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Effect of feeding rice gluten meal with and without enzymes on immunity of broiler chickens

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Abstract
A biological experiment was conducted to evaluate the effect of feeding rice gluten meal (RGM) as soybean replacement without or with different enzymes on immunity in broiler chickens for 42 days. The experiment was conducted as per 3x4 factorial completely randomized design. A total of 384 broiler chicks of same hatch with uniform weight were used. There were twelve different treatments with 4 replicates for each treatment and each replicate consisted of 8 chicks. Two levels of RGM were taken (15 and 17.5%). Protease, xylanase and multienzymes supplementation under different treatments were done. Effect of feeding different levels of RGM (0, 15 and 17.5%) without or with enzymes (xylanase, protease and multienzymes) and their interaction with enzymes did not exhibit any significant (P>0.05) difference between control and other dietary treatments on CMI and humoral immunity. Thus, it may be concluded that 15% and 17.5% RGM levels with or without enzyme supplementation had not any adverse effect on immune response in broiler chickens.

Keywords: Enzyme, immunity, protease, rice gluten meal

Introduction
Poultry industry is the fastest growing sector in Indian agriculture. Feed is the major constituent in the poultry production accounts for about 70% of total cost. Feed costs are primarily driven by the cost of protein sources. Substitution of expensive protein sources with lower cost ingredients would potentially reduce the cost of the feed. Poultry industry depends on soybean meal as a source of dietary protein due to its uniform quality and ideal amino acid profile. Instability in its production, indiscriminate exports and higher demand has resulted in its shortage for the poultry industry leading to its higher price. Soybean meal is used 40% towards poultry and livestock feeding, so there is net shortage per year is about 2.5 MMT during 2014-2025. As there is scarcity of soybean meal at reasonable price, there is need to utilize locally available alternate protein sources [8].

Asia is the primary production region for rice with over 90% of global production. China tops the list of rice production followed by India. Now days, certain newer rice by products are available in appreciable quantities and cheaper rate that can be utilized as protein sources from rice processing industries such as rice gluten meal (RGM). Rice gluten meal (RGM) is a by-product of wet-milling of rice obtained during starch extraction and syrup preparation. It is relatively a new feedstuff having brownish coloured and coarse powdery texture. Commercial traders categorise RGM as a high crude protein and energy ingredient which is priced lower than SBM. Regarding the feeding value of RGM, scanty studies were done in broiler [12, 8, 16] and Cattle [6, 7].

Enzyme supplementations leads to increase feeding value of the dietary raw materials, reduction in the variation of nutrient quality of the diet, increased nutrient digestibility, reduction in water content of the excreta, reduced viscosity of intestinal contents and accelerated rate of passage of digesta through the gastrointestinal tract [9] .

Rice as subtract for RGM is increasing due to relative lower price, increased production and easy availability. However, limited information is available on the appropriate enzyme or their combination that are specific for broiler diets based on corn-soya diet and soybean meal partially replaced with RGM. Very scanty researches were done in RGM regarding its effect on immunity [16] in poultry. In view of above an experiment was conducted to study the effect of feeding RGM with and without enzymes on immunity of broiler chickens.

Materials and Methods

The research work was carried out at the Division of Avian Nutrition and Feed Technology, ICAR-Central Avian Research Institute (CARI), Izatnagar, India. Broilers of CARIBRO-VISHAL (white variety) were used in the study. The study was carried out as per the guidelines and approval of institute animal ethical committee (IAEC) and committee for the purpose of control and supervision of experiments on animals (CPCSEA).The IAEC/CPCSEA number is 452/01/ab/CPCSEA.

Procurement of feed ingredients

The required quantities of the feed ingredients and supplements for formulation of experimental diets including RGM and enzyme supplements xylanase, protease and multienzymes were procured from the feed storage and processing section of ICAR-CARI, Izatnagar.

Procurement of experimental eggs

In the study required eggs of CARI BRO VISHAL (white variety) were obtained from the Experimental Broiler Farm, ICAR-Central Avian Research Institute (CARI), Izatnagar, India and incubated at Experimental Hatchery Unit of the institute. Day old broiler chicks of same hatch with uniform weight wing banded were used in the experiments.

Housing and management

Experimental broilers day old chicks were randomly divided into different groups as per experimental plan. The birds were housed in specially designed battery brooder cages with watering and feeding facilities and were reared under standard management conditions. Experimental diets were offered *ad libitum*, randomly as mash to all groups of broiler birds for an experimental period of six weeks. Weighted amount of respective diets were offered to birds daily with every attempt to minimize feed spillage/wastage. Fresh and wholesome water were always made available to the birds throughout the experimental period. The control as well as test diets and drinking water were provided *ad libitum* to the birds during the entire experimental period. All management practices including feeding, watering, lighting and vaccination practices were kept identical for all the birds under different dietary treatments.

Basal diets and Laboratory analysis

Corn-soya meal based basal diets to meet ICAR standard [5] for broiler chickens were formulated as prestarter (Table 1), starter (Table 2) and finisher (Table 3). Energy, protein, major minerals and limiting amino acids will be kept constant. *Isonitrogenous* and *isocalorific* diets were used for all experiments. The three commercial enzyme preparations protease (P), xylanase (X) and multienzymes(M) were analyzed for different enzyme activities as per standard methods and used as per manufacturer's instruction.

Experimental design

The experiment was conducted as per 3x4 factorial completely randomized design (CRD). A total of 384 broiler chicks (CARIBRO vishal) of same hatch with uniform weight were used in the experiment. The birds were randomly divided into 48 replicates of eight birds each. There were twelve different treatments with 4 replicates for each treatment. So, each treatment was allocated 32 birds. The allocation of birds in each treatment was based on the similar initial body weight. Two levels of RGM were taken, the best inclusion level from earlier experiments as first level (15%) and then adding over and above the best level of 2.5 % RGM to this level with enzymes. Experimental layout for feeding different level of RGM with or without enzymes is presented in Table 4 and analyzed chemical composition of dietary ingredients (%) on as such basis is presented in Table 5.

Immunological parameters

Humoral immune (HI) response

Humoral immune response estimated by method of Siegel and Gross [14] was followed for slight modified by Saxena *et.al*. [12] assaying the immune response to sheep red blood cells (SRBCs).Blood from jugular vein of healthy sheep was collected in Alsever's solution. Blood stored in Alsever's solution was taken in a sterile test tube after removing the supernatant Alsever's solution. PBS was added to the tube then centrifuged at 2500 rpm for 10 minutes and washed thrice in PBS till the supernatant become clear. After washing, 1 ml of SRBC (PCV) was added in 99 ml of PBS to make 100 ml of 1 % SRBC (1% v/v) suspension and stored in refrigerator at 4^0C until its use.

Eight broilers (4 males and 4 females) per treatment were injected with 1 ml of 1% SRBC suspension intravenously at 28th day post hatch in broilers other than used for assessing CMI. On 34th day (5 days post immunization) two ml of blood was collected from jugular vein. The blood was collected in sterile test tube without any anticoagulant and allows clotting at room temperature for two hours at slanting position. The blood was then centrifuged at 2000 rpm for 15 minutes and immune serum was harvested and stored at -20oC for subsequent testing. Antibody titer was determined by haemagglutination test (HA) using U bottom 96 well micro titer plates. The reciprocal of highest dilution which show clear agglutination was the end titer. Titers were expressed as log 2.

Cell mediated immune response

The *in vivo* cell mediated immune response to PHA-P was evaluated by the method of Cheng and Lamont [2] on 29th day post hatch. At 29th day post hatch, 8 birds (4 males and 4 females) from each treatment were selected for assessing CMI. Phytohaemagglutinin type P (PHA-P) provokes responses, influenced by subpopulation of T-helper and T-suppressor cells. Good responder to PHA-P means a higher general level of cellular immunity influencing T-cell mechanisms restricting or preventing lymphoma formation.

Statistical analysis

Data subjected to test of significance as per 3x4 factorial completely randomized design (CRD) were analyzed for mean, standard errors and analysis of variance by Snedecor and Cochran [15] using statistical package for social sciences (SPSS) 16.0 version and comparison of means were done using Tukey test [16] .

Results and Discussion

Influence of different levels of RGM with or without enzymes on humoral and cell mediated immunity (CMI) are tabulated in Table 6.

Humoral immune response: Main effect of RGM for humoral immunity in terms of haemagglutination (HA) titer (log2) was numerically higher in 15 and 17.5% RGM groups

as compared to control (0%RGM). It was reported 2.60 in 15 %RGM group, 2.63 in 17.5% RGM group and 2.58 in control group (HA titer). Effect of enzyme supplementation showed that numerically improvement in humoral immunity but it did not showed significant (P>0.05) difference as compared to control. Humoral immunity in xylanase, protease and multienzymes supplemented groups were 2.63, 2.68 and 2.59, respectively but in control it was found 2.50 (HA titer). Effect of feeding different levels of RGM (0, 15 and 17.5%) and their interaction with enzymes (xylanase, protease and multienzymes) did not exhibit any significant (P>0.05) difference between control and other dietary treatments in humoral immunity.

Cell mediated immune response: Main effect of RGM for cell mediated immunity in terms of footpad index (in mm) did not differ significant (P>0.05) as compared to control due to inclusion level of 15 and 17.5% RGM levels as compared to control. It was reported 58.38 in 15% RGM level and 57.25 in 17.5% RGM level. Effect of enzyme supplementation showed no significant (P>0.05) difference as compared to control in cell mediated immunity. The CMI in xylanase, protease and multienzymes supplemented groups were reported 58.54, 57.33 and 58.33, respectively while in control it was found 58.08 (foot pad index response in mm). Effect of feeding different levels of RGM (0, 15 and 17.5%) and their interaction with enzymes (xylanase, protease and multienzymes) did not exhibit any significant (P>0.05) difference between control and other dietary treatments in cell mediated immunity.

Thus, it may be concluded that effect of feeding different levels of RGM (0, 15 and 17.5%) without or with enzymes (xylanase, protease and multienzymes) and their interaction with enzymes did not exhibit any significant (P>0.05) difference between control and other dietary treatments on CMI and humoral immunity. Feeding of RGM at 15 or 17.5% level has no adverse effect in broiler chickens as an alternative of soybean meal.

Our results are in agreement with researchers [17, 4] but disagreement with Barekatain *et al*. [1]. Wani [17] reported no significant (P>0.05) difference on dietary inclusion of RGM up to 20% level, with or without protease enzyme and there interaction on humoral immunity and cell mediated immunity. No other reference is available regarding effect of feeding RGM on immunity. Similarly another rice based product distiller's dried grains with solubles (DDGS) reported by Gupta [4] that no significant (P>0.05) difference in humoral and cell mediated immunity by feeding up to 10% inclusion of rice DDGS. But contrary to this, Barekatain *et al*. [1] found that incorporation of 20% level of sorghum DDGS in the diets significantly (P<0.01) improved the IgA and IgG titer in broiler. A functional immune system is a requirement of a healthy life in modern animal production as infectious diseases still represent a serious drain on the economics of poultry production. Almost all the nutrients in the diet play a fundamental role in sustaining an optimal immune response and the deficient and excessive intakes can have negative consequences on immune status and susceptibility to a variety of pathogens [3,11] Immune response of birds evaluated in terms of humoral and cell mediated immune response can be correlated with the effect of test ingredient, as dietary components can regulate physiological functions of the body, interacting with the immune system is one of the most important functions of nutrients [2].

Table 1: Ingredients and nutrient composition (%) of pre-starter diets with or without enzymes for different level of RGM

Ingredients	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12
Maize	54.42	54.42	54.42	54.42	59.40	59.40	59.40	59.40	60.00	60.00	60.00	60.00
SBM	38.40	38.40	38.40	38.40	20.70	20.70	20.70	20.70	17.80	17.80	17.80	17.80
RGM	0.00	0.00	0.00	0.00	15.00	15.00	15.00	15.00	17.50	17.50	17.50	17.50
Oil	3.00	3.00	3.00	3.00	0.70	0.70	0.70	0.70	0.40	0.40	0.40	0.40
LSP	1.40	1.40	1.40	1.40	1.30	1.30	1.30	1.30	1.30	1.30	1.30	1.30
DCP	1.82	1.82	1.82	1.82	1.95	1.95	1.95	1.95	1.95	1.95	1.95	1.95
Lysine	0.00	0.00	0.00	0.00	0.12	0.12	0.12	0.12	0.18	0.18	0.18	0.18
Methionine	0.20	0.20	0.20	0.20	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Constant*	0.765	0.765	0.765	0.765	0.765	0.765	0.765	0.765	0.765	0.765	0.765	0.765
Enzyme	-	+	+	+	-	+	+	+	-	+	+	+
Total	100.01	100.01	100.01	100.01	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Nutrient composition												
CP	21.99	21.99	21.99	21.99	22.06	22.06	22.06	22.06	22.07	22.07	22.07	22.07
Lysine	1.19	1.19	1.19	1.19	1.20	1.20	1.20	1.20	1.21	1.21	1.21	1.21
Methionine	0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.54	0.54	0.54	0.54
Threonine	0.81	0.81	0.81	0.81	0.83	0.83	0.83	0.83	0.82	0.82	0.82	0.82
Ca	1.03	1.03	1.03	1.03	1.01	1.01	1.01	1.01	1.01	1.01	1.01	1.01
P	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.44	0.44	0.44	0.44
ME (kcal/kg)**	2998	2998	2998	2998	3001	3001	3001	3001	3001	3001	3001	3001
Cost (Rs./kg)	28.52	29.03	29.13	28.93	24.68	25.19	25.29	25.09	24.31	24.82	24.92	24.72

In prestarter diet *Constant 0.765 includes salt 0.4%, trace mineral premix 0.1%, vitamin premix 0.15%, vit. B complex 0.015%, choline chloride 0.05% and Toxin binder 0.05%. Trace mineral premix supplied mg / kg diet: Mn, 55; I, 1; Fe, 75; Zn, 60; Cu, 10;Se,0.15 and Cr,0.2. The vitamin premix supplied per kg diet: Vit.A, 5000 IU; Vit.D3, 2400 IU; Vit.E,15 and Vit.K, 1mg. Vitamin B complex supplied per kg diet: Vit. B1, 5 mg; Vit. B2, 6 mg; Vit. B6 5 mg; Vit.B12, 15 mcg; nicotinic acid, 35 mg; pantothenic acid, 12 mg; biotin 0.15 mg and folic acid 0.5 mg. Choline chloride supplied per kg diet: choline, 1300 mg.(As per ICAR,2013) **calculated value

Table 2: Ingredients and nutrient composition (%) of starter diets with or without enzymes for different level of RGM

Ingredients	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12
Maize	55.63	55.63	55.63	55.63	60.70	60.70	60.70	60.70	61.62	61.62	61.62	61.62
SBM	37.10	37.10	37.10	37.10	19.20	19.20	19.20	19.20	16.20	16.20	16.20	16.20
RGM	0.00	0.00	0.00	0.00	15.00	15.00	15.00	15.00	17.50	17.50	17.50	17.50
Oil	3.50	3.50	3.50	3.50	1.20	1.20	1.20	1.20	0.80	0.80	0.80	0.80
LSP	1.35	1.35	1.35	1.35	1.32	1.32	1.32	1.32	1.32	1.32	1.32	1.32
DCP	1.55	1.55	1.55	1.55	1.70	1.70	1.70	1.70	1.70	1.70	1.70	1.70
Lysine	0.00	0.00	0.00	0.00	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Methionine	0.10	0.10	0.10	0.10	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Constant*	0.765	0.765	0.765	0.765	0.765	0.765	0.765	0.765	0.765	0.765	0.765	0.765
Enzyme	-	+	+	+	-	+	+	+	-	+	+	+
Total	100.00	100.00	100.00	100.00	100.0	100.0	100.0	100.0	100.00	100.00	100.00	100.00
Nutrient composition												
CP	21.52	21.52	21.52	21.52	21.51	21.51	21.51	21.51	21.50	21.50	21.50	21.50
Lysine	1.38	1.38	1.38	1.38	1.09	1.09	1.09	1.09	1.04	1.04	1.04	1.04
Methionine	0.48	0.48	0.48	0.48	0.49	0.49	0.49	0.49	0.50	0.50	0.50	0.50
Threonine	0.80	0.80	0.80	0.80	0.79	0.79	0.79	0.79	0.81	0.81	0.81	0.81
Ca	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95	0.95
P	0.41	0.41	0.41	0.41	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
ME (kcal/kg)**	3050	3050	3050	3050	3050	3050	3050	3050	3051	3051	3051	3051
Cost (Rs./ kg)	28.03	28.53	28.63	28.43	24.43	25.03	24.93	24.83	23.86	24.46	24.36	24.26

In starter diet *Constant 0.765 includes salt 0.4%, trace mineral premix 0.1%, vitamin premix 0.15%, vit. B complex 0.015%, choline chloride 0.05% and Toxin binder 0.05%. Trace mineral premix supplied mg / kg diet: Mn, 55; I, 1; Fe, 60; Zn, 60; Cu, 10;Se,0.15 and Cr,0.2. The vitamin premix supplied per kg diet: Vit.A, 5000 IU; Vit.D3, 2400 IU; Vit.E,15 and Vit.K, 1mg. Vitamin B complex supplied per kg diet: Vit. B1, 4 mg; Vit. B2, 6 mg; Vit. B6 5 mg; Vit.B12, 15 mcg; nicotinic acid, 35 mg; pantothenic acid, 10 mg; biotin 0.15 mg and folic acid 0.5 mg. Choline chloride supplied per kg diet: choline, 1200 mg.(As per ICAR,2013) **calculated value

Table 3: Ingredients and nutrient composition (%) of finisher diets with or without enzymes for different level of RGM

Ingredients	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12
Maize	62.00	62.00	62.00	62.00	67.07	67.07	67.07	67.07	67.97	67.97	67.97	67.97
SBM	31.30	31.30	31.30	31.30	13.40	13.40	13.40	13.40	10.40	10.40	10.40	10.40
RGM	0.00	0.00	0.00	0.00	15.00	15.00	15.00	15.00	17.50	17.50	17.50	17.50
Oil	3.22	3.22	3.22	3.22	0.90	0.90	0.90	0.90	0.50	0.50	0.50	0.50
LSP	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
DCP	1.45	1.45	1.45	1.45	1.60	1.60	1.60	1.60	1.60	1.60	1.60	1.60
Lysine	0.00	0.00	0.00	0.00	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12
Methionine	0.06	0.06	0.06	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Marbal Chips	1.20	1.20	1.20	1.20	1.14	1.14	1.14	1.14	1.14	1.14	1.14	1.14
Constant*	0.765	0.765	0.765	0.765	0.765	0.765	0.765	0.765	0.765	0.765	0.765	0.765
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Nutrient composition CP	19.51	19.51	19.51	19.51	19.50	19.50	19.50	19.50	19.50	19.50	19.50	19.50
Lysine	1.20	1.20	1.20	1.20	0.98	0.98	0.98	0.98	0.92	0.92	0.92	0.92
Methionine	0.41	0.41	0.41	0.41	0.43	0.43	0.43	0.43	0.44	0.44	0.44	0.44
Threonine	0.86	0.86	0.86	0.86	0.81	0.81	0.81	0.81	0.80	0.80	0.80	0.80
Ca	0.86	0.86	0.86	0.86	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85
P	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.37	0.37	0.37	0.37
ME**	3100.3	3100.3	3100.3	3100.3	3099.1	3099.1	3099.1	3099.1	3099.3	3099.3	3099.3	3099.3
Cost (Rs./kg)	2672	2672	2672	2672	2584	2584	2584	2584	2526	2526	2526	2526

In finisher diet *Constant 0.77 includes salt 0.4%, trace mineral premix 0.1%, vitamin premix 0.15%, vit. B complex 0.015%, choline chloride 0.05% and Toxin binder 0.05%. Trace mineral premix supplied mg / kg diet: Mn, 50; I, 1; Fe, 50; Zn, 60; Cu, 8;Se,0.15 and Cr,0.2. The vitamin premix supplied per kg diet: Vit.A, 5000 IU; Vit.D3, 2400 IU; Vit.E,15 and Vit.K, 0.8 mg. Vitamin B complex supplied per kg diet: Vit. B1, 4 mg; Vit. B2, 6 mg; Vit. B6 5 mg; Vit.B12, 15 mcg; nicotinic acid, 30 mg; pantothenic acid, 10 mg; biotin 0.15 mg and folic acid 0.5 mg. Choline chloride supplied per kg diet: choline, 900 mg.(As per ICAR,2013) **calculated value

Table 4: Experimental layout for feeding different level of RGM with or without enzymes

Experimental design			3x4 factorial CRD		
Treatments	Rice gluten meal (%)	No. of replicates	Birds/ replication	Total	Enzymes
T1	0.0	4	8	32	-
T2	0.0	4	8	32	Xylanase
T3	0.0	4	8	32	Protease
T4	0.0	4	8	32	Multienzymes
T5	15	4	8	32	-
T6	15	4	8	32	Xylanase
T7	15	4	8	32	Protease
T8	15	4	8	32	Multienzymes

T9	17.5	4	8	32	-
T10	17.5	4	8	32	Xylanase
T11	17.5	4	8	32	Protease
T12	17.5	4	8	32	Multienzymes

Table 5: Analyzed chemical composition of dietary ingredients (%) on as such basis

Ingredients	Moisture	DM	CP	EE	CF	TA	NFE	Ca	P	GE (kcal/kg)	*ME (kcal/kg)
Maize	8.6	91.3	9	3.9	1.8	1.4	83.8	0.03	0.29	4447	3350
SBM	9.1	90.9	44.5	0.9	6.2	3.1	45.2	0.32	0.68	4097	2400
DORB	10.1	91.8	14	1.6	15.9	5.8	62.6	0.3	1.54	3854	2000
RGM	7.6	92.3	49.9	5.7	7.4	3.3	33.5	0.84	0.98	4742	3031
Soybean oil	-	-	-	- -	-	-	-		-	8900	8450
Lime stone powder	1.4	98.6	-	-	-	-	-	33.89	-	-	-
Marbal chip	1.3	98.7	-	-	-	-	-	33.84	-	-	-
DCP	7.2	92.7	-	-	-	-	-	22.92	16.04	-	-

*calculated value

Table 6: Effect of feeding different level of RGM with or without enzymes on immunological parameters

Treatments	RGM %	Enzyme	HA (log 2)	CMI (mm)
T1	0	-	2.48	59.63
T2	0	X	2.64	57.75
T3	0	P	2.66	57.88
T4	0	M	2.57	59.13
T5	15	-	2.52	56.88
T6	15	X	2.58	59.88
T7	15	P	2.66	58.38
T8	15	M	2.64	58.38
T9	17.5	-	2.52	57.75
T10	17.5	X	2.69	58.00
T11	17.5	P	2.75	55.75
T12	17.5	M	2.58	57.50
		Pooled SEM	0.02	0.35
		RGM		
		0	2.58	58.59
		15	2.60	58.38
		17.5	2.63	57.25
		Enzyme		
		-	2.50	58.08
		X	2.63	58.54
		P	2.68	57.33
		M	2.59	58.33
		Significance		
		RGM	NS	NS
		Enzyme	NS	NS
		Interaction	NS	NS

NS: Non-significant (P>0.05)

Conclusion

The RGM feeding and enzyme supplementation or their interaction had no significant (P>0.05) effects on the immunological parameters of broiler chicken. Thus, it may be concluded that 15% and 17.5% RGM levels with or without enzyme supplementation had not any adverse effect on immune response in broiler chickens.

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