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Effect of Feeding Rice Gluten meal on gut health, immunity and intestinal Histomorphometry in broilers

O.P.Dinani*, Pramod Kumar Tyagi, A. B. Mandal, Praveen Kumar Tyagi, Mukesh Singh, M.A.Wani And Dukare Sagar Popat**
ICAR-Central Avian Research Institute, Izatnagar, Bareilly, U.P, 243122
*Corresponding author:O.P.Dinani,Ph.D.scholar, dr_dinani@rediffmail.com
**ICAR-IVRI,Izatnagar-243122

ABSTRACT
A biological experiment of six weeks was undertaken with completely randomized design (CRD) on broiler chickens to investigate the effects of rice gluten meal (RGM) feeding on gut health, immunity and intestinal histomorphometry in broilers. A total of 192 day old chicks were taken and divided into six treatments with 32 chicks with four replicates per treatment. Six experimental diets as per ICAR (2013) were prepared by incorporating five different levels of RGM (0, 10, 15, 20, 25 and 30 %). Chemical analysis on as such basis indicated that RGM contained crude protein 49.94% and gross energy 4742 kcal/kg. In crop, total viable count (TVC) decreased significantly (P<0.01) at 30% RGM inclusion level as compared to control and other dietary treatments. Lactobacillus count were significantly (P<0.01) increased in 25 and 30% RGM levels. In jejunum, no significant (P>0.05) difference were observed in TVC and Lactobacillus count in control and other dietary treatments. Humoral immunity was significantly (P<0.05) better in 30% RGM group as compared to control but cell mediated immunity did not show any significant (P>0.05) difference between control and other dietary treatments. Villus height (VH) decreased significantly (P<0.01) in 20, 25 and 30% RGM levels but villus depth (VD) and VH/VD did not show any significant(P>0.05) difference between control and other dietary treatments. Thus, it is concluded that RGM can safely can be incorporated in broiler diet at the inclusion level of 15% without any adverse effect on humoral immunity, gut health and intestinal histomorphometry.
Keywords: rice gluten meal, gut health, immunity, intestinal histomorphometry

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INTRODUCTION

Poultry industry is the fastest growing sector in Indian agriculture. Feed is the major constituent in the poultry production accounts for 65-75% of total recurring expenditure. 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. Soybean meal (SBM) is the major protein source used in poultry diet. Instability in its production, indiscriminate exports and higher demand has resulted in its shortage for the poultry industry leading to its higher price. Substitution of SBM at reasonable price will lead to economic broiler production [3, 4, 13].

India is second largest producers of rice in world after China, producing approximately 106.65 MT rice in 2013-14 [1]. Now days, certain newer rice by products are available in appreciable quantities and cheaper rate that can be utilized from rice processing industries such as rice gluten meal (RGM). Rice gluten meal is a by-product of wet-milling of rice obtained after starch extraction and syrup preparation. It is relatively a new feedstuff having brownish color and coarse powdery texture. Initial research finding showed that RGM can be included up to 10% level in broiler chicken without affecting feed efficiency and dressing percentage [20]. Metwally and Farahat [18] found that broiler fed RGM with different inclusion rates up to 12.5% had the same growth performance. Kumar *et al*. [15] found that RGM could replace ground nut cake (GNC) in the concentrate mixture of growing calves up to 75% level without any adverse effect on growth performance and nutrient utilization. Malik *et al*. [16] reported that replacement of ground nut cake (GNC) by RGM and maize gluten meal (MGM) at 75% level did not differ in dry matter intake, feed efficiency, average daily gain (ADG) in growing Sahiwal cattle [17].

No research is available regarding effect of feeding rice gluten meal on gut health, immunity and intestinal histomorphometry in broiler. So, there is need to evaluate RGM effective safe inclusion level in broiler diet due to its increased availability as low cost feed source.

MATERIALS AND METHODS

The research work carried out at the Division of Avian Nutrition and Feed Technology, ICAR-Central Avian Research Institute (CARI), Izatnagar, India in the year 2018. 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).

Experimental design: A biological experiment of six weeks was undertaken with completely randomized design (CRD) on broiler chickens (CARIBRO Vishal) to investigate the effects of rice gluten meal (RGM) feeding on gut health, immunity and intestinal histomorphometry in broilers. A total of 192 day old chicks were taken and divided into six groups with 32 chicks in each treatment. Each replicate consists of 8 chicks housed together in battery cages and 4 replicates allocated for each treatment.

Experimental diets: Six experimental broiler diets *iso-caloric* and *iso-nitrogenous* were prepared by incorporating different levels of rice gluten meal (0, 10, 15, 20, 25 and 30 %) with as per ICAR [14] standard. The diets along with all the used ingredients including rice gluten meal were analyzed for proximate [2], calcium [23] and fibre fractions [24]. *In vitro* pepsin-pancreatin digestibility of RGM and soybean meal was measured according to the method of Gopalkrishnan and Prakash [11]. Mycotoxin (aflatoxin B1 and ochratoxin) screening has been done by thin layer chromatography [2] for RGM.

Statistical analysis: Data was subjected to test of significance as per complete randomized design [9].Treatments means were separated using Duncan's multiple range test [22]. The SPSS (Statistical Package for the Social Sciences) software program (IBM Corporation, Somers, NY, USA) version 16.0 used for analysis of data.

Microbial status: At the end of trial (42 days), eight birds from each dietary treatment were sacrificed by cervical dislocation; crop and jejunum scraping were collected in sterile vials for evaluation of total microbial load colonization. Microbial populations were determined by serial dilution (10^4 to 10^6) of crop and jejunum samples in anaerobic diluents before inoculation onto petri dishes of sterile agar as described by Bryant and Burkey [5]. Total bacterial count and *Lactobacilli* was grown on nutrient agar and rogosa agar respectively [8]. Colony forming units were defined as distinct colonies measuring at least 1 mm in diameter expressed in cfu/g.

Histometry: Samples from jejunum will be taken from four birds per treatment at the end of experiment.All the light microscopic variables will be 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 in 1 will be embedded in paraffin and sections of 5-micron thickness of each sample will be placed on a glass slide and stained with hematoxyline and eosine for examination [7].

Immunity: Humoral immune response estimated by method of Siegel and Gross [21] will be followed for slight modified by Saxena *et al*. [19] assaying the immune response to sheep red blood cells (SRBCs).The *in vivo* cell mediated immune response to PHA-P will be evaluated by the method of Cheng and Lamont [6]on 29th day post hatch. Eight broilers (4 males and 4 females) per treatment were used for assaying immune response.

Statistical analysis: Data was subjected to test of significance as percomplete randomized design [22].Treatments means were separated using Duncan's multiple range test [9]. The SPSS (Statistical Package for the Social Sciences) software program (IBM Corporation, Somers, NY, USA) version 16.0 used for analysis of data.

RESULTS AND DISCUSSION

Chemical composition of test material and diets: Experimental diets ingredients and nutrient composition as prestarter (0-2 wk) and starter (2-3 wk) diets has been given in the table no.1 and finisher diets (3-6 wk) in the table no.2 as per ICAR (2013) feeding standard.RGM used in this experiment was analyzed and contained (%) Moisture 7.64, Dry matter (DM) 92.36,Crude protein (CP) 49.94, Ether extract (EE) 5.79,Crude fibre (CF) 7.43, Total ash (TA) 3.31,Acid insoluble ash (AIA) 0.89,Nitrogen free extract (NFE) 33.53, Calcium 0.84, Phosphorus 0.98, Neutral detergent fibre (NDF) 43.41, Acid detergent fibre (ADF) 16.24, Acid detergent soluble (ADS) 27.17, Acid detergent lignin (ADL) 1.54 and Gross energy (kcal/kg) 4742 on as basis. *In vitro* pepsin-pancreatic digestibility (IVPPD) of RGM was found 81.92%

while IVPPD of soybean meal was observed 88.15%. No detectable aflatoxin B1 and ochratoxin has been found in RGM.

Metwally and Farhat [18] reported higher value of RGM in terms of protein (57.60%), but lower values of RGM in terms of EE (3.16%) and CF (1.45%) as compared to our results. Similarly Kumar *et al*. [15] reported lower values of RGM in terms of protein (46.40%) and EE (3.40%) as compared to our results. Furthermore, the drying process can have crucial influence not only on variability of nutrients but also on concentration and availability of nutrients in different samples.

Microbial status: The data pertaining to influence of different levels of RGM on total viable count (TVC) and *Lactobacillus* count in crop and jejunum have been presented in Tables 3. In crop, total viable count (TVC) decreased significantly (P<0.01) at 30% RGM inclusion level as compared to control and other dietary treatments. *Lactobacillus* count were significantly (P<0.01) increased in 25 and 30% RGM levels. In jejunum, no significant (P>0.05) difference were observed in TVC and *Lactobacillus* count in control and other dietary treatments.

Table 1. Ingredients and nutrient composition of pre-starter and starter diets for different level of RGM

Pre-Starter Diets (0-2 wk)								Starter Diets (2-3 wk)					
Ingredients	D1	D2	D3	D4	D5	D6		D1	D2	D3	D4	D5	D6
Maize	54.32	58.0	59.8	61.0	59.0	57.5		55.63	59	59.92	61.42	62.7	60.75
SBM	38	26.50	20.40	14.60	8.60	2.20		37.1	25.2	19.3	13.24	7.2	1.2
DORB	0	0	0.00	0.00	2.90	5.70		0	0	0.5	0.7	1	3.8
Oil	3.10	1.40	0.60	0.00	0.00	0.00		3.5	2	1.4	0.65	0	0
RGM	0	10	15.00	20.00	25.00	30.00		0	10	15	20	25	30
LSP	1.40	1.30	1.30	1.30	1.30	1.30		1.35	1.37	1.32	1.3	1.27	1.26
DCP	1.82	2	2	2	2	2		1.55	1.6	1.7	1.76	1.8	1.85
Lysine	0	0	0.1	0.2	0.3	0.5		0	0	0.06	0.16	0.26	0.37
Methionine	0.2	0.1	0.1	0.0	0.0	0.0		0.1	0.06	0.03	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	100.00	100.00	99.995	99.975	100.015	100.015		100.00	100.00	100.00	100.00	100.00	100.00
Nutrient composition (%)													
CP	21.97	22.01	21.97	21.98	22.04	21.98		21.51	21.52	21.53	21.51	21.49	21.53
ME (kcal/kg)**	3003.2	3000.4	2998.2	3000.1	2998.6	3002.3		3049.7	3053.4	3053.4	3050.4	3051.0	3049.2
Ca	1.03	1.00	1.00	1.02	1.03	1.03		0.95	0.95	0.95	0.95	0.95	0.96
P	0.45	0.45	0.44	0.45	0.45	0.45		0.40	0.39	0.40	0.40	0.40	0.40
Lysine	1.18	1.20	1.19	1.19	1.19	1.18		1.31	1.15	1.09	1.08	1.07	1.07
Methionine	0.52	0.52	0.52	0.51812	0.52	0.54		0.48	0.48	0.48	0.48	0.50	0.51
Threonine	0.81	0.83	0.82	0.81	0.81	0.81		0.80	0.79	0.78	0.79	0.78	0.78
Cost (Rs.per kg)	28.60	25.67	24.58	23.67	23.00	22.32		28.03	25.60	24.56	23.47	22.62	22.07

In prestarter diet *Constant 0.765 includes salt 0.4%, trace mineral premix 0.10%, vitamin premix 0.15%, vit. B complex 0.015%, choline chloride 0.05% and toxin binder 0.05%.(As per ICAR,2013) **calculated value

Table 2. Ingredients and nutrient composition of finisher diets (3-6 wk)

Ingredients	D1	D2	D3	D4	D5	D6
Maize	62	65.36	67.07	68.51	67.82	67.82
SBM	31.3	19.4	13.4	7.5	1.4	1.4
DORB	0	0	0	0	1.9	1.9
oil	3.2	1.7	0.9	0.2	0	0
RGM	0	10	15	20	25	25
Marbal Chips	0.7	0.63	0.64	0.6	0.56	0.56
LSP	0.5	0.5	0.5	0.5	0.5	0.5
DCP	1.45	1.6	1.6	1.7	1.75	1.75

Lysine	0	0.02	0.12	0.22	0.3	0.3
Methionine	0.06	0.02	0	0	0	0
Constant*	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
Nutrient composition (%)						
CP	19.50	19.51	19.49	19.50	19.49	19.49
ME (kcal/kg)**	3100.2	3101.9	3099.1	3098.1	3101.3	3101.3
Ca	0.86	0.85	0.85	0.85	0.85	0.85
P	0.38	0.38	0.38	0.38	0.39	0.38
Lysine	0.98	0.97	0.98	0.97	0.96	0.96
Methionine	0.42	0.42	0.43	0.45	0.46	0.46
Threonine	0.68	0.67	0.68	0.69	0.67	0.68
Cost (Rs./ kg)	26.71	26.89	25.83	25.00	24.31	24.31

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%. (As per ICAR,2013) **calculated value

Table 3. Effect of feeding different levels of RGM on microbiological parameters (cfu/g)

		Crop		Jejunum	
Treatment	**RGM (%)**	**TVC**	**Lactobacillus count**	**TVC**	**Lactobacillus count**
T1	**0**	6.56^{bc}	3.71^{a}	6.54	3.75
T2	**10**	6.62^{bc}	3.74^{a}	6.74	3.52
T3	**15**	6.79^{c}	3.80^{ab}	6.47	3.56
T4	**20**	6.50^{ab}	3.86^{abc}	6.63	3.68
T5	**25**	6.38^{ab}	3.96^{bc}	6.58	3.57
T6	**30**	6.25^{a}	4.00^{c}	6.60	3.54
	Pooled SEM	0.046	0.0302	0.0344	0.0421
	P value	(P<0.01)	(P<0.01)	NS	NS

Values bearing different superscripts within the column differ significantly

Table 4. Effect of feeding different levels of RGM on immunological parameters

Treatment	**RGM (%)**	**HA (log2)**	**CMI (mm)**
T1	**0**	2.59^{ab}	0.60
T2	**10**	2.51^{a}	0.58
T3	**15**	2.53^{ab}	0.60
T4	**20**	2.57^{ab}	0.57
T5	**25**	2.69^{bc}	0.56
T6	**30**	2.77^{c}	0.57
	Pooled SEM	0.024	0.005
	P value	(P<0.05)	NS

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

Table 5. Effect of feeding different levels of RGM on intestinal morphometry

Treatment	**RGM (%)**	**Villus height (VH)**	**Villus depth (VD)**	**VH:CD**	**Villus width**
T1	**0**	1194^{d}	167	7.2	92b
T2	**10**	1124^{cd}	176	6.5	90b
T3	**15**	1113^{cd}	150	7.5	84ab
T4	**20**	1058^{bc}	151	7.1	80a
T5	**25**	988^{ab}	152	6.5	80a
T6	**30**	941^{a}	134	7.0	78a
	Pooled SEM	20.952	4.354	0.117	1.518
	P value	(P<0.01)	NS	NS	(P<0.05)

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

Lactobacillus is the major component of the microbial barrier to infection. It is suggested that the composition of RGM in that it is more fibrous offers more nutrients to *Lactobacillus.* Our results are in

agreement with Giannenas *et al.*[10], who reported no change in the lactobacillus spp. populations in gut on feeding low quality protein, corn gluten meal in broiler chicken upto 20% inclusion level.

Immunity: The data pertaining to influence of different levels of rDDGS on humoral and cell mediated immunity have been presented in Table 3. Humoral immunity was significantly (P<0.05) better in 30% RGM group as compared to control but cell mediated immunity did not show any significant (P>0.05) difference between control and other dietary treatments. Better humoral immunity in 30% RGM diets may be associated with type and composition of amino acids particularly methionine present in higher level in RGM.

Intestinal histomorphometry: **T**he histometrical changes of villus height, width, crypt depth and villus height/crypt depth of duodenum as influenced by dietary addition of different levels of rice in broiler diets are presented in Table 4. Villus height (VH) decreased significantly(P<0.01) in 20, 25 and 30% RGM levels but villus depth (VD) and VH/VD did not show any significant(P>0.05) difference between control and other dietary treatments. Villus width (VW) decreased significantly (P<0.05) in 20, 25 and 30% RGM levels. Our results are in agreement with Giannenas *et al.* (2017) who reported no significant (P>0.05) difference on VH and CD values on feeding corn gluten protein up to 20 % in the diet of broiler chicken. Contrary to this Gupta [12] reported improvement in intestinal morphology by incorporating rice based dry distiller's grains with soluble. Decrease in villus length in 20% and above RGM groups may be associated with poor digestibility at this inclusion level.

CONCLUSIONS

Thus, it is concluded that RGM can be incorporated safely in broiler diet at the inclusion level of 15% without any adverse effect on humoral immunity, gut health and intestinal histomorphometry.

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