



Original Research

Effect of Feeding Rice Gluten Meal without or with Different Enzymes on Gut Health 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 gut health in broiler chickens. The experiment was conducted as per 3x4 factorial completely randomized design. A total of 384 broiler chicks were divided into 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%). Mixing ratio 50 g per 100 kg feed for protease, 10 g per 100 kg feed for xylanase and 25 g per 100 kg feed for multienzymes were used as per manufacturer instructions. The study concluded that RGM feeding and their interaction with protease, xylanase and multienzymes revealed no significant ($P>0.05$) difference on intestinal histomorphometry and microbiological parameter of broiler chicken. However, xylanase enzyme supplementation improved gut health in rice gluten meal based diet in terms of improvement in villus height and reduction in total viable count in jejunum.

Key words: Broiler, Enzyme, Gut Health, Histomorphometry, Rice Gluten Meal

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Introduction

The gut is a fundamental organ system which makes up two equally important functions, *i.e.* the digestion and host defence. To elicit the well-functioning and healthy gut, the dynamic balance of gut ecosystem is important. Most feed ingredients of plant origin contain considerable amounts of fibre (non-starch polysaccharides), with the majority being insoluble. Insoluble fibre has traditionally been regarded as inert nutrient diluents with little or no nutritive value in monogastric animals, but plays various roles in improving gut health and enhancing nutrient digestion (Hetland *et al.*, 2003). Jejunum portion of small intestine is the main site of digestion and absorption of nutrients in avian species. Histological changes and



microbiological status in gut are indicators of the physiological, nutritional and pathological status of birds and can be correlated to identify the impact of nutritional factors and additives supplied in the diet. Different factors such as diet, age as well as health status of the birds influence the establishment of a particular bacterial colony in the gut and histomorphometry (Barnes, 1979). In healthy chickens, *Lactobacilli* are beneficial bacteria and play good role in inhibiting pathogens. Feeding chickens with a plant protein based diet resulted in the production of high levels of short chain fatty acid (SCFA) compared to when birds were fed on normal protein based diet (Tsukahara and Ushida, 2000). By dietary means, it is possible to modify the gut microbial population, concomitant with the growth of favourable bacteria in the gut of chicken (Choct, 2009).

Feed costs are primarily driven by the cost of protein sources. Substitution of expensive protein sources like soybean meal with low cost ingredients would potentially reduce the cost of feed. The rice production in our country was about 106.65 MT in 2013-14 and rice tops the list of total cereal production in the country (Agriculture statistics, 2015-16). 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 rice gluten meal (Wani *et al.*, 2017). Despite the acceptance of fibre hydrolyzing enzymes concept, highly variable *In vivo* responses to non-starch polysaccharide (NSP) enzyme supplementation have been observed. Thus, strategic development of suitable NSP enzyme combination based on the composition of NSP in diet in terms of substrate specific preparation should enhance the nutritive value of diets (Chesson, 2001; Lazaro *et al.*, 2004).

Information on effect of feeding diets containing various levels of RGM on histological changes and microbiological status in gut is very scanty in literature. Based on these facts the research work entitled “Effect of feeding rice gluten meal without or with different enzymes on gut health of broiler chickens” was carried out with *in vivo* and *in vitro* experiments. The response criteria were assessed in terms of intestine histomorphometry and gut microbial status in broiler production.

Material and Methods

Experimental Location

The research work carried out at the Division of Avian Nutrition and Feed Technology, ICAR-Central Avian Research Institute (CARI), Izatnagar, Bareilly, India in the year 2018. All the procedures carried out on the birds were approved by the Institutional Animal Ethics Committee of ICAR-Central Avian Research Institute Izatnagar, Bareilly, U.P. 243122 (452/01/ab/CPCSEA).

Experimental Design and Diets

Experimental layout for feeding different level of RGM with or without enzymes is presented in Table 1. The experiment was conducted as per 3x4 factorial completely randomized design (CRD).

Table 1: Experimental layout for feeding different level of RGM with or without enzymes

Experimental Design			3x4 Factorial CRD		
Treatment	Rice Gluten Meal (%)	No. of Replicates	Birds/ Replication	Total	Enzymes
T1	0	4	8	32	-
T2	0	4	8	32	Xylanase
T3	0	4	8	32	Protease
T4	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
	Total	48		384	

A total of 384 broiler chicks of same hatch with uniform weight (42 to 45 g) were used. There were twelve different treatments with 4 replicates for each treatment and each replicate consisted of 8 chicks. Two levels of rice gluten meal were taken (15 and 17.5%) along with maize soybean based control diet were taken. Protease activity was estimated $600,000 \pm 849$ units per g. Xylanase activity was estimated $150,000 \pm 683$ units per g. Multienzymes activity were estimated cellulase 15,000, xylanase $18,500 \pm 328$, Beta glucanase $12,500 \pm 128$, amylase 1500 ± 46 , pectinase 150 ± 16 , protease 5000 ± 136 , lipase 15 ± 3.8 and Beta mannanase 400 ± 31 . Mixing ratio 50 g per 100 kg feed for protease, 10 g per 100 kg feed for xylanase and 25 g per 100 kg feed for multienzymes were used as per manufacturer instructions. The feeding trial was conducted for six weeks and the feed as well as drinking water were provided ad libitum to the birds during the entire experimental period. The broiler chicken ration was formulated as per ICAR (2013) guideline by using RGM as replacement of soybean meal in the basal diets along with enzyme supplementation. *Isonitrogenous* and *isocalorific* diets were used for this experiment. The feed ingredients and the nutrient composition of diets represented in Table 2, 3 and 4.

Intestinal Histomorphometry

Samples from jejunum were taken from 8 birds per treatment at the end of experiment on 42nd day. All the light microscopic variables were measured for jejunum of each bird using optical microscope (Motic Inverted microscope, Honkong), at a 10 X magnification, a camera (Motic cam, CMOS, Honkong), and image analysis software (Motic Image 2.0, Honkong). Pieces of 2-3 mm thickness at the midpoint of jejunum was removed, the segment was washed with physiological saline solution and fixed in 10% buffered formalin. Each jejunum segment collected were embedded in paraffin and sections of 5-micron thickness of each sample were placed on a glass slide and stained with hematoxyline and eosine for examination (Culling, 1974).

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

Ingredients	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
Maize	54.42	54.42	54.42	54.42	59.4	59.4	59.4	59.4	60	60	60	60
SBM	38.4	38.4	38.4	38.4	20.7	20.7	20.7	20.7	17.8	17.8	17.8	17.8
RGM	0	0	0	0	15	15	15	15	17.5	17.5	17.5	17.5
Oil	3	3	3	3	0.7	0.7	0.7	0.7	0.4	0.4	0.4	0.4
LSP	1.4	1.4	1.4	1.4	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3
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	0	0	0	0.12	0.12	0.12	0.12	0.18	0.18	0.18	0.18
Methionine	0.2	0.2	0.2	0.2	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	100	100	100	100	100	100	100	100	100	100	100
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	1.2	1.2	1.2	1.2	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. SBM (Soybean meal) and LSP (Lime stone powder).

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

Ingredients	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
Maize	55.63	55.63	55.63	55.63	60.7	60.7	60.7	60.7	61.62	61.62	61.62	61.62
SBM	37.1	37.1	37.1	37.1	19.2	19.2	19.2	19.2	16.2	16.2	16.2	16.2
RGM	0	0	0	0	15	15	15	15	17.5	17.5	17.5	17.5
oil	3.5	3.5	3.5	3.5	1.2	1.2	1.2	1.2	0.8	0.8	0.8	0.8
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	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7
Lysine	0	0	0	0	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
Methionine	0.1	0.1	0.1	0.1	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	100	100	100	100	100	100	100	100	100	100	100
Nutrient Composition												
CP	21.52	21.52	21.52	21.52	21.51	21.51	21.51	21.51	21.5	21.5	21.5	21.5
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	0.5	0.5	0.5	0.5
Threonine	0.8	0.8	0.8	0.8	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	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
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. SBM (Soybean meal) and LSP (Lime stone powder).

Table 4: Ingredients and nutrient composition (%) of finisher diets with or without enzymes for different level of RGM

Ingredients	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
Maize	62	62	62	62	67.07	67.07	67.07	67.07	67.97	67.97	67.97	67.97
SBM	31.3	31.3	31.3	31.3	13.4	13.4	13.4	13.4	10.4	10.4	10.4	10.4
RGM	0	0	0	0	15	15	15	15	17.5	17.5	17.5	17.5
Oil	3.22	3.22	3.22	3.22	0.9	0.9	0.9	0.9	0.5	0.5	0.5	0.5
LSP	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Marbal Chips	0.7	0.7	0.7	0.7	0.64	0.64	0.64	0.64	0.64	0.64	0.64	0.64
DCP	1.45	1.45	1.45	1.45	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6
Lysine	0	0	0	0	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	0	0	0	0	0	0	0
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	100	100	100	100	100	100	100	100	100	100	100
Nutrient Composition (%)												
CP	19.51	19.51	19.51	19.51	19.5	19.5	19.5	19.5	19.5	19.5	19.5	19.5
Lysine	1.2	1.2	1.2	1.2	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	0.8	0.8	0.8	0.8
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 (kcal/kg)*	3100	3100	3100	3100	3100	3100	3100	3100	3100	3100	3100	3100
Cost (Rs.per kg)	26.72	27.22	27.32	27.12	25.84	26.34	26.44	26.24	25.26	25.76	25.86	25.76

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. SBM (Soybean meal) and LSP (Lime stone powder).

Microbiological Analysis

At the end of experiment jejunum samples were collected from 8 birds per treatment. Total viable count (TVC) and *Lactobacillus* spp. counts in jejunum samples were enumerated as per the methods described by Speck (1984). The 10 fold serial dilutions of the samples collected were formed before inoculation in the agar containing petri dishes. Duplicate plates were prepared and the counts were expressed as log₁₀ value colony forming units (cfu/g). The total viable count (TVC) and *Lactobacilli* count were determined by using nutrient agar and Rogosa SL agar, respectively (Deman *et al.*, 1960).

Statistical Analysis

The data collected was subjected to general linear model (GLM) as per factorial CRD to present the results as means and standard errors described by Snedecor and Cochran (1989) by using statistical package for social sciences (SPSS) 16.0 version and the comparison of significant mean differences was as per Tukey (1949).

Results and Discussion

Intestinal Histomorphometry

The data pertaining feeding different levels of RGM with or without enzymes on intestinal histomorphometry (in μm) are tabulated in Table 5.

Table 5: Effect of feeding different level of RGM with or without enzymes on intestinal morphometry (in μm)

Treatment	RGM %	Enzyme	Villus Height (VH)	Crypt Depth (CD)	VH:CD	Villus Width
T1	0	-	1187	179	6.67	108
T2	0	X	1280	169	7.55	110
T3	0	P	1310	174	7.53	111
T4	0	M	1266	176	7.56	105
T5	15	-	1204	174	6.93	120
T6	15	X	1311	176	7.59	108
T7	15	P	1230	169	7	111
T8	15	M	1216	176	7.01	110
T9	17.5	-	1214	174	7.05	111
T10	17.5	X	1314	176	7.79	108
T11	17.5	P	1251	169	6.96	119
T12	17.5	M	1224	176	7.13	107
		Pooled SEM	9.59	2.03	0.09	1.33
		RGM				
		0	1261	176	7.33	108
		15	1240	175	7.13	112
		17.5	1251	174	7.23	111
		Enzyme				
		-	1201 ^a	175	6.88 ^a	113
		X	1301 ^c	174	7.64 ^c	109
		P	1263 ^{bc}	171	7.15 ^{ab}	114
		M	1235 ^{ab}	176	7.23 ^{ab}	107
		Significance				
		RGM	NS	NS	NS	NS
		Enzyme	P<0.01	NS	P<0.05	NS
		Interaction	NS	NS	NS	NS

Values bearing different superscripts within the column differ significantly, NS: Non-significant ($P>0.05$)

Effect of feeding different levels of RGM (0, 15 and 17.5%) and their interaction with xylanase, protease and multienzymes or without enzymes did not exhibit any significant ($P>0.05$) difference on intestinal histomorphometry in terms of villus height (VH), crypt depth (CD), their ratio (VH: CD) and villus width. Enzymes (xylanase, protease and multienzymes) supplementation on intestinal histomorphometry did not exhibit any significant ($P>0.05$) difference on crypt depth and villus width. Xylanase enzyme supplemented groups significantly ($P<0.01$) increased villus height as compared to multienzymes and without enzyme levels, it did not show any significant ($P>0.05$) difference from protease supplemented groups. Xylanase enzyme supplemented groups significantly ($P<0.01$) increased VH: CD ratio as compared to without enzyme, protease and multienzymes enzymes groups. However, without and protease and multienzymes enzymes groups did not exhibit any significant ($P>0.05$) difference on VH: CD ratio.

Nabuurs *et al.* (1994) reported that shorter villi and deeper crypts have been associated with the presence of toxins. Shortening of the villi decreases the surface area for nutrient absorption. The crypt can be regarded as the villus factory and a large crypt indicates rapid tissue turnover and a high demand for new tissue. Shorter villus and deeper crypt resulted in fewer absorptive and more secretory cells. Changes in intestinal morphology can lead to poor nutrient absorption, increased secretion in the gut, diarrhoea, reduced disease resistance and impaired overall performance. Our results are in agreement with Giannenas *et al.* (2017), Wani *et al.* (2017) and Dinani *et al.* (2018). Giannenas *et al.* (2017) reported no significant ($P>0.05$) difference on villus height and villus depth values on feeding corn gluten protein up to 20 % in the diet of broiler chicken. Wani *et al.* (2017) reported that protease supplementation significantly ($P<0.05$) increased villus height than without enzyme groups in RGM diets up to 20% level on intestine morphometry at 42 days post hatch of broiler chicken. Dinani *et al.* (2018) reported RGM can be incorporated safely in broiler diet at the inclusion level of 15% without any adverse effect on intestinal histomorphometry without any enzyme supplementation, but villus height decreased significantly ($P<0.01$) in 20, 25 and 30% RGM levels.

Microbiological Parameters

The data pertaining to influence of different levels of RGM with or without enzymes on total viable count (TVC) and *Lactobacillus* count ($\log_{10}\text{cfu/g}$) in crop and jejunum are presented in Table 6. Feeding different levels of RGM (0, 15 and 17.5%) and their interaction with xylanase, protease and multienzymes did not exhibit any significant ($P>0.05$) difference on microbiological parameter in crop and jejunum. Feeding RGM without or with enzymes (xylanase, protease and multienzymes) on microbiological parameter did not exhibit any significant ($P>0.05$) difference on TVC in jejunum and *Lactobacillus* count in crop and jejunum. Xylanase enzyme supplemented groups significantly ($P<0.05$) decreased TVC in crop as compared to without enzyme and protease groups, but it did not show any significant ($P>0.05$) difference from multienzymes group. However, multienzymes and protease groups did not show any significant difference ($P>0.05$) from without enzymes groups in TVC in crop.

Our results are in agreement with Wani *et al.* (2017), Giannenas *et al.* (2017) and Dinani *et al.* (2018) in terms of microbiological parameter. Wani *et al.* (2017) reported inclusion of RGM up to 20% level with and without protease enzyme supplementation in the diet had no adverse effects on microbial count of broiler chicken in gut. Giannenas *et al.* (2017) reported no change in the *Lactobacillus* spp. populations in gut on feeding low quality protein, corn gluten meal in broiler chicken up to 20% inclusion level. Hence, in our study no adverse effect of the test ingredient *i.e.*, RGM with or without enzymes and their interaction was observed in the treatment groups on gut microbial count. Dinani *et al.* (2018) reported no significant ($P>0.05$) difference in TVC and *Lactobacillus* count in control and up to 30% RGM level in jejunum without any enzyme supplementation.

Conclusion

The study concluded that rice gluten meal feeding and their interaction with protease, xylanase and multienzymes revealed no adverse effect on intestinal histomorphometry and microbiological parameter of broiler chicken up to 17.5% inclusion level. However, xylanase enzyme supplementation at the rate of 10 g per 100 kg feed having activity of 1,50,000 units per g improved gut health in rice gluten meal based diet at 15 and 17.5% inclusion level.

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