Effect of *Psidium guajava* leaf meal supplementation on production performance, biochemical profile and immune response of broiler chickens

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Effect of PGLM on performance of broilers

Abstract: Study was carried out to investigate the effect of *Psidium guajava* leaf meal (PGLM) supplementation on production performance, biochemical profile and immune response of broiler chickens. Two hundred and forty unsexed day old broiler chicks (average body weight 49.50± 0.25 g) were procured from local hatchery and randomly divided into 4 dietary treatments (T_0, T_1, T_2 and T_3), with 4 replicates of 15 chicks in each deep litter pen with similar managerial conditions in a completely randomized block design for a period of 42 days. T_0 group was fed basal diet, while T_1, T_2 and T_3 groups were fed basal diet supplemented with PGLM @ 1.5, 2.5 and 3.5 percent, respectively. Three phase feeding (pre-starter, starter and finisher) was followed for feeding of experimental chicks. Chicks were provided feed and water *ad-libitum* throughout the feeding trial. Production performance viz. body weight gain (g), feed (g) and water (ml) intakes and feed conversion ratio (FCR) were measured weekly on bird bases, however, carcass characteristics, biochemical profiles and economics were determined at the end of the feeding trial. Humoral and cell mediated immune responses were assessed between 4th to 5th weeks of age. Feed (g) and water (ml) intake, body weight gain (g) and FCR were significantly ($P<0.05$) higher in T_1, T_2 and T_3 groups as compared to T_0 group. PGLM supplementation significantly ($P<0.001$) reduced blood glucose, cholesterol and triglyceride levels and improved globulin level in T_1, T_2 and T_3 groups compared to T_0 group. PGLM supplementation did not exert any adverse effect on carcass characteristics. Though, PGLM supplementation significantly ($P<0.001$) reduced abdominal fat content and improved net return. PGLM supplementation (upto 3.5%) in broilers' diets is cost effective, environmental friendly, natural alternative production performance enhancer, immune-modulator without affecting carcass characteristics and produce low cholesterol healthy broiler meat.

Keywords: Broilers, carcass characteristics, immunity, net profit, *Psidium guajava* leaf meal

Introduction

Broiler production is one of the fast-growing segments of agribusiness in India, with an annual growth rate of about 6 percent. Chicken production appears to be bright but main hurdles to future growth are high feed cost, morbidity and mortality caused by various diseases, ban on use of synthetic feed additive as growth promoters due to development of drug resistance and their residual effect in the food chain and increased demand for organic low fat meat [1-2]. Importance of maize grain as feed ingredient for livestock in many parts of the world is well documented. However, day to day hike in price, restrict its use in the ration, which is one of the most important components, accounting 70% of total cost of production, which will further increase total production cost and thus decrease the profit margin of broiler farming [3]. Attempt has been made to reduce the feed cost by incorporating locally available unconventional feed resources (leaf meal; LM) in broiler diets [2, 4].

The LM and leaf meal mixture (LMM) as feed ingredients in broiler rations have been incorporated by Abdulsalam et al. [5], Aroche et al. [6], Daing et al. [7] and Zargar et al. [8]. It has been reported that LM/ LMM of tropical trees are potential sources of condensed tannins (CT) [7-8]. It is water soluble poly-phenolic compounds that are beneficial for health of livestock including poultry in small amount [2, 7]. Incorporation of LM/LMM in the ration of broiler chickens may not only reduce feed cost, but also elicit health-promoting effect [7-8]. Despite their benefits, use of LM/LMM in broiler ration may be limited due to high content of crude fibre (CF) and CT [2, 7-9]. Generally, broilers showed low tolerance to CF and CT, and therefore feeding diets containing higher levels of LM/ LMM may restrict their use in broilers' ration.

Applications of LM and LMM as feed ingredients in the ration have been reported to improve health status and physiological conditions of broilers. Hence, they are having great potential for use as natural alternative to synthetic feed additives for broiler chicken production. Presence of CT in LM/ LMM has been reported to promote the immune organ development as well as stimulate immune response [10-11]. It may also serve as antibacterial [1], anthelmintics [11], anticoccidial [12], and antioxidants [1, 11-12] that eventually protect the immune system from more dangerous free radicals [1, 11-12]. With regard to CT, although at higher level it may act as anti-nutritional factors, but at lower level it has been reported to improve performance and immune response of broiler chickens [2, 13].

Among locally available tropical tree leaves, *Psidium guajava* leaf meal (PGLM) is a good source of CT as well as showing nutritional and functional feed potential in livestock and poultry [1-2, 10-13]. Keeping all these points in view, the present study was planned to investigate the effect of PGLM as functional feed on production performance, biochemical profiles, immune response, carcass characteristics and economics of broiler chickens.

Materials and Methods

This study was carried out at Division of Animal Nutrition, Faculty of Veterinary Sciences and Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, India. It was planned and conducted as per approved protocols of Animal Nutrition Division and the prescribed guidelines of Institute Animal Ethics Committee (Permitted Number: 12/IAEC 2020). Before the start of this study we got ethical clearance from the committee for care and supervision of experimental study on birds (CPCSEA).

Collection and processing of *Psidium guajava* leaves

Fresh *Psidium guajava* leaves were harvested directly from the branches of trees within the faculty premises, R. S. Pura, Jammu. Leaves were air-dried in the shed for 30 days after ensuring perfect drying, milled in electric grinder. The PGLM was stored in cool and dry place until used for feeding of chicks. A representative sample of PGLM was brought to laboratory for nutritional analysis and presence of CT content.

Experimental birds, design, feeding and management

Experimental birds' care and approved procedures were used for conducting research work. A total of 240 day-1 old unsexed broiler chicks (Cobb-K strain) weighing 49.50± 0.25 g were procured from Kashmir Valley Poultry breeding farms and hatcheries, Jammu. They were used in a study which lasted for 42 days. They were housed in 16 deep litter pens with rice husk as litter material. Before arrival of chicks, the pens were cleaned, washed and disinfected using standard protocol. Temperature and relative humidity of poultry house were maintained at 32^0C and 68-70 percent, respectively with the provision of 1.3 Sq. ft. floor space per bird, 23 hrs light and 1 hr darkness. The poultry house temperature was maintained 32^0C for first 7 days followed by every 2^0C temperature fall per week till the end of feeding trial. To ensure clean bedding material at all times, rice husks were spread regularly at weekly interval.

The chicks were divided into 4 groups of 60 chicks each. Each treatment group was further subdivided into 4 replicates of 15 chicks each in a completely randomized block design (CRD). Chicks were assigned to 0%, 1.5%, 2.5% and 3.5% PGLM of diets representing treatments T_0, T_1, T_2 and T_3 respectively. Chicks in each pen were provided *ad libitum* feed and water. A three phase feeding (Pre-starter, 1-14 days of age; Starter, 15-21 days of age and Finisher, 22-42 days of age) was followed for feeding of experimental chicks as per ICAR [14] nutrient requirements. For formulation and preparation of basal diets, all feed ingredients were procured from local market of Jammu except PGLM, which was processed in our division. PGLM was mixed manually with basal diet at the rate of 1.5, 2.5 and 3.5 percent of feed in T_1, T_2 and T_3 groups, respectively, of all the three phases, while, T_0 group served as control (0 % PGLM) which was fed only the basal diet.

Measurement and sample analysis

Bird performance viz. body weight gain (g), feed (g), water intake (ml) and feed conversion ratio (FCR) were determined weekly on bird bases. All chicks were weighed at the start of the experiment (day 1) and weekly thereafter. Chicks of each pen were weighed individually with a digital weighing scale and then the previous live weights were subtracted from the current live weights to calculate body weight gain. Measured amount of respective feed and water was offered to the experimental birds replicate wise. Average daily feed and water intakes per bird were recorded at weekly interval by dividing number of days and birds from the total amount of feed and water after subtracting the residual amounts. The FCR was calculated by dividing the feed consumed by the live weight gain. Samples of respective feeds, residues and PGLM were brought to the laboratory for estimation of chemical composition as per AOAC [15] and CT content as per butanol Hcl method [16].

Blood collection and analysis

Blood samples were collected from 32 birds (2 from each replicate) at the end of feeding trial of 42 days. About 4 ml of blood from slaughtered birds was collected in sterile centrifuge tubes without anti-coagulant for separation of serum to determine various biochemical profiles and humoral immune response. All the serum samples were stored in a deep freeze at – 20 ^{0}C until processed for analysis. Biochemical profiles such as glucose, cholesterol, triglycerides, total serum protein and albumin levels were determined by using readymade diagnostic kits (Erba Diagnostics Mannheim GmbH, Germany). Glucose (mg/dl) was estimated by glucose-oxidase peroxidise (GOD-POD) end-point assay [17]. Serum cholesterol (mg/dl) and triglyceride (mg/dl) levels were analysed as per the methods described by Roeschlan et al. [18] and Cole et al. [19], respectively. Total serum protein and albumin values were quantified by direct biuret method [20] and bromocresol green dye [21], respectively. However, globulin (g/dl) level was calculated as the difference between total protein and albumin, although, A:G ratio was then determined using albumin and globulin values.

Cell mediated immune (CMI) response

The CMI stimulating activity of CT containing PGLM in broiler diets was assessed through *in vivo* delayed type hypersensitivity (DTH) reaction against phytohaemagglutin-p (PHA-p) as described by Blecha et al. [22] and Kornegay et al. [23]. Towards the beginning of 5^{th} week, foot pad area of 2 birds from each pen were cleaned with sprit containing cotton swab and thickness of foot pad was measured with the help of digital vernier calipers, which would represent the basal (0 hr) value. All tested birds were injected 25 µl of PHA-p intradermally with the help of insulin syringe. Thickness of foot pad was subsequently measured at 24, 36, 48 and 72 hrs post inoculation. The extent of thickness was taken in to consideration to determine CMI response. This was assessed by measuring the hypersensitivity reaction of cutaneous basophills to PHA-p.

Humoral immune response

It was assessed through antibody titre (Haem-agglutination; HA titre $\log_2$ value) against sheep red blood corpuscles (SRBC) in 32 experimental birds, Two birds per pen were selected randomly at 31^{st} day of age. For immunization, 100 µl (0.5 %) SRBC suspension was injected intravenously in wing vein of each tested bird. After 10 days post immunization, blood samples from immunized birds were collected and processed for serum harvesting. Serum samples were heated in water bath at 56 ^{0}C for 30 minutes to inactivate the complement fraction. For determination of antigen-antibody reaction, haem-agglutination assay was performed in 96 well micro titre plates as per method described by Hudson and Hay [24]. Thus, humoral immune response was determined through antibody titre (log_2 values) against SRBCs.

Carcass characteristics and economics

At the end of experimental period (42 days), 8 birds from each group, which had the body weights close to the mean replicate weights, were slaughtered for carcass characteristics evaluation. After complete bleeding by hanging the birds in a head down position, carcass was de-feathered and dressed out. The internal organs were removed from the abdomen and the hot carcass weight was recorded. Weights of internal organs like heart, spleen, kidneys, liver, gizzard, bursa, lungs and weights of cuts up parts, etc. were recorded.

The economics of experimental broiler chickens were evaluated by using cost parameters, return parameters and net profits. The cost parameters included the price of purchased chicks, cost/kg feed, cost of total feed consumed per bird, cost/kg weight gain, revenue from sales, net benefit, cost benefit ratio and cost of feed per bird in relation to total variable costs. The cost was estimated in Indian Rupees (INR) over the course of the study. The cost/kg feed was obtained by adding the cost of procuring the various feed ingredients in a particular treatment and dividing with the total feed consumed in that treatment. Cost of total feed consumed was calculated by multiplying total feed consumed in each treatment with the cost/kg feed. The cost/kg weight gain was obtained by multiplying the cost/kg feed with feed conversion ratio (feed: gain). Net benefit was obtained as revenue less the production cost.

Statistical analysis

Experimental data generated were analyzed using statistical package SPSS 16.0. Results obtained were subjected to analysis of variance and treatment means were ranked using Duncan's multiple range test. Degree of freedom of the treatments was portioned into orthogonal polynomial, depicting linear and quadratic trends associated with increasing levels of CT containing PGLM supplementation. Periodic alterations in feed and water intake, body weight gain, FCR and CMI response were analyzed using General linear model (GLM), Multivariate. Significance was declared at $P<0.05$ unless otherwise stated. All statistical procedures were followed as per Snedecor and Cochran [25].

Results

Experimental diets

Ingredient and chemical compositions of broiler pre-starter, starter and finisher diets are presented in tabular form (Tables 1 and 2), respectively. Nutrient composition viz. organic matter (OM), crude protein (CP), ether extract (EE), crude fibre (CF), acid insoluble ash (AIA), calcium (Ca), phosphorus (P) (% DM bases) and calculated metabolisable energy of experimental diets in all the three phases of T_0, T_1, T_2 and T_3 groups were within the normal range. CT contents of experimental diets of T_0, T_1, T_2 and T_3 were 0.0, 0.098, 0.16 and 0.23 percent, respectively of all three phases.

Productive performance, carcass characteristics and cost benefit analysis

Feed (g) and water intake (ml), body weight gain (g) and feed conversion ratio (FCR) of experimental broiler chickens are presented at weekly interval (Table 3). Mean weight gain (g) was significantly ($P<0.05$) higher in T_3 as compared to T_0, whereas T_1 and T_2 have an intermediate values between T_0 and T_3. The mean body weight gain differ significantly ($P<0.05$) between different periods. The overall mean of feed intake was significantly ($P<0.05$) higher in T_1 and T_3 groups followed by T_2 and the lowest feed intake was recorded in T_0 group. As the age of birds increased the mean feed and water intakes increased significantly ($P<0.05$) from 1st week to 6th weeks. PGLM supplementation significantly improved the FCR. As the PGLM in broiler diets increased (from 0.0 to 3.5 %) overall mean FCR decreased significantly ($P<0.05$) in T_2 and T_3 except T_1 and the highest FCR in T_0 group.

The PGLM supplementation (up to 3.5 % of diet) did not exert any adverse effect on carcass traits (dressing percentage, weight of cut up parts, abdominal fat, immunological and visceral organs) except abdominal fat content (Table 4). Dietary incorporation of PGLM significantly ($P<0.001$) reduced abdominal fat content in T_1, T_2 and T_3 groups as compared to T_0 group. However, weights of bursa, liver, heart, kidney and length of duodenum, jejunum, ileum and cecum were found to be statistically similar but spleen weight was significantly ($P<0.036$) higher in T_3 than that of T_0 and T_1, whereas, T_2 showed intermediate value.

The economic analyses of experimental birds fed diets with and without PGLM comprises of total feed intake (kg/bird), and total body weight (kg/bird) were significantly ($P<0.001$, $P<0.011$) higher in T_1, T_2 and T_3 groups as compared to T_0 group. Feed cost per kilogram diet was found to be lowest in T_3 followed by T_2 and T_1, and the highest feed cost per kilogram was recorded in T_0. The maximum net return (Rs) was obtained in T_3 group followed by T_2 and T_1 and the lowest net return was obtained in T_0 group (Table 5).

Biochemical profiles

The PGLM supplementation significantly ($P<0.001$) decreased the blood glucose, serum cholesterol and triglyceride levels in T_1, T_2 and T_3 groups than that of T_0 group (Table 6), but the values of all the four groups were within the normal physiological range as suggested for broiler birds. Total serum proteins did not differ significantly among groups, whereas, serum albumin and A:G ratio were significantly ($P<0.001$) lower in supplemented groups as compared to control. Although, PGLM supplementation significantly ($P<0.027$) improved globulin levels in T_1, T_2 and T_3 than that of T_0.

Immune responses

Experimental birds fed CT containing PGLM supplemented diets significantly ($P<0.05$) enhanced CMI response (foot pad thickness; in mm) when compared to un-supplemented control (Figure 1). The maximum CMI response was recorded in T_2 followed by T_3 and T_1 and minimum CMI response was recorded in T_0 against PHA-P. CMI response when compared between different periods, the highest ($P<0.05$) CMI response was recorded on 48 hrs post inoculation followed by 36 hrs and 72 hrs, while 24 hrs post inoculation showed statistically similar findings to 0 hr period. Dietary incorporation of CT containing PGLM significantly ($P<0.001$) improved humoral immune response (HA titre log_2 value) against sheep-RBCs when compared to control.

Humoral immune response was found to be highest (P<0.001) in T_3 followed by T_2 and the lowest response was observed in T_0 group, while, T_1 has an intermediate value between T_0 and T_2 (Figure 2).

Discussion

The present results showed that the nutrient composition of all the experimental diets were adequate for broiler chicks in all three phases as recommended by ICAR [14]. It was observed that as the level of PGLM increased the EE, CF and Ca levels slightly increased, whereas, CP, P and ME contents slightly decreased but they remained within the normal range as suggested by ICAR [14]. This may be due to the adequate nutrient profile of PGLM containing diets in the present study.

Performance, carcass traits and cost benefit analysis

Present results are in agreement with the observation of Rahman et al. [26] who also reported no deleterious effect on body weight gain up to 4.5 % dietary inclusion of PGLM in broiler chickens. As the PGLM level in diets increased, intakes of feed and drinking water and body weight gain were increased. Similarly, as the age of birds increased, mean feed and water intakes and body weight gain increased significantly (P<0.05) from 1st week to 6th weeks. This clearly indicated that PGLM supplemented diets in each phase (up to 3.5 percent of feed) were palatable, acceptable, non-hazardous and showing growth promoting potential to broiler birds. In another studies of Daing et al. [7] and R*ahman* et al. [26] higher body weight gain was observed in guava leaf meal supplemented group as compared to control. Thus it could be attributed from the present study that PGLM supplementation (1.5 to 3.5 %) in the diets of broilers provided all the nutritional components vis-a-vis growth promoting potential of CT containing PGLM that improved feed and water intake and production performance of broiler birds. Increased water consumption in PGLM supplemented birds could be attributed to the dry nature of processed PGLM, as the experimental birds tend to consume more water. The better FCR in supplemented groups might be due to functional feed potential of CT containing PGLM [7].

The PGLM supplementation in broiler chickens did not set forth any undesirable effect on the carcass traits. Present results are in harmony with the findings of Daing et al. [7] and Zargar et al. [8]. They conducted experiments on broiler chicks fed 1-4 % LM of guava and 2.5-7.5 LMM of *Eugenia jambolana* and *P. guajava* and observed non-significant differences in carcass characteristics up to 5% level of LMM. On the other hand, abdominal fat content was significantly (P<0.05) decreased as the CT containing PGLM increased. Present results are in line with the findings of Daing et al. [7] and Zargar et al. [8], they also reported significant reduction of abdominal fat deposition in broiler chickens fed LM and LMM supplemented diets. Zargar et al. [8] also explained that the presence of CT in LMM supplemented diets had been associated with reduced carcass fat. Moreover, a possible explanation for fat reduction had been associated with lower concentration of growth hormone (GH), which may be responsible for increase nitrogen retention and reduced fat deposition with an increase in fat turn over. A possible explanation for reduction of GH may be related to inactivation of gut wall proteins by CT. Furthermore, a gradual decrease in abdominal fat content in PGLM supplemented groups compared to control might be attributed to the reduction in serum cholesterol and triglycerides levels due to presence of CT. Thus there was a clear cut relationship between the abdominal fat weight and the levels of serum cholesterol and triglycerides.

Dietary incorporation of PGLM significantly (P<0.05) reduced the cost of producing a kilogram of feed (total feed cost/kg diet) and this was reflected in the total body weight (kg/bird) and total feed cost per bird. The higher revenues (Rs.) per bird were obtained in PGLM supplemented groups compared to control which suggested that the material was economically viable alternative feed ingredient. This was in agreement with the reports of Onunkwo and George [27] who reported that non-conventional feed ingredients having functional feed ingredients properties, often reduces feed cost. The present results confirm that there is better economic gain by feeding PGLM supplemented diets to broiler chickens since it has the potential of reducing feeding cost of broilers with better production performance and better health status. The higher weight gain and better FCR on PGLM supplementation up to 3.5 percent level may have accounted for higher revenue per bird (Rs. 189.70, 190.34 and 193.31) and net return (Rs. 58.07, 61.06 and 63.38) in T_1, T_2 and T_3 groups compared to T_0 (Rs. 182.23 and Rs. 52.52), respectively. This supports the conclusion and recommendation of various researchers that leaf meal supplementation in broiler ration has proved as means of reducing feed cost with better returns.

Biochemical profiles

Dietary supplementation of CT containing PGLM significantly (P<0.001) reduced glucose, cholesterol and triglyceride levels in birds, these are in line with the findings of Daing et al. [7], who conducted an experiment in broiler chickens and affirmed that dietary supplementation of 1-4 % of guava declined blood cholesterol and triglyceride levels. Similarly, Zargar et al. [8] also reported reduced glucose, cholesterol and triglyceride concentrations in broiler chickens fed *E. jambolana* and *P. guajava* LMM supplemented diets. The presence of CT in PGLM could manage and prevent vascular complications by lowering blood glucose levels and lipid profiles. Previous studies had shown that supplementation of CT containing LMM decreased blood glucose via enhanced insulin secretion from pancreas beta cells. In another study of Cheng and Shen [28] who also suggested that aqueous CT extract of *P. guajava* leaves promotes glucose uptake in liver cells and consequently contributes to the alleviation of hypoglycemia in diabetic rats. Reduced cholesterol in present study may reflect the hypocholesterolemic properties of CT containing PGLM which may block intestinal cholesterol absorption. Moreover, decrease in serum triglyceride is associated with the change in total serum magnesium concentration. Triglycerides are secreted from liver into blood by triglyceride-rich lipoproteins, therefore, impaired hepatic lipogenesis results in decreased triglyceride concentrations in plasma [7]. Total protein, albumin and A:G ratio of broiler chickens under different treatments were within the normal physiological range [29], which clearly indicated that up to 3.5 % PGLM did not exert any adverse effect on total protein, albumin and A:G ratio. However, elevated globulin levels of T_1, T_2 and T_3 groups as compared T_0 showed immune stimulating property of CT containing PGLM.

Immune responses

Improved CMI response is in agreement with the findings of Daing et al. [7] and Zargar et al. [8]. They also reported higher CMI response in birds fed diets supplemented with LM and LMM in broiler chickens. Increased CMI response in PGLM supplemented groups may be attributed to the better health status and immune stimulating properties of CT [17]. The role of PGLM on CMI response in the present study can be understood as CT has been suggested to act as natural antioxidant and exert its antioxidant activity [7, 17] because cellular integrity is imperative for receiving and responding to messages needed to coordinate an immune response.

Better humoral immune response against sheep-RBCs in supplemented groups clearly indicated that CT containing PGLM had immune-modulating properties in broiler chickens. Present results are in agreement with Daing et al. [7], who fed guava LM supplemented diets to broiler chickens (1-4%), which significantly enhanced the antibody titers against SRBCs as antigens. Durrani et al. [30] reported higher antibody titre against IBD in broilers given neem leaves extract in drinking water (50 ml/litre) group than all other groups suggesting better immune-modulating effect against IBD virus. Similarly, Zargar et al. [8] also observed immune-stimulatory property of *E. jambolana* and *P. guajava* LMM against SRBCs in broiler chickens.

Conclusion

On the basis of results obtained it may be concluded that dietary supplementation of CT containing PGLM in broilers' ration displayed production performance enhancing potential (better weight gain and FCR) and lowering blood glucose, cholesterol and triglyceride levels and abdominal fat content. Further study indicated significant improvement in CMI and humoral immune responses in broiler birds. Hence, the ration of broiler chickens containing upto 3.5 percent of PGLM (up to 3.5%) can be considered as a potential, cost effective, environmental friendly, functional feed with attributes of production performance enhancer, immune-modulator and production of low cholesterol healthy broiler meat.

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Conflict of interest

We certify that there is no conflict of interest with my financial organization regarding the material discussed in the manuscript.

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Table 1. Ingredient composition of experimental diets of broiler chickens (as such basis)

Feed ingredients	Pre-starter (0-14 days)	Starter (15-21 days)	Finisher (22-42 days)
Maize	54.75	53.00	55.00
Rice polish	4.00	6.50	10.00
Soybean meal	33.75	32.30	26.45
Meat cum bone meal	3.00	3.00	3.00
Soybean oil	1.50	2.25	2.50
Lysine-Hcl	0.325	0.31	0.30
DL-Methionine	0.15	0.11	0.10
Di-calcium phosphate	1.00	1.00	1.125
Lime stone powder	1.00	1.00	1.00
Common salt	0.40	0.40	0.40
Mineral premix[1]	0.10	0.10	0.10
Vitamin premix[2]	0.025	0.025	0.025
PGLM[3]	0.00	0.00	0.00

[1]Contained the following per gram of vitamin premix: vitamin A, 40,000 IU; vitamin D_3, 10,000 IU; vitamin E, 32 mg; vitamin K_3, 4 mg; vitamin B_1, 3.2 mg; vitamin B_2, 20 mg; vitamin B_6, 6.4 mg; vitamin B_{12}, 82 mcg; niacin, 48 mg; calcium pantothinate, 32 mg; choline chloride, 247 mg and folic acid, 3.2 mg.

[2]Contained the following per gram of trace mineral premix: Mn, 90 mg; Zn, 80 mg; Fe, 90 mg; I, 2 mg; Cu, 15 mg and Se, 0.3 mg.

[3]Incorporated at the rate of 1.5, 2.5 and 3.5 percent of basal feed in T_1, T_2 and T_3 groups, respectively, while T_0 group serve as control i.e. 0 % PGLM of each pre-starter, starter and finisher feeds.

Table 2. Proximate composition of broiler chick diets (% on DM basis)

Attributes	Experimental diets			
	T_0	T_1	T_2	T_3
	Pre-starter (0-14 days)			
Organic matter	89.69	89.35	89.22	89.08
Crude protein	21.92	21.83	21.71	21.59
Ether extract	2.27	2.35	2.3	2.73
Crude fibre	4.38	4.53	4.87	4.99
Acid insoluble ash	2.43	2.54	2.75	2.86
Calcium	1.57	1.63	1.53	1.58
Phosphorus	0.70	0.68	0.69	0.67

ME* (Kcal/kg)	3099.35	3086.2	3067.25	3079.65
Condensed tannins*	0.00	0.098	0.16	0.23
	Starter (15-21 days)			
Organic matter	89.18	88.98	89.24	88.88
Crude protein	21.53	21.34	21.22	21.11
Ether extract	3.01	3.25	3.47	3.51
Crude fibre	4.49	5.04	5.22	5.51
Acid insoluble ash	2.48	2.64	2.36	2.73
Calcium	1.55	1.60	1.70	1.75
Phosphorus	0.68	0.68	0.65	0.66
ME* (Kcal/kg)	3114.65	3100.4	3114.2	3093.45
Condensed tannins*	0.00	0.098	0.16	0.23
	Finisher (22-42 days)			
Organic matter	88.85	88.52	88.44	88.66
Crude protein	19.51	19.36	19.25	19.16
Ether extract	4.32	4.46	4.43	4.21
Crude fibre	5.33	5.39	5.76	5.91
Acid insoluble ash	2.67	2.79	2.87	2.91
Calcium	1.65	1.62	1.69	1.72
Phosphorus	0.61	0.59	0.59	0.57
ME* (Kcal/kg)	3139.2	3132.55	3115.3	3106.75
Condensed tannins*	0.00	0.098	0.16	0.23

*Calculated values; T_0: 0 % PGLM of feed; T_1: 1.5 % PGLM of feed; T_2: 2.5 % PGLM of feed; T_3: 3.5 % PGLM of feed.

Table 3. Effect of PGLM on feed and water intake, weight gain and FCR in broiler chickens

Groups	Periods (weeks)						GM±SE
	1st	2nd	3rd	4th	5th	6th	
			Body weight gain (g)				
T_0	86	226	399	337	521	576	357.67^a±11.20
T_1	104	243	418	329	519	622	372.74^{ab}±11.18
T_2	103	250	421	308	527	635	373.87^{ab}±10.80
T_3	108	259	408	322	523	659	379.96^b±11.12
PM ±SE	100.46^a ±1.21	244.53^b ±3.11	411.97^c ±6.32	323.68^d ±7.05	522.59^e ±10.25	623.13^f ±12.24	
			Feed intake (g)				
T_0	128	324	559	637	844	1040	588.56^a±16.10
T_1	127	357	561	658	871	1080	609.12^c±16.61
T_2	126	355	514	659	868	1074	599.28^b±16.63
T_3	124	359	525	690	869	1087	608.98^c±16.83
PM ±SE	126.33^a ±0.13	348.46^b ±1.36	539.80^c ±2.33	660.93^d ±1.94	863.03^e ±1.72	1070.35^f ±2.39	
			Water intake (ml)				
T_0	284	896	1150	1623	2326	2732	1501.80^a±44.14
T_1	259	946	1281	1715	2391	2803	1565.74^b±45.28
T_2	254	943	1262	1727	2421	2833	1573.30^c±46.08
T_3	293	961	1295	1788	2485	3070	1648.74^d±49.08
PM ±SE	272.46^a ±1.25	936.42^b ±2.71	1246.96^c ±4.86	1713.31^d ±6.00	2405.67^e ±4.57	2859.54^f ±8.68	
Feed conversion ratio (FCR)							

T_0	1.57	1.51	1.52	2.28	1.83	2.15	$1.81^c \pm 0.04$
T_1	1.25	1.54	1.60	2.28	2.01	1.99	$1.78^b \pm 0.05$
T_2	1.25	1.50	1.28	2.36	1.77	1.83	$1.66^a \pm 0.03$
T_3	1.18	1.42	1.36	2.32	1.82	1.84	$1.66^a \pm 0.03$
PM ±SE	1.31^a ±0.02	1.49^b ±0.02	1.44^{ab} ±0.05	2.31^d ±0.06	1.86^c ±0.05	1.95^c ±0.06	

[abcd&abcdef]Means with different superscript within column and rows differ significantly (P<0.05), respectively; T_0: 0 % PGLM of feed; T_1: 1.5 % PGLM of feed; T_2: 2.5 % PGLM of feed; T_3: 3.5 % PGLM of feed; GM±SE: Group mean ± standard error; PM±SE: Period mean ± standard error.

Table 4. Effect of PGLM supplementation on carcass Characteristics of broiler chickens

Attributes	Groups				SEM	P values
	T_0	T_1	T_2	T_3		
Live weight (g)	2420	2378	2406	2437	31.44	0.796
De-feathered (g)	1894	1850	1880	1926	28.77	0.646
Feathers wt (g)	526	528	526	511	11.49	0.663
Eviscerated (g)	1585	1566	1582	1625	27.21	0.593
Carcass/ organ weight % of live weight						
GIT	8.46	8.59	8.81	8.88	0.14	0.255
Gizzard	3.15	3.24	3.25	3.28	0.03	0.169
Head	2.55	2.81	2.86	2.79	0.07	0.242
Shank	4.25	4.41	4.02	4.54	0.11	0.647
Neck	3.33	3.23	3.29	3.21	0.11	0.778
Leg	4.49	4.71	4.43	4.70	0.07	0.585
Thigh	5.54^b	4.83^a	5.10^{ab}	5.24^{ab}	0.11	0.505
Back muscles	9.05	8.73	9.18	9.13	0.34	0.827
Breast	17.41^a	19.58^{ab}	19.40^{ab}	21.32^b	0.46	0.004
Abdominal fat	0.53^c	0.44^b	0.35^a	0.29^a	0.02	0.000
Bursa	0.31	0.34	0.35	0.35	0.01	0.279
Spleen	0.14^a	0.16^a	0.19^{ab}	0.22^b	0.01	0.004
Liver	1.98	1.88	1.87	1.83	0.05	0.342
Heart	0.50	0.51	0.52	0.54	0.01	0.149
Lungs	0.41^a	0.52^{ab}	0.54^b	0.59^b	0.02	0.007
Kidney	0.34	0.34	0.31	0.33	0.01	0.486

[abc]Means with different superscript within rows differ significantly (P<0.05); C: Combined; L: Linear; Q: Quadratic; T_0: 0 % PGLM of feed; T_1: 1.5 % PGLM of feed; T_2: 2.5 % PGLM of feed; T_3: 3.5 % PGLM of feed.

Table 5. Economics (cost benefit analysis) of broiler chickens fed with and without PGLM supplemented diets

Parameters	Groups				SEM	P values
	T_0	T_1	T_2	T_3		
Feed intake (kg/b)	3.53^a	3.66^c	3.60^b	3.65^c	0.01	0.000
Body weight (kg/b)	2.20^a	2.29^b	2.29^b	2.33^c	0.01	0.002
Feed cost / kg diet	26.25^d	25.89^c	25.66^b	25.43^a	0.02	0.000
Feed cost / bird	92.71^b	94.63^c	92.28^a	92.93^b	0.13	0.116
Price @ Rs. /Chick	37.00	37.00	37.00	37.00	-	-
Price @ Rs. /kg bird	83.00	83.00	83.00	83.00	-	-
Revenue / bird	182.23^a	189.70^b	190.34^b	193.31^b	1.24	0.002
Investment/bird	129.71^b	131.63^c	129.28^a	129.93^b	0.13	0.000
Net return (Rs.)	52.52^a	58.07^b	61.06^{bc}	63.38^c	1.22	0.001

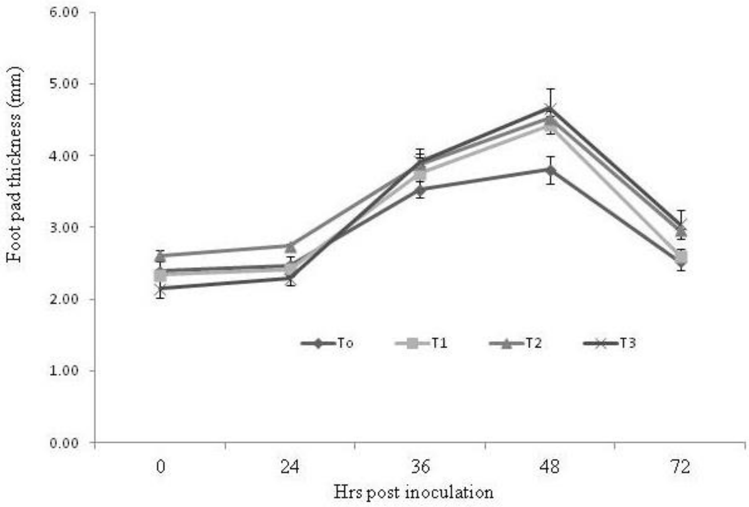
Benefit: cost ratio	1.40:1^{a}	1.44:1^{b}	1.47:1^{bc}	1.49:1^{c}	0.01	0.000

^{abc}Means with different superscript within rows differ significantly (P<0.05);
T_0: 0 % PGLM of feed; T_1: 1.5 % PGLM of feed; T_2: 2.5 % PGLM of feed; T_3: 3.5 % PGLM of feed.

Table 6. Effect of PGLM supplementation on biochemical profiles of experimental broiler chickens

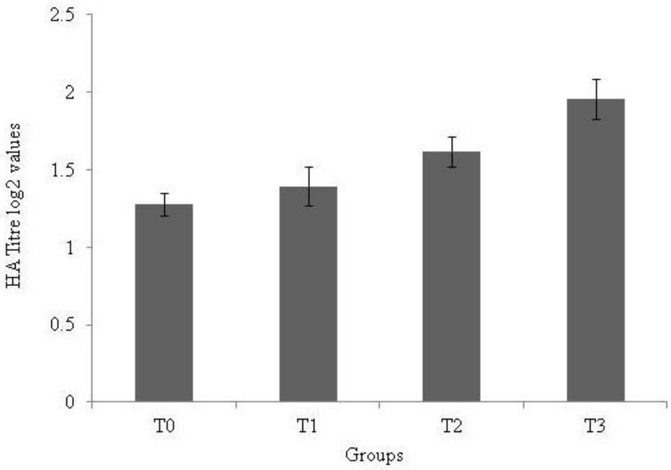
Parameters	Groups				SEM	P Values
	T_0	T_1	T_2	T_3		
Glucose (mg/dl)	200.77^{c}	191.10^{b}	185.62^{ab}	181.17^{a}	1.57	0.000
Cholesterol (mg/dl)	112.89^{c}	103.13^{bc}	96.49^{b}	83.21^{a}	2.35	0.000
Triglyceride (mg/dl)	61.79^{c}	56.13^{bc}	51.42^{b}	43.87^{a}	1.58	0.000
Total Protein (g/dl)	4.12	4.08	3.88	4.00	0.06	0.287
Albumin (g/dl)	2.34^{b}	2.05^{ab}	1.87^{a}	1.76^{a}	0.06	0.000
Globulin (g/dl)	1.78^{a}	2.04^{b}	2.01^{b}	2.24^{c}	0.07	0.027
A/G ratio	1.38^{b}	1.05^{ab}	0.95^{a}	0.81^{a}	0.06	0.000

^{abc}Means with different superscript within rows differ significantly (P<0.05); C: Combined; L: Linear; Q: Quadratic; T_0: 0 % PGLM of feed; T_1: 1.5 % PGLM of feed; T_2: 2.5 % PGLM of feed; T_3: 3.5 % PGLM of feed.



T_0: 0 % PGLM of feed; T_1: 1.5 % PGLM of feed; T_2: 2.5 % PGLM of feed; T_3: 3.5 % PGLM of feed.

Figure 1. Effect of CT containing PGLM supplementation on CMI response (Foot pad thickness) in broiler chickens



T_0: 0 % PGLM of feed; T_1: 1.5 % PGLM of feed; T_2: 2.5 % PGLM of feed; T_3: 3.5 % PGLM of feed.

Figure 2. Effect of CT containing PGLM supplementation on humoral immune response in broiler chickens