyzed using a completely randomized design (CRD) and the analysis of variance (ANOVA) method, following the F-test (variance ratio) approach as described by Snedecor and Cochran (1994). The analysis was performed using the one-way ANOVA function within the General Linear Model (GLM) framework of SPSS software version 16.0 (2008).

3. Results

The effects of different dietary treatments on the serum biochemical parameters of broiler chickens are presented in the Table-1. Glucose levels were significantly higher ($p \leq 0.05$) in the control group (T0: 206.11 mg/dL) compared to all supplemented groups (T1–T4), which exhibited slightly but significantly lower values ranging from 205.14 to 205.52 mg/dL. Total cholesterol and triglyceride concentrations were notably reduced in all treatment groups compared to the control. The control group (T0) had the highest cholesterol (157.56 mg/dL) and triglyceride (98.38 mg/dL) levels, whereas the lowest values were observed in the enzyme-supplemented group T3 (149.84 mg/dL and 94.93 mg/dL, respectively).

Urea and creatinine levels were also significantly influenced by supplementation. The highest urea (9.88 mg/dL) and creatinine (0.95 mg/dL) concentrations were found in the control group, while all supplemented groups (T1–T4) showed reduced levels, with the lowest creatinine observed in group T3 (0.63 mg/dL). Correspondingly, the BUN/Creatinine ratio was significantly higher in the control group (4.62) compared to all other treatments, which ranged between 3.44 and 3.75. These findings suggest that supplementation with organic minerals, probiotics, enzymes, and acidifiers positively influenced the metabolic profile of broilers by lowering serum cholesterol, triglycerides, and markers of nitrogen metabolism, indicating improved renal function and nutrient utilization.

4. Discussion

The present study demonstrates that dietary supplementation with organic mineral mixtures,

Table 1: Effect of organic minerals, probiotics, enzymes, and acidifiers on blood biochemistry in broiler chickens

Treatment Groups	Glucose (mg/dL)	Total cholesterol (mg/dL)	Triglycerides (mg/dL)	Urea (mg/dL)	Creatinine (mg/dL)	BUN/Creatinine ratio
T0	$206.11^{b} \pm 0.16$	$157.56^{a} \pm 0.05$	$98.38^{a} \pm 0.04$	$9.88^{a} \pm 0.00$	$0.95^{a} \pm 0.00$	$4.62^{a} \pm 0.03$
T1	$205.14^{a} \pm 0.07$	$150.74^{b} \pm 0.30$	$95.02^{b} \pm 0.24$	$9.20^{b} \pm 0.11$	$0.82^{b} \pm 0.01$	$3.44^{b} \pm 0.11$
T2	$205.40^{a} \pm 0.17$	$151.57^{b} \pm 0.23$	$95.05^{b} \pm 0.23$	$9.17^{b} \pm 0.05$	$0.73^{bc} \pm 0.01$	$3.44^{b} \pm 0.11$
T3	$205.52^{a} \pm 0.01$	$149.84^{b} \pm 0.76$	$94.93^{b} \pm 0.5$	$9.25^{b} \pm 0.07$	$0.63^{d} \pm 0.00$	$3.60^{b} \pm 0.14$
T4	$205.43^{a} \pm 0.06$	$151.33^{b} \pm 0.17$	$94.99^{b} \pm 0.19$	$9.25^{b} \pm 0.02$	$0.65^{cd} \pm 0.02$	$3.75^{b} \pm 0.09$

Different superscript letters in the same column indicate statistically significant differences ($p < 0.05$)

probiotics, enzymes, and acidifiers significantly influence the serum biochemical profile of broiler chickens, indicating improvements in metabolic health and nutrient utilization. The observed reduction in serum glucose levels in all supplemented groups compared to the control, though marginal, was statistically significant. This suggests that the inclusion of feed additives may improve glucose homeostasis, possibly by enhancing nutrient absorption and metabolic efficiency (Lin et al., 2024). Similar findings have been reported by earlier studies, where feed additives improved glycemic control through better gut health and enzyme activity, leading to efficient carbohydrate metabolism (Lee et al., 2024). Total cholesterol and triglyceride levels were significantly reduced in all treated groups, with the most pronounced effect observed in the enzyme-supplemented group (T3). This indicates that exogenous enzyme supplementation may play a pivotal role in lipid metabolism, possibly by enhancing fat digestion and absorption (Gao et al., 2021). Probiotics and acidifiers, which are known to modulate gut microbiota, may also contribute to improved lipid profiles by reducing intestinal synthesis and absorption of cholesterol (Wu et al., 2022). These results are in agreement with previous findings that reported a hypocholesterolemic effect of probiotics and enzymes in poultry diets (Sedghi et al., 2024). A notable decline in serum urea and creatinine levels across all treatment groups compared to the control suggests improved protein metabolism and renal function (Abd El-Hack et al., 2025). The lowest creatinine level observed in the enzyme-supplemented group (T3) further supports the role of feed enzymes in improving nitrogen utilization and reducing metabolic waste (Lian et al., 2024). Urea levels followed a similar trend, indicating reduced protein catabolism and enhanced amino acid assimilation in supplemented birds (Liang et al., 2023). These findings align with the reports of better nitrogen retention and kidney function in broilers fed diets containing nutritional additives such as probiotics and enzymes (Such et al., 2023). The BUN/Creatinine ratio, a reliable indicator of renal function and protein metabolism, was significantly lower in all treatment groups than in the control. This indicates that supplementation contributed to a more favorable metabolic state, with better utilization of dietary protein and reduced renal burden (Ghanima et al., 2023). The lowest ratio in the T1 and T2 groups further supports the beneficial effects of organic mineral mixtures and probiotics in maintaining metabolic efficiency (Selim et al., 2022). These findings underscore the positive impact of targeted nutritional interventions on the physiological health of broilers. The improved biochemical parameters suggest enhanced nutrient assimilation, optimized metabolic processes, and potential alleviation of stress on vital organs such as the liver and kidneys. These results reinforce the utility of feed additives not only as performance enhancers but also as tools to promote systemic health in poultry.

5. Conclusion

The findings of the present study clearly indicate that dietary supplementation with organic mineral mixtures, probiotics, enzymes, and acidifier has a significant positive impact on the serum biochemical profile of broiler chickens. All supplemented groups demonstrated improved metabolic indicators compared to the control, including reduced levels of glucose, total cholesterol, triglycerides, urea, and creatinine, along with a lower BUN/creatinine ratio. Among the treatments, enzyme supplementation showed the most pronounced improvements in lipid and nitrogen metabolism. These results suggest enhanced nutrient absorption, better protein utilization, and improved renal function in birds receiving dietary additives. Overall, the study supports the strategic use of feed supplements as an effective means to promote physiological health, metabolic efficiency, and potentially better growth performance in broiler chickens, offering a viable alternative to antibiotic-based growth promoters in modern poultry production.

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Conflicts of interest and financial disclosures

The authors state that there are no conflicts of interest to disclose.

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