Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2020; 8(4): 85-89

Received: 28-05-2020
Accepted: 30-06-2020

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# Effect of feeding rice based distillers dried grains solubles with and without enzymes on immunity of broiler chicken

**Om Prakash Dinani, Pramod Kumar Tyagi, Rokade JJ and SK Bhanja, JS Tyagi, Narayan Dutta and Gopi M**

## Abstract
A biological experiment of 384 broiler chicks was conducted to evaluate the effect of feeding rice based distillers dried grain with solubles (rDDGS) as soybean replacement without or with different enzymes on immunity in broiler chickens for 42 days as per 3x4 factorial completely randomized design. There were twelve different treatments with 4 replicates for each treatment and each replicate consisted of 8 chicks. Two levels of rDDGS were taken (12.5 and 15%). Protease, xylanase and multienzymes supplementation under different treatments were done. The humoral immunity was significantly ($P$<0.01) better at 12.5% and 15% rDDGS levels compared to control. Protease enzyme supplementation improved significantly ($P$<0.05) humoral immunity at 15% rDDGS level. Thus, it may be concluded that 12.5% and 15% rDDGS levels with or without protease supplementation improved immune response in broiler chickens in terms of humoral immunity.

**Keywords:** Enzyme, immunity, protease, rice based distillers dried grain with solubles

## Introduction
Poultry sector is one of the fastest growing sub-sector in agriculture of our country. Feed is the major recurring constituent in the poultry farming accounts for about 65-70% of total cost of production. Feed costs are mainly decided by the cost of protein ingredients in the diet used. Replacement of high cost protein ingredients with lower cost ingredients would potentially reduce the cost of the poultry production. 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 2020-2025. As there is scarcity of soybean meal at reasonable price, there is need to utilize locally available alternate protein sources [7].
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 distillers dried grain with solubles (DDGS). The DDGS is co-product of the ethanol industry produced during dry milling process. Its availability is increasing due to higher demand for ethanol as biofuel. Clean fuel policy in the country has given the guideline to incorporate 10% ethanol in petrol along with Ministry of Petroleum has given the mandate of using 20% ethanol in petrol by the year 2025 [2]. It will further lead to increase availability of DDGS. It contained 65% distillers grains and 35% distillers solubles on dry matter basis [1]. The DDGS contain all the nutrients from grain in a concentrated form except for the majority of the starch, which has been utilized in the fermentation process during ethanol production. So, it concentrates all nutrients about three fold present in the cereal since two third to three fourth portion of cereal content is starch. The enhanced availability and potential cost-benefit of DDGS represents a substantial economic value as it is less expensive than other protein sources like soybean [13].
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 DDGS is increasing due to relative lower price, increased production and easy availability. Nutritional status is positively related with immunity of the individual. 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 rice DDGS. Most of the researches are limited to corn, wheat, sorghum, barley based DDGS. Very scanty researches were done in rice DDGS regarding its effect on immunity [6] in poultry but few studies were done by some researchers in other than rice DDGS on immunity [3, 4, 6, 8]. In view of above an experiment was conducted to study the effect of feeding rice based distillers dried grains solubles 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 rice based distiller's dry grains with solubles (DDGS) 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 (2013) standard 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.

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

| Ingredients | T1 | T2 | T3 | T4 | T5 | T6 | T7 | T8 | T9 | T10 | T11 | T12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Maize | 54.42 | 54.42 | 54.42 | 54.42 | 55.94 | 55.94 | 55.94 | 55.94 | 56.40 | 56.40 | 56.40 | 56.40 |
| SBM | 38.40 | 38.40 | 38.40 | 38.40 | 25.50 | 25.50 | 25.50 | 25.50 | 22.90 | 22.90 | 22.90 | 22.90 |
| DDGS | 0.00 | 0.00 | 0.00 | 0.00 | 12.50 | 12.50 | 12.50 | 12.50 | 15.00 | 15.00 | 15.00 | 15.00 |
| Oil | 3.00 | 3.00 | 3.00 | 3.00 | 1.80 | 1.80 | 1.80 | 1.80 | 1.52 | 1.52 | 1.52 | 1.52 |
| LSP | 1.40 | 1.40 | 1.40 | 1.40 | 1.30 | 1.30 | 1.30 | 1.30 | 1.20 | 1.20 | 1.20 | 1.20 |
| DCP | 1.82 | 1.82 | 1.82 | 1.82 | 1.83 | 1.83 | 1.83 | 1.83 | 1.83 | 1.83 | 1.83 | 1.83 |
| Lysine | 0.00 | 0.00 | 0.00 | 0.00 | 0.23 | 0.23 | 0.23 | 0.23 | 0.27 | 0.27 | 0.27 | 0.27 |
| Methionine | 0.20 | 0.20 | 0.20 | 0.20 | 0.13 | 0.13 | 0.13 | 0.13 | 0.11 | 0.11 | 0.11 | 0.11 |
| 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 | 22.01 | 22.01 | 22.01 | 22.01 | 22.02 | 22.02 | 22.02 | 22.02 |
| Lysine | 1.19 | 1.19 | 1.19 | 1.19 | 1.23 | 1.23 | 1.23 | 1.23 | 1.23 | 1.23 | 1.23 | 1.23 |
| Methionine | 0.52 | 0.52 | 0.52 | 0.52 | 0.53 | 0.53 | 0.53 | 0.53 | 0.52 | 0.52 | 0.52 | 0.52 |
| Threonine | 0.83 | 0.83 | 0.83 | 0.83 | 0.82 | 0.82 | 0.82 | 0.82 | 0.82 | 0.82 | 0.82 | 0.82 |
| Ca | 1.03 | 1.03 | 1.03 | 1.03 | 1.05 | 1.05 | 1.05 | 1.05 | 1.03 | 1.03 | 1.03 | 1.03 |
| P | 0.45 | 0.45 | 0.45 | 0.45 | 0.45 | 0.45 | 0.45 | 0.45 | 0.45 | 0.45 | 0.45 | 0.45 |
| ME (kcal/kg)** | 2998 | 2998 | 2998 | 2998 | 2998 | 2998 | 2998 | 2998 | 2999 | 2999 | 2999 | 2999 |
| Cost (Rs./ kg) | 28.52 | 29.03 | 29.13 | 28.93 | 26.36 | 26.86 | 26.96 | 26.76 | 25.86 | 26.37 | 26.47 | 26.27 |

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 rDDGS

| Ingredients | T1 | T2 | T3 | T4 | T5 | T6 | T7 | T8 | T9 | T10 | T11 | T12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Maize | 55.63 | 55.63 | 55.63 | 55.63 | 57.66 | 57.66 | 57.66 | 57.66 | 58.10 | 58.10 | 58.10 | 58.10 |
| SBM | 37.10 | 37.10 | 37.10 | 37.10 | 24.10 | 24.10 | 24.10 | 24.10 | 21.40 | 21.40 | 21.40 | 21.40 |
| DDGS | 0.00 | 0.00 | 0.00 | 0.00 | 12.50 | 12.50 | 12.50 | 12.50 | 15.00 | 15.00 | 15.00 | 15.00 |
| Oil | 3.50 | 3.50 | 3.50 | 3.50 | 2.15 | 2.15 | 2.15 | 2.15 | 1.90 | 1.90 | 1.90 | 1.90 |
| LSP | 1.35 | 1.35 | 1.35 | 1.35 | 1.20 | 1.20 | 1.20 | 1.20 | 1.17 | 1.17 | 1.17 | 1.17 |
| DCP | 1.55 | 1.55 | 1.55 | 1.55 | 1.58 | 1.58 | 1.58 | 1.58 | 1.58 | 1.58 | 1.58 | 1.58 |
| Lysine | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.05 | 0.05 | 0.05 | 0.05 |
| Methionine | 0.10 | 0.10 | 0.10 | 0.10 | 0.04 | 0.04 | 0.04 | 0.04 | 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 | 21.54 | 21.54 | 21.54 | 21.54 | 21.50 | 21.50 | 21.50 | 21.50 |
| Lysine | 1.38 | 1.38 | 1.38 | 1.38 | 1.11 | 1.11 | 1.11 | 1.11 | 1.10 | 1.10 | 1.10 | 1.10 |
| Methionine | 0.48 | 0.48 | 0.48 | 0.48 | 0.48 | 0.48 | 0.48 | 0.48 | 0.49 | 0.49 | 0.49 | 0.49 |
| Threonine | 0.78 | 0.78 | 0.79 | 0.79 | 0.81 | 0.80 | 0.80 | 0.81 | 0.81 | 0.81 | 0.81 | 0.81 |
| Ca | 0.95 | 0.95 | 0.95 | 0.95 | 0.96 | 0.96 | 0.96 | 0.96 | 0.96 | 0.96 | 0.96 | 0.96 |
| P | 0.41 | 0.41 | 0.41 | 0.41 | 0.41 | 0.41 | 0.41 | 0.41 | 0.41 | 0.41 | 0.41 | 0.41 |
| ME (kcal/kg)** | 3050 | 3050 | 3050 | 3050 | 3052 | 3052 | 3052 | 3052 | 3053 | 3053 | 3053 | 3053 |
| Cost (Rs./kg) | 28.03 | 28.53 | 28.63 | 28.43 | 25.34 | 25.85 | 25.95 | 25.75 | 24.92 | 25.42 | 25.52 | 25.32 |

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 for with or without enzymes different level of rDDGS

| Ingredients | T1 | T2 | T3 | T4 | T5 | T6 | T7 | T8 | T9 | T10 | T11 | T12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Maize | 62.00 | 62.00 | 62.00 | 62.00 | 64.18 | 64.18 | 64.18 | 64.18 | 64.38 | 64.38 | 64.38 | 64.38 |
| SBM | 31.30 | 31.30 | 31.30 | 31.30 | 18.20 | 18.20 | 18.20 | 18.20 | 15.70 | 15.70 | 15.70 | 15.70 |
| DDGS | 0.00 | 0.00 | 0.00 | 0.00 | 12.50 | 12.50 | 12.50 | 12.50 | 15.00 | 15.00 | 15.00 | 15.00 |
| Oil | 3.22 | 3.22 | 3.22 | 3.22 | 1.80 | 1.80 | 1.80 | 1.80 | 1.60 | 1.60 | 1.60 | 1.60 |
| LSP | 1.20 | 1.20 | 1.20 | 1.20 | 1.00 | 1.00 | 1.00 | 1.00 | 0.96 | 0.96 | 0.96 | 0.96 |
| DCP | 1.45 | 1.45 | 1.45 | 1.45 | 1.50 | 1.50 | 1.50 | 1.50 | 1.50 | 1.50 | 1.50 | 1.50 |
| Lysine | 0.00 | 0.00 | 0.00 | 0.00 | 0.05 | 0.05 | 0.05 | 0.05 | 0.10 | 0.10 | 0.10 | 0.10 |
| 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 |
| Enzyme | - | + | + | + | - | + | + | + | - | + | + | + |
| 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 | 19.50 | 19.50 | 19.50 | 19.50 | 19.53 | 19.53 | 19.53 | 19.53 |
| Lysine | 1.20 | 1.20 | 1.20 | 1.20 | 0.98 | 0.98 | 0.98 | 0.98 | 0.98 | 0.98 | 0.98 | 0.98 |
| Methionine | 0.41 | 0.41 | 0.41 | 0.41 | 0.42 | 0.42 | 0.42 | 0.42 | 0.43 | 0.43 | 0.43 | 0.43 |
| Threonine | 0.68 | 0.68 | 0.68 | 0.68 | 0.69 | 0.69 | 0.69 | 0.69 | 0.70 | 0.70 | 0.70 | 0.70 |
| 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 | 0.38 | 0.38 | 0.38 | 0.38 |
| ME (kcal/kg)** | 3100 | 3100 | 3100 | 3100 | 3099 | 3099 | 3099 | 3099 | 3101 | 3101 | 3101 | 3101 |
| Cost (Rs./kg) | 26.71 | 27.22 | 27.32 | 27.12 | 24.08 | 24.58 | 24.68 | 24.48 | 23.78 | 24.28 | 24.38 | 24.18 |

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

## 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 rDDGS were taken, the best inclusion level from earlier experiments as first level (12.5%) and then adding over and above the best level of 2.5% rDDGS to this level with enzymes. Experimental layout for feeding different level of rDDGS with or without enzymes is presented in Table 4.

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

| Experimental design | | | 3x4 factorial CRD | | |
|---|---|---|---|---|---|
| **Treatment** | **rDDGS (%)** | **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 | 12.5 | 4 | 8 | 32 | - |
| T6 | 12.5 | 4 | 8 | 32 | Xylanase |
| T7 | 12.5 | 4 | 8 | 32 | Protease |
| T8 | 12.5 | 4 | 8 | 32 | Multienzymes |
| T9 | 15 | 4 | 8 | 32 | - |
| T10 | 15 | 4 | 8 | 32 | Xylanase |
| T11 | 15 | 4 | 8 | 32 | Protease |
| T12 | 15 | 4 | 8 | 32 | Multienzymes |

## Immunological Parameters

### Humoral Immune (HI) Response

Humoral immune response estimated by method of Siegel and Gross [11] (1980) were followed for slight modified by Saxena *et.al*. [10] 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 $^0$C until its use.

Eight broilers (4 males and 4 females) per treatment were injected with 1 ml of 1% SRBC suspension intravenously at 28$^{th}$ day post hatch in broilers other than used for assessing CMI. On 34$^{th}$ 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 [5] on 29$^{th}$ day post hatch. At 29$^{th}$ day post hatch, 8 birds (4 male and 4 female) 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 [12] using statistical package for social sciences (SPSS) 16.0 version and comparison of means were done using Tukey's test [14] .

## Results and Discussion

Influence of different levels of rDDGS with or without enzymes on humoral and cell mediated immunity are tabulated in tables 5. Feeding different levels of rDDGS (0, 12.5 and 15%) levels revealed that no significant ($P$>0.05) difference was found on cell mediated immune (CMI) immunity by incorporating rDDGS. Humoral immune response in 0, 12.5 and 15% rDDGS in terms of haemagglutination (HA) titer (log2) value were 2.76, 2.86 and 2.90 respectively. Humoral immunity was significantly ($P$<0.01) better in 12.5 and 15% rDDGS levels as compared to control. Humoral immune response in no enzyme, xylanase, protease and multienzymes supplemented groups in terms of haemagglutination (HA) titer (log2) value were reported 2.86, 2.81, 2.89 and 2.83 respectively. Feeding rDDGS with enzymes (xylanase, protease and multienzymes) supplementation on humoral immunity revealed that no significant ($P$>0.05) difference was found on CMI and humoral immunity with or without enzymes. Interaction of rDDGS and enzymes did not show any significant ($P$>0.05) difference in CMI, but humoral immunity was significantly ($P$<0.05) better in T2, T5, T7, T8, T9, T10, T11 and T12 groups as compared to control. Humoral immunity was also significantly ($P$<0.05) better in 15% rDDGS with protease as compared to control and other dietary treatments (T2, T3, T4, T6 and T10).

Our results are in agreement with researchers [3, 4, 6, 8]. No significant ($P$>0.05) difference in humoral and cell mediated immunity was found by feeding up to 10% inclusion of rDDGS [6]. Incorporation of 20% level of sorghum DDGS in the diets significantly ($P$<0.01) improved the IgA and IgG titer in broiler [4]. Better humoral immunity in rDDGS diets may be associated with type and composition of amino acids particularly higher level of methionine present in DDGS. DDGS reduced serum superoxide dismutase (SOD), and total antioxidant activity, whereas increased IgA, IgG and malondialdehyde (MDA) of 21 days old broiler. Thus, 15% dietary DDGS inclusion has the beneficial effects on immune functions for broilers [8]. Diet containing 10% of DDGS stimulated cell-mediated immune response indicating the immunomodulatory activities of these products following immunization with non-inflammatory antigens in broiler chickens [3].

**Table 5:** Effect of feeding different level of rDDGS with or without enzymes on immunological parameters

| Treatment | rDDGS% | Enzyme | HA (log 2) | CMI (mm) |
|---|---|---|---|---|
| T1 | 0 | - | 2.65[a] | 57.88 |
| T2 | 0 | X | 2.82[bc] | 57.25 |
| T3 | 0 | P | 2.79[abc] | 58.50 |
| T4 | 0 | M | 2.76[ab] | 58.13 |
| T5 | 12.5 | - | 2.90[bcd] | 56.50 |
| T6 | 12.5 | X | 2.79[abc] | 59.14 |
| T7 | 12.5 | P | 2.85[bcd] | 60.63 |
| T8 | 12.5 | M | 2.88[bcd] | 58.22 |
| T9 | 15 | - | 2.95[cd] | 54.50 |
| T10 | 15 | X | 2.82[bc] | 59.88 |
| T11 | 15 | P | 3.00[d] | 60.38 |
| T12 | 15 | M | 2.84[bcd] | 58.50 |
| | | Pooled SEM | 0.02 | 0.41 |
| | | rDDGS | | |
| | | 0 | 2.76[a] | 57.94 |
| | | 12.5 | 2.86[b] | 58.59 |
| | | 15 | 2.90[b] | 58.31 |
| | | Enzyme | | |
| | | - | 2.84 | 56.29 |
| | | X | 2.81 | 58.58 |
| | | P | 2.89 | 59.83 |
| | | M | 2.83 | 58.41 |
| | | Significance | | |
| | | rDDGS | $P<0.01$ | NS |
| | | Enzyme | NS | NS |
| | | Interaction | $P<0.05$ | NS |

Values bearing different superscripts within the column differ significantly *($P<0.01$), ** ($P<0.05$) and NS: Non-significant ($P>0.05$)

## Conclusion
Thus, it may be concluded that 12.5% and 15% rDDGS levels with or without protease supplementation improved immune response in broiler chickens in terms of humoral immunity.

## Acknowledgement
ICAR-Central Avian Research Institute, Izatnagar, Utter Pradesh-243122, India for providing all necessary inputs and facilities.

## Conflict of Interest
All authors declare no conflicts of interest. All authors participated and approved the article for publication.

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