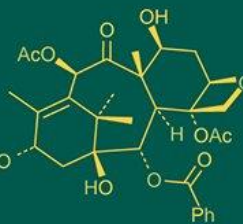
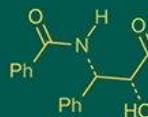
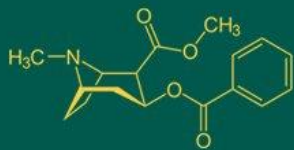


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Seasonal and nutritional modulation of serum gamma-glutamyl transferase, calcium and phosphorus in broiler chickens

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Abstract

The present investigation was conducted to evaluate the influence of season and dietary plane of nutrition on selected blood biochemical parameters of broiler chickens. A total of 480-day-old commercial broiler chicks were used in two independent seasonal feeding trials, comprising 240 birds during winter and 240 birds during summer. In each season, birds were randomly allotted to four dietary treatments (T₁, T₂, T₃ and T₄), with four replications of 15 birds each, following a Randomized Block Design. The control treatment (T₁) received standard protein and standard energy (SPSE) diets according to ICAR specifications. T₂ received a diet containing 1% lower crude protein with xylanase (GH11) and serine protease supplementation; T₃ received a diet with 100 kcal/kg lower metabolizable energy with the same enzyme supplementation; and T₄ received a diet with both 1% lower crude protein and 100 kcal/kg lower metabolizable energy along with xylanase and serine protease. Serum gamma-glutamyl transferase (GGT), calcium and phosphorus were evaluated at different stages of growth. The results showed clear numerical differences among dietary treatments, particularly during the later growth stages. GGT values generally declined with increasing nutritional modification, with T₄ showing the lowest values during several sampling stages. In winter, GGT declined from 11.0 U/L in T₁ to 9.02 U/L in T₄ at day 42, whereas in summer the corresponding values were 11.99 and 9.53 U/L. Serum calcium generally increased with dietary modification, particularly in T₃ and T₄. At day 42 during summer, calcium increased from 9.61 mg/dL in T₁ to 10.63 mg/dL in T₄. Phosphorus also showed a favourable numerical response to nutritional modification. Overall, the findings indicate that modification of dietary protein and energy levels with enzyme supplementation may influence hepatic enzyme activity and mineral metabolism in broiler chickens under contrasting seasonal conditions.

Keywords: Broiler chicken, blood biochemistry, GGT, calcium, phosphorus, season, dietary nutrition, xylanase, serine protease

1. Introduction

Blood biochemical parameters provide useful information about the physiological and metabolic condition of poultry and can reflect nutritional adequacy, hepatic function and mineral homeostasis. Among the biochemical indicators, gamma-glutamyl transferase (GGT), calcium and phosphorus are important parameters associated with metabolic health. Gamma-glutamyl transferase is a membrane-associated enzyme involved in the metabolism of glutathione and is used as an indicator of hepatic and metabolic status. Changes in GGT activity may occur in response to environmental stress, nutritional imbalance and alterations in hepatic function. Calcium is an essential mineral involved in skeletal development, neuromuscular transmission, enzyme activation and several other physiological processes. Phosphorus works closely with calcium in bone mineralization and is also essential for energy metabolism and enzymatic reactions. The bioavailability of phosphorus may be influenced by dietary composition, phytate and phytase activity.

Seasonal environmental conditions can modify feed intake, nutrient utilization and metabolic responses of broiler chickens. High ambient temperature during summer may increase physiological stress and alter nutrient and mineral metabolism. Conversely, comparatively favourable environmental conditions during winter may support better nutrient utilization and metabolic stability.

Dietary manipulation of crude protein and metabolizable energy, particularly when combined with exogenous enzymes such as xylanase and serine protease, may improve nutrient utilization and influence biochemical responses. The present study was therefore undertaken to evaluate the effect of different planes of dietary protein and energy, with enzyme supplementation, on selected blood biochemical parameters of broiler chickens during winter and summer seasons.

The specific objective was to study the response of GGT, calcium and phosphorus under four dietary treatments at different stages of broiler growth.

2. Materials and Methods

2.1 Experimental site and duration

The experiment was conducted at the Experimental Poultry Shed of the Department of Animal Husbandry, Sam Higginbottom University of Agriculture, Technology and Sciences (SHUATS), Prayagraj, Uttar Pradesh, India. Two feeding trials of 42 days each were conducted separately during winter and summer seasons.

2.2 Experimental birds and design

A total of 480-day old commercial broiler chicks were procured from a reliable local hatchery. For each season, 240 chicks were randomly distributed into four dietary treatments (T₁, T₂, T₃ and T₄), with 60 birds per treatment and four replications of 15 birds each. The experiment followed a Randomized Block Design.

2.3 Dietary treatments

The basal ration was formulated for pre-starter, starter and finisher phases according to ICAR (2013) nutrient specifications. The experimental treatments were:

T₁-SPSE: Standard Protein and Standard Energy diet according to ICAR specifications.

T₂-LPSE: Diet containing 1% lower crude protein than the ICAR specification, supplemented with xylanase (GH11) and serine protease at the recommended dose.

T₃-SPLE: Diet containing 100 kcal/kg lower metabolizable energy than the ICAR specification, supplemented with xylanase (GH11) and serine protease.

T₄-LPLE: Diet containing 1% lower crude protein and 100 kcal/kg lower metabolizable energy than the ICAR specification, supplemented with xylanase (GH11) and

serine protease.

The basal diets contained maize, soybean meal, mustard deoiled cake, meat and bone meal, vegetable oil, amino acids, vitamin-mineral premix and other feed additives. The basal diet contained 22.0, 21.5 and 19.5% crude protein and 3000, 3050 and 3100 kcal/kg metabolizable energy during pre-starter, starter and finisher phases, respectively.

2.4 Feeding and management

The feeding trial was conducted for 42 days in each season. Feed offered and residual feed were recorded during the experimental period. Birds were maintained in a well-ventilated experimental poultry shed, and clean drinking water was supplied ad libitum. A standard vaccination schedule was followed.

2.5 Blood sample collection

Blood samples were collected from 10 birds from each group through the wing vein and heart at the initial stage and at the end of the pre-starter, starter and finisher phases during both seasons.

2.6 Estimation of biochemical parameters

The blood biochemical parameters evaluated in the present study were GGT, calcium and phosphorus.

GGT activity was estimated using an enzymatic kinetic assay on a biochemical autoanalyzer and expressed in U/L. Calcium concentration was estimated by the o-cresol phthalein complex one (OCPC) method and expressed in mg/dL. Serum phosphorus was determined by the molybdate UV method and expressed in mg/dL.

2.7 Statistical analysis

Experimental data were statistically analysed using SPSS software (version 20). Analysis of variance (ANOVA) was conducted according to the procedures described by Snedecor and Cochran (2004) under the Randomized Block Design. Mean and standard error were calculated for treatment comparisons.

3. Results

3.1 Serum GGT

GGT showed a progressive numerical decline with the modified nutritional treatments in both seasons. At the beginning of the winter trial, GGT values were similar among treatments, ranging from 12.98 U/L in T₁ to 13.01 U/L in T₃ and T₄. The treatment effect was reported as non-significant.

Table 1: Serum GGT (U/L) in broiler chickens under different dietary treatment during winter and summer.

Season	Day	T ₁	T ₂	T ₃	T ₄
Winter	0	12.98 ± 0.017	13.00 ± 0.032	13.01 ± 0.019	13.01 ± 0.009
	14	12.14 ± 0.013	11.19 ± 0.032	10.73 ± 0.018	9.52 ± 0.017
	25	11.60 ± 0.025	10.66 ± 0.021	10.31 ± 0.021	9.25 ± 0.022
	42	11.00 ± 0.025	10.25 ± 0.011	9.8 ± 0.031	9.03 ± 0.028
Summer	0	12.96 ± 0.027	13.00 ± 0.031	12.97 ± 0.041	13.01 ± 0.019
	14	13.28 ± 0.042	12.11 ± 0.042	11.54 ± 0.023	10.15 ± 0.013
	25	12.60 ± 0.010	11.66 ± 0.034	11.12 ± 0.021	9.82 ± 0.023
	42	11.99 ± 0.032	11.20 ± 0.031	10.73 ± 0.025	9.53 ± 0.015

Values are expressed as Mean ± SE.

T₁ = Standard Protein Standard Energy (SPSE); T₂ = Low Protein Standard Energy (LPSE); T₃ = Standard Protein Low Energy (SPLE); T₄ = Low Protein Low Energy (LPLE).

By day 14 of the winter trial, GGT values were 12.14, 11.19, 10.73 and 9.52 U/L in T₁, T₂, T₃ and T₄, respectively. At day 25, corresponding values were 11.60, 10.66, 10.31 and 9.25 U/L. By day 42, GGT declined further to 11.00, 10.25, 9.81 and 9.02 U/L in T₁, T₂, T₃ and T₄, respectively. The reported ANOVA results for these stages were non-significant, despite the clear numerical treatment differences.

A similar pattern was observed during summer. At day 14, mean GGT values were 13.28, 12.11, 11.54 and 10.15 U/L

in T₁-T₄, respectively. At day 25, values were 12.60, 11.66, 11.12 and 9.82 U/L. At day 42, the corresponding values were 11.99, 11.20, 10.73 and 9.53 U/L. Thus, T₄ consistently recorded the lowest numerical GGT value, followed generally by T₃.

3.2 Serum calcium

Serum calcium demonstrated a generally favourable numerical response to dietary modification.

Table 2: Effect of dietary treatments on serum calcium (mg/dL) of broiler chickens during winter and summer

Season	Day	T ₁	T ₂	T ₃	T ₄
Winter	0	9.50 ± 0.013	9.54 ± 0.019	9.50 ± 0.019	9.46 ± 0.002
	14	9.52 ± 0.010	9.78 ± 0.011	10.14 ± 0.027	10.57 ± 0.015
	25	9.74 ± 0.039	10.02 ± 0.027	10.39 ± 0.014	10.68 ± 0.017
	42	9.94 ± 0.018	10.20 ± 0.037	10.58 ± 0.028	10.74 ± 0.028
Summer	0	9.48 ± 0.037	9.47 ± 0.011	9.50 ± 0.042	9.49 ± 0.030
	14	9.28 ± 0.016	9.55 ± 0.033	9.83 ± 0.032	10.14 ± 0.015
	25	9.42 ± 0.023	9.79 ± 0.038	10.03 ± 0.017	10.38 ± 0.042
	42	9.61 ± 0.012	9.86 ± 0.021	10.27 ± 0.017	10.63 ± 0.013

Values are expressed as Mean ± SE.

T₁ = Standard Protein Standard Energy (SPSE); T₂ = Low Protein Standard Energy (LPSE); T₃ = Standard Protein Low Energy (SPLE); T₄ = Low Protein Low Energy (LPLE).

During winter, calcium at day 0 ranged from 9.46 to 9.54 mg/dL and the treatment effect was reported as non-significant. By day 14, calcium increased from 9.52 mg/dL in T₁ to 10.57 mg/dL in T₄, with T₃ recording 10.14 mg/dL. During summer, the numerical advantage of the modified treatments was also evident. At day 25, calcium values were 9.42, 9.79, 10.03 and 10.38 mg/dL in T₁, T₂, T₃ and T₄, respectively. At day 42, calcium increased from 9.61 mg/dL in T₁ to 10.63 mg/dL in T₄, while T₂ and T₃ recorded 9.86 and 10.27 mg/dL, respectively.

3.3 Serum phosphorus

Serum phosphorus also exhibited numerical differences among dietary treatments.

Table 3: Effect of dietary treatments on serum phosphorus (mg/dL) of broiler chickens during winter and summer season.

Season	Day	T ₁	T ₂	T ₃	T ₄
Winter	0	5.21 ± 0.037	5.18 ± 0.026	5.18 ± 0.029	5.14 ± 0.025
	14	5.24 ± 0.032	5.51 ± 0.027	5.84 ± 0.038	6.15 ± 0.012
	25	5.44 ± 0.013	5.68 ± 0.016	6.01 ± 0.020	6.31 ± 0.029
	42	5.59 ± 0.002	5.87 ± 0.027	6.20 ± 0.039	6.52 ± 0.005
Summer	0	5.22 ± 0.029	5.20 ± 0.019	5.26 ± 0.012	5.15 ± 0.012
	14	5.07 ± 0.018	5.32 ± 0.015	5.67 ± 0.014	5.92 ± 0.026
	25	5.26 ± 0.027	5.54 ± 0.041	5.86 ± 0.029	6.13 ± 0.031
	42	5.42 ± 0.022	5.75 ± 0.021	6.02 ± 0.012	6.27 ± 0.055

Values are expressed as Mean ± SE.

T₁ = Standard Protein Standard Energy (SPSE); T₂ = Low Protein Standard Energy (LPSE); T₃ = Standard Protein Low Energy (SPLE); T₄ = Low Protein Low Energy (LPLE).

During winter at day 0, treatment means were approximately 5.21, 5.18, 5.18 and 5.14 mg/dL for T₁, T₂, T₃ and T₄, respectively, and the treatment effect was reported as non-significant. At day 14, phosphorus values increased progressively from 5.24 mg/dL in T₁ to 6.15 mg/dL in T₄.

The overall seasonal summary in the thesis indicates that phosphorus showed treatment-associated differences across the later sampling stages, with T₃ and T₄ generally showing

more favourable numerical profiles than the control. The reported summary indicates significant treatment effects at days 14, 25 and 42 during both seasons, while the day-0 effect was non-significant in winter and significant in summer.

3.4 Overall biochemical response

The combined interpretation of the biochemical results indicated that T₃ and T₄ generally produced more favourable biochemical profiles than T₁. The thesis reported lower GGT and improved mineral profiles in the modified dietary treatments. Winter was generally associated with a more favourable biochemical condition, whereas summer was associated with greater metabolic stress.

4. Discussion

The present study demonstrated that modifying dietary protein and energy levels, in combination with exogenous enzymes, was associated with numerical changes in serum biochemical parameters of broiler chickens. The most consistent pattern was the reduction in GGT under T₃ and particularly T₄ compared with the standard diet.

GGT is associated with hepatic and metabolic function. The lower numerical GGT values observed in the modified nutritional groups may indicate a comparatively favourable metabolic response to the altered nutrient plane. The effect was particularly apparent after the initial stage of growth. For example, during winter at day 42, GGT declined from 11.00 U/L in T₁ to 9.02 U/L in T₄. A comparable pattern was observed during summer, where GGT decreased from 11.99 U/L in T₁ to 9.53 U/L in T₄.

The numerical response of calcium was different from that of GGT. Calcium tended to increase with nutritional modification, particularly under T₃ and T₄. The increase was prominent during the later stages of growth. During summer at day 42, T₄ recorded 10.63 mg/dL compared with 9.61 mg/dL in the control treatment. This pattern may reflect improved mineral utilization associated with the modified nutrient plane and enzyme supplementation, although the

present data should be interpreted within the statistical significance reported in the thesis.

Phosphorus followed a generally similar nutritional trend. During winter at day 14, the phosphorus concentration increased from 5.24 mg/dL in T₁ to 6.15 mg/dL in T₄. Phosphorus metabolism is closely associated with calcium metabolism and dietary mineral utilization. The presence of phytase in the experimental diets may also be relevant to mineral availability, although the present study did not isolate the effect of phytase from the other dietary modifications.

Seasonal conditions also appeared to influence the biochemical response. The thesis interpretation suggests that summer conditions were associated with higher GGT and comparatively lower mineral values, whereas winter conditions showed a more favourable biochemical profile. Such seasonal variation may reflect differences in environmental stress and nutrient utilization. However, the present experiment was designed to evaluate the combined effect of season and dietary treatments rather than to establish a specific physiological mechanism.

The results are broadly consistent with the references already cited in the thesis, including Holsheimer and Veerkamp (1992) and Chattopadhyay and Mondal (2006), which were used in the thesis to support the interpretation that balanced nutrition can contribute to biochemical stability.

An important observation is that the numerical differences among treatments were not uniformly statistically significant. Therefore, the numerical superiority of T₃ and T₄ should not be interpreted as a statistically proven treatment effect for every sampling stage. The condensed ANOVA summary in the thesis indicates that significant responses occurred at several later sampling stages, while someday-0 comparisons remained non-significant.

5. Conclusion

The present study demonstrated that seasonal conditions and dietary plane of nutrition were associated with changes in selected blood biochemical parameters of broiler chickens. Dietary treatments involving reduced protein and/or energy levels with xylanase and serine protease supplementation generally produced more favourable numerical biochemical profiles than the standard diet.

GGT showed a consistent numerical reduction in T₃ and particularly T₄ during the later stages of growth. In contrast, serum calcium and phosphorus generally showed improved numerical values under the modified nutritional treatments. The response was evident in both winter and summer, although seasonal differences in biochemical status were also observed.

Overall, the findings suggest that strategic modification of dietary protein and metabolizable energy, together with enzyme supplementation, may influence hepatic enzyme activity and mineral metabolism in broiler chickens. However, treatment effects should be interpreted according to the statistical significance reported for each sampling stage.

6. Practical Implication

The findings may be useful in developing season-specific nutritional strategies for broiler production. The use of enzyme-supported diets with carefully adjusted protein and energy levels could potentially support metabolic stability

while reducing dependence on conventional nutrient density. Further studies involving larger sample sizes and factorial statistical analysis of season × diet interactions would be useful to establish the independent and interactive effects of environmental and nutritional factors.

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