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# Effect of feeding rice distillers dried grains with solubles (rDDGS) and rice gluten meal (RGM) based diet on the gut health of broiler chicken

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## Abstract
A biological experiment was conducted to evaluate the effect of feeding rice distillers dried grains with solubles (rDDGS) and rice gluten meal (RGM) combination as a replacement of soybean along with exogenous enzyme supplementation on the intestinal morphometry and microbiology of broiler chicken at 21 days of age. Following a 2x3 factorial design, the experimental diets were: T1 (no rDDGS/RGM/enzyme), T2 (no rDDGS/RGM, with multienzymes), T3 (12.5% rDDGS, 15% RGM, no enzyme), T4 (12.5% rDDGS, 15% RGM, with protease enzyme), T5 (10% rDDGS, 12.5% RGM, no enzyme), and T6 (10% rDDGS, 12.5% RGM, with protease enzyme). Each treatment was allocated 5 replicates of broiler chicken with 8 birds in each. The feeding of the dietary combination of 12.5% rDDGS and 15% RGM significantly ($P<0.05$) decreased the villus height (VH), crypt depth (CD), VH: CD ratio and villus width compared to the control and other rDDGS & RGM combination. The microbiological parameters revealed that TVC in crop and jejunum were increased significantly ($P<0.01$) in 12.5% rDDGS & 15% RGM as compared to control and other dietary combination. *Lactobacillus* count in crop was decreased significantly ($P<0.05$) in12.5% rDDGS & 15% RGM as compared control. It was concluded that the simultaneous inclusion of rDDGS and RGM in broiler chicken ration with or without enzyme supplementation had adverse effects on the intestinal histomorphometry and microbiology.

**Keywords:** Rice distillers dried grains with solubles (rDDGS), rice gluten meal (RGM), broiler chicken, intestinal morphometry, microbiology

## Introduction
Feed is the major constituent in the poultry production which accounts for 65-75% of total recurring expenditure. Therefore, efforts are being made to reduce the cost of production by employing alternative feed ingredients in the broiler chicken ration. India is one of the largest producers of rice in world producing approximately 105 million tons of rice in 2015-16 [6]. The rice processing industries provides us a variety of by products, particularly rice distillers dried grains with solubles (rDDGS) and rice gluten meal (RGM) which potential economic feed ingredients to be used in poultry ration. This rising cost of production in broiler chicken production can be overcome by the use of 'rice DDGS and rice gluten meal', protein rich alternative feed ingredients. The rDDGS is an excellent low cost alternative feed ingredient that continues to be produced in large quantities by the dry-grind fuel ethanol industry. An increase in ethanol production over the last 5-10 years, due to increasing prices of conventional oil and limited underground reserves, has led to an increased supply of DDGS that is available as livestock feed [14]. However, the lower amount of available lysine in DDGS, which occurs due to drying process while DDGS production, can hamper the growth and efficiency of broiler chicken. On the other hand, the RGM, a by-product of wet-milling of rice, is available in appreciable amounts at the lower cost compared to soybean. RGM contains 3152 kcal ME/kg, 46.45% crude protein, 3.4% ether extract and a favorable amino acid profile with a relatively higher abundance of methionine [11]. It has been also designated as a source of rumen undegradable protein with the highest known metabolizable protein value among plant proteins [9]. Further, the exogenous enzyme supplementations in poultry diets, nutritionally, economically and environmentally justified, increase nutrient digestibility, reduce water content and viscosity of the excreta, and accelerate the rate of passage of digesta through the gastrointestinal tract [12, 15].

Thus, it is pertinent that the use of rDDGS, RGM along with enzyme supplementation will have a significant impact on the gut morphology, physiology, and microbial environment. Keeping these facts in view the current study was aimed to evaluate the effects of feeding different combinations of rDDGS and RGM levels along with enzyme supplementation on the intestinal histology and microbiology of broiler chicken.

## Materials and Methods

### Birds, experimental diets and design

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). A total of 240 broiler chicks of the same hatch and uniform weight were used in the study. The birds were housed in specially designed battery brooder cages with watering and feeding facilities, and were reared under standard management conditions. The broiler chicken diets were formulated as per the recommendations of ICAR [10] by employing rDDGS and RGM as replacement of soybean meal in the basal diets along with enzyme supplementation. The feed ingredients and the nutrient composition of the pre-starter, starter and finisher diets have been given in Table 1. A preliminary trial was conducted to select the suitable levels of rDDGS & RGM and suitable enzyme. The rDDGS levels of 10% & 12.5 %, and RGM levels of 12.5% & 15% were selected and multienzyme & protease enzymes were selected to formulate the experimental diets. Following a 2x3 factorial design, the experimental diets were: T1 (no rDDGS/RGM/enzyme), T2 (no rDDGS/RGM, with multienzymes), T3 (12.5% rDDGS, 15% RGM, no enzyme), T4 (12.5% rDDGS, 15% RGM, with protease enzyme), T5 (10% rDDGS, 12.5% RGM, no enzyme), and T6 (10% rDDGS, 12.5% RGM, with protease enzyme). Each treatment was allocated 5 replicates of chicks, with 8 birds in each replicate. 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.

### Sampling and measurement

For the purpose of sampling five birds from each treatment were sacrificed. At 21 days of age the samples from jejunum were taken from five birds per treatment, and two cross-sections per sample with four measurements per cross-section (40 measurements per treatment) were undertaken. The measurements were in the form of villus height (VH), villus width (VW), crypt depth (CD), and VH: CD ratio. The histological slides of cross-sections were prepared by microtome and stained with hematoxyline and eosine for examination [2]. The examination of the stained histological slides was done by optical microscope (Motic Inverted microscope, Honkonga), at 10X magnification.

For evaluation of microbiological status of broiler chicken at 21 days of age, the crop and jejunum samples from five birds per treatment were collected in sterile vials. The 10 fold serial dilutions of the samples collected were formed before inoculation in the agar containing petri dishes. The Total viable count (TVC) and *Lactobacilli* count were determined by using nutrient agar and Rogosa SL agar, respectively [3]. Each sample was replicated three times. For TVC, the inoculated petri plates were incubated at 37oC for 24 h and for *Lactobacillus* count plates were incubated anaerobically 37oC for 24 h. The bacterial count was measured as following:

$$\text{CFU / g} = \frac{\text{Total No. of colony counted x Dilution factor}}{\text{Volume of aliquot taken}}$$

The data collected was subjected to two way ANOVA to present the results as means and standard errors [10] by using statistical package for social sciences (SPSS) 16.0 version and the comparison of significant mean differences was as per Duncan's multiple range test [4].

## Results and Discussion

### Intestinal histomorphometry

The effects of feeding different levels of rDDGS and RGM combinations with enzyme supplementation on the intestinal histomorphometry of broiler chicken at 21 days of age have been given in Table 2. The results revealed that 0% rDDGS +0% RGM (DR1), 12.5% rDDGS +15% RGM (DR2) and 10 % rDDGS +12.5 % RGM (DR3) combination levels showed villus height and villus width decreased significantly ($P$<0.01) in DR2 and DR3 as compared to DR1 level, however there was no significant ($P$>0.05) difference in DR2 as compared to DR3 level. Crypt depth decreased significantly ($P$<0.01) in DR3 as compared to DR2, but DR3 did not show any significant ($P$>0.05) difference from DR1 in crypt depth. Villus height and crypt depth (VH: CD) ratio decreased significantly ($P$<0.01) in DR2 as compared to DR1 and DR3 levels. Villus height and crypt depth (VH: CD) ratio increased significantly ($P$<0.01) in DR3 as compared to DR2, but it was decreased significantly ($P$<0.01) as compared to DR1 level. Enzymes supplementation did not exhibit any significant ($P$>0.05) difference on intestinal histomorphometry. Interaction of rDDGS and RGM combination with or without enzymes did not show any significant (P>0.05) difference on crypt depth, VH: CD ratio and villus width. Villus height was decreased significantly ($P$<0.05) in T3, T4 and T5 groups as compared to control, T2 and T6 groups. Also, villus height was decreased significantly ($P$<0.05) in T6 group as compared to control and T2 groups.

Not with standing our knowledge there is no literature available dealing with the combination of rDDGS& RGM along with enzyme supplementation in broiler chicken ration. However, there are many reports where DDGS and RGM have been used individually in poultry ration. It has been reported that intestinal histomorphology revealed no adverse effects of feeding DDGS containing ration to broiler chicken up to 28 days post hatch [13]. Similarly, in layers no negative effects on the intestinal morphology was observed by DDGS inclusion up to 10% level in ration [7]. No significant differences have been reported in VH and CD values by feeding of corn gluten protein up to 20% in the diet of broiler chicken [5]. However, on the other hand enzyme supplementation was shown to cause a significant ($P$<0.05) increase in VH and decrease in CD, whereas RGM up to 20% level of inclusion with or without enzyme supplementation in the diet had no adverse effects on intestine morphometry of broiler chicken [18].

**Table 1:** Ingredient and nutrient composition of broiler chicken diets

| Ingredients (%) | Pre-starter diet (0-14days) | | | | | | Starter diet (14-28days) | | | | | | Finisher diet (28-42days) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **T1** | **T2** | **T3** | **T4** | **T5** | **T6** | **T1** | **T2** | **T3** | **T4** | **T5** | **T6** | **T1** | **T2** | **T3** | **T4** | **T5** | **T6** |
| Maize | 54.42 | 54.42 | 58.58 | 58.58 | 57.78 | 57.78 | 55.63 | 55.63 | 60.97 | 60.97 | 59.81 | 59.81 | 62.00 | 62.00 | 66.77 | 66.77 | 65.61 | 65.61 |
| SBM | 38.40 | 38.40 | 8.80 | 8.80 | 14.30 | 14.30 | 37.10 | 37.10 | 7.40 | 7.40 | 13.00 | 13.00 | 31.30 | 31.30 | 2.00 | 2.00 | 7.50 | 7.50 |
| DORB | 0.00 | 0.00 | 0.70 | 0.70 | 0.90 | 0.90 | 0.00 | 0.00 | 12.50 | 12.50 | 10.00 | 10.00 | 3.22 | 3.22 | 0.00 | 0.00 | 0.70 | 0.70 |
| DDGS | 0.00 | 0.00 | 12.50 | 12.50 | 10.00 | 10.00 | 0.00 | 0.00 | 15.00 | 15.00 | 12.50 | 12.50 | 0.00 | 0.00 | 12.50 | 12.50 | 10.00 | 10.00 |
| RGM | 0.00 | 0.00 | 15.00 | 15.00 | 12.50 | 12.50 | 3.50 | 3.50 | 0.20 | 0.20 | 0.80 | 0.80 | 0.00 | 0.00 | 15.00 | 15.00 | 12.50 | 12.50 |
| Oil | 3.00 | 3.00 | 0.00 | 0.00 | 0.40 | 0.40 | 1.35 | 1.35 | 1.15 | 1.15 | 1.23 | 1.23 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| LSP | 1.40 | 1.40 | 1.30 | 1.30 | 1.10 | 1.10 | 1.55 | 1.55 | 1.70 | 1.70 | 1.67 | 1.67 | 0.70 | 0.70 | 0.40 | 0.40 | 0.33 | 0.33 |
| DCP | 1.82 | 1.82 | 2.00 | 2.00 | 2.00 | 2.00 | 0.00 | 0.00 | 0.32 | 0.32 | 0.22 | 0.22 | 1.45 | 1.45 | 1.70 | 1.70 | 1.64 | 1.64 |
| Lysine | 0.00 | 0.00 | 0.35 | 0.35 | 0.25 | 0.25 | 0.10 | 0.10 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.36 | 0.36 | 0.30 | 0.30 |
| Methionine | 0.20 | 0.20 | 0.00 | 0.00 | 0.00 | 0.00 | 0.765 | 0.765 | 0.765 | 0.765 | 0.765 | 0.765 | 0.06 | 0.06 | 0.00 | 0.00 | 0.00 | 0.00 |
| Constant* | 0.765 | 0.765 | 0.765 | 0.765 | 0.765 | 0.765 | 55.63 | 55.63 | 60.97 | 60.97 | 59.81 | 59.81 | 0.77 | 0.77 | 0.77 | 0.77 | 0.77 | 0.77 |
| Enzyme | -- | M | -- | P | -- | P | -- | M | -- | P | -- | P | -- | M | -- | P | -- | P |
| **Nutrient composition** | | | | | | | | | | | | | | | | | | |
| CP (%) | 21.99 | 21.99 | 22.02 | 22.02 | 22.02 | 22.02 | 21.52 | 21.52 | 21.50 | 21.50 | 21.49 | 21.49 | 19.51 | 19.51 | 19.52 | 19.52 | 19.49 | 19.49 |
| ME (kcal/kg) | 2998 | 2998 | 3002 | 3002 | 2998 | 2998 | 3050 | 3050 | 3051 | 3051 | 3050 | 3050 | 3100 | 3100 | 3099 | 3099 | 3104 | 3104 |
| Ca (%) | 1.03 | 1.03 | 1.08 | 1.08 | 1.00 | 1.00 | 0.95 | 0.95 | 0.95 | 0.95 | 0.96 | 0.96 | 0.86 | 0.86 | 0.85 | 0.85 | 0.86 | 0.86 |
| Available P (%) | 0.45 | 0.45 | 0.45 | 0.45 | 0.46 | 0.46 | 0.41 | 0.41 | 0.40 | 0.40 | 0.40 | 0.40 | 0.38 | 0.38 | 0.39 | 0.39 | 0.39 | 0.39 |
| Lysine (%) | 1.19 | 1.19 | 1.19 | 1.19 | 1.20 | 1.20 | 1.38 | 1.38 | 1.12 | 1.12 | 1.13 | 1.13 | 1.20 | 1.20 | 1.00 | 1.00 | 1.04 | 1.04 |
| Methionine (%) | 0.52 | 0.52 | 0.53 | 0.53 | 0.51 | 0.51 | 0.48 | 0.48 | 0.53 | 0.53 | 0.50 | 0.50 | 0.41 | 0.41 | 0.50 | 0.50 | 0.48 | 0.48 |
| Threonine (%) | 0.80 | 0.80 | 0.81 | 0.81 | 0.82 | 0.82 | 0.79 | 0.79 | 0.81 | 0.81 | 0.80 | 0.80 | 0.86 | 0.86 | 0.79 | 0.79 | 0.81 | 0.81 |
| Cost (Rs./kg) | 28.52 | 28.93 | 23.02 | 23.63 | 23.68 | 24.29 | 28.03 | 28.43 | 22.88 | 23.48 | 23.65 | 24.25 | 26.72 | 26.72 | 22.03 | 22.03 | 22.93 | 22.93 |

SBM: Soybean meal,, DORB: Deoiled rice bran, DDGS: Dried distillers grains with solubles, RGM: Rice gluten meal, LSP: Limestone powder, DCP: Di-calcium phosphate, CP: Crude protein, ME: Metabolizable energy
*Constant (0.4% salt, 0.1% trace mineral premix, 0.15% vitamin premix, 0.015% vit. B complex, 0.05% choline chloride and 0.05% Toxin binder)
1: Trace mineral premix supplied (mg/kg diet): Mg 300; Mn 55; I 0.4; Fe 56; Zn 30; Cu 4.
2: Vitamin premix supplied (per kg diet): Vitamin A 8250 IU; Vitamin D3 1200 IU; Vitamin K 1mg; Vitamin E 40 IU.
3: B complex: Vitamin B1 2 mg; Vitamin B2 4 mg; Vitamin B12 10 µg; niacin 60 mg; pantothenic acid 10 mg; choline 500mg.

**Table 2:** Effect of feeding different level of rDDGS and RGM combinations on histomorphometryin broiler chicken

| rDDGS | RGM | Enzyme | VH (µ) | CD (µ) | VH:CD | VW (µ) |
|---|---|---|---|---|---|---|
| 0.0 % | 0.0 % | - | 6.75$^{c}$ | 130 | 6.75$^{c}$ | 117 |
| 0.0 % | 0.0 % | M | 6.67$^{c}$ | 122 | 6.67$^{c}$ | 115 |
| 12.5 % | 15.0 % | - | 4.92$^{a}$ | 132 | 4.92$^{a}$ | 100 |
| 12.5 % | 15.0 % | P | 4.55$^{a}$ | 135 | 4.55$^{a}$ | 94 |
| 10.0 % | 12.5 % | - | 4.77$^{a}$ | 122 | 4.77$^{a}$ | 98 |
| 10.0 % | 12.5 % | P | 5.85$^{b}$ | 109 | 5.85$^{b}$ | 92 |
| Pooled SEM | | | 24.41 | 3.25 | 0.20 | 3.25 |
| **Main effects** | | | | | | |
| rDDGS/RGM combination | | | | | | |
| 0.0 % | 0.0 % | | 836$^{b}$ | 125$^{ab}$ | 6.71$^{c}$ | 115$^{b}$ |
| 12.5 % | 15.0 % | | 632$^{a}$ | 133$^{b}$ | 4.73$^{a}$ | 97$^{a}$ |
| 10.0 % | 12.5 % | | 605$^{a}$ | 115$^{a}$ | 5.31$^{b}$ | 94$^{a}$ |
| Enzyme supplementation | | | | | | |
| Without enzyme | | | 699 | 128 | 5.48 | 105 |
| With enzyme | | | 683 | 122 | 5.69 | 100 |
| **Significance** | | | | | | |
| rDDGS/RGM combination | | | P<0.01 | P<0.01 | P<0.01 | P<0.01 |
| Enzyme supplementation | | | NS | NS | NS | NS |
| Interaction | | | NS | NS | P<0.05 | NS |

Values bearing different superscripts within the column differ significantly
NS: Non-significant (P>0.05)
VH: Villus height, CD: Crypt depth, VW: Villus width
M: Multienzyme, P: Protease

**Table 3:** Effect of feeding different level of rDDGS and RGM combinations on intestinal microbiology (log 10 cfu/g)

| rDDGS | RGM | Enzyme | Crop | | Jejunum | |
|---|---|---|---|---|---|---|
| | | | TVC | *Lactobacillus* | TVC | *Lactobacillus* |
| 0.0 % | 0.0 % | - | 5.13 | 3.16 | 4.88 | 2.81 |
| 0.0 % | 0.0 % | M | 5.06 | 3.31 | 5.08 | 2.58 |
| 12.5 % | 15.0 % | - | 5.60 | 3.08 | 4.60 | 2.87 |
| 12.5 % | 15.0 % | P | 5.18 | 3.05 | 4.62 | 2.99 |
| 10.0 % | 12.5 % | - | 5.20 | 3.09 | 4.49 | 2.85 |
| 10.0 % | 12.5 % | P | 4.94 | 3.16 | 4.65 | 2.82 |
| Pooled SEM | | | 24.41 | 3.25 | 0.05 | 0.02 |
| **Main effects** | | | | | | |
| rDDGS/RGM combination | | | | | | |
| 0.0 % | 0.0 % | | 5.09$^{a}$ | 3.24$^{b}$ | 4.98$^{a}$ | 2.70 |
| 12.5 % | 15.0 % | | 5.39$^{b}$ | 3.07$^{a}$ | 4.61$^{b}$ | 2.93 |
| 10.0 % | 12.5 % | | 5.08$^{a}$ | 3.12$^{ab}$ | 4.57$^{a}$ | 2.83 |
| Enzyme supplementation | | | | | | |
| Without enzyme | | | 5.06$^{a}$ | 3.11 | 4.65 | 2.84 |
| With enzyme | | | 5.30$^{b}$ | 3.17 | 4.78 | 2.79 |
| **Significance** | | | | | | |
| rDDGS/RGM combination | | | P<0.01 | P<0.05 | P<0.01 | NS |
| Enzyme supplementation | | | P<0.01 | NS | NS | NS |
| Interaction | | | NS | NS | NS | NS |

Values bearing different superscripts within the column differ significantly
NS: Non-significant (P>0.05)
TVC: Total viable count

## Intestinal microbiology

The effects of feeding different levels of rDDGS and RGM combinations with enzyme supplementation on the intestinal microbiology of broiler chicken at 21 days of age have been given in Table 3. The results revealed that 0% rDDGS +0% RGM (DR1), 12.5% rDDGS +15% RGM (DR2) and 10 % rDDGS +12.5 % RGM (DR3) combination levels showed TVC in crop was increased significantly (*P*<0.01) in DR2 as compared to DR1 and DR3, but TVC in crop did not show any significant (*P*>0.05) difference in DR3 as compared to DR1 level. *Lactobacillus* count in crop was decreased significantly (*P*<0.05) in DR2 as compared to DR1, but *Lactobacillus* count in crop did not show any significant (*P*>0.05) difference in DR3 as compared to DR1 level. Total viable count in jejunum was increased significantly (*P*<0.01) in DR2 as compared to DR1 and DR2 levels, but TVC in jejunum did not show any significant (*P*>0.05) difference in DR3 as compared to DR1 level. *Lactobacillus* count in jejunum did not show any significant (*P*>0.05) difference in rDDGS and RGM combinations. Effect of enzyme supplementation revealed that TVC in jejunum, *Lactobacillus* count in crop and jejunum did not show any significant (*P*>0.05) difference with or without enzyme supplementation. Total viable count in crop was decreased significantly (*P*<0.01) in enzyme supplemented group as compared to without enzyme groups. Interaction of rDDGS and RGM combination with or without enzymes did not show any significant (*P*>0.05) difference on microbiological parameters in crop and jejunum.

There is no literature available regarding the use of rDDGS& RGM jointly along with enzyme supplementation in broiler chicken to substantiate the results of the present study. However, there are many reports where rDDGS and RGM have been used individually in broiler chicken ration along with enzyme supplementation. The corn DDGS diet is reported to have a greater ($P < 0.05$) lactic acid concentration than the wheat DDGS diet which has been implicated in inhibition of the growth of pathogens [1, 17]. The diets containing corn DDGS had a significantly higher count of *Lactobacillus* sp. compared to control diet [8]. The rDDGS in layer diet up to the inclusion level of 10% decreased TVC and improved *Lactobacillus* count [7]. This could be because DDGS provides more fiber to be used by the *Lactobacillus* as source of nutrients. On the other hand the inclusion of RGM up to 20% level with or without protease enzyme supplementation in the diet revealed no significant effects on the intestinal microbiology of broiler chicken in gut [18].

## Conclusion

From the results of this study, it can be concluded that the simultaneous inclusion of rDDGS and RGM in broiler chicken ration with or without enzyme supplementation had adverse effects on the intestinal histomorphometry and microbiology.

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