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# Effect of Feeding Rice Based Distillers Dried Grains Solubles with and without Enzymes on Haemato-biochemical profile of Broiler Chickens

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**ABSTRACT**

A biological experiment of 42 days duration was undertaken in which 384 day old chicks were divided into 12 dietary treatments as per 3×4 factorial design having 4 replicates per treatment with 8 birds in each. Twelve experimental diets were prepared by incorporating control (maize-soya based), two different levels of rice Distillers Dried Grains (rDDGS) 12.5 and 15%, without and with three different types of enzymes (xylanase, protease and multienzymes). The inclusion of rice based distillers dried grains with solubles (rDDGS), enzyme supplementation and their interaction revealed no significant ($P>0.05$) difference on the haematological parameters of broiler chicken. The serum cholesterol decreased significantly ($P<0.01$) at 15% rDDGS level as compared to control and 12.5% rDDGS level. The serum triglyceride decreased significantly ($P<0.01$) at 12.5 and 15% rDDGS levels compared to control diet. The serum glucose significantly ($P<0.05$) increased by multienzymes supplementation compared to xylanase and control diet. The serum total protein, albumin and globulin significantly ($P<0.01$) increased in protease supplemented groups as compared to control and other enzyme supplemented groups. The interaction of rDDGS levels and enzyme supplementation revealed no significant ($P>0.05$) effect on any of the serum parameters. Thus, it may be concluded that feeding of (rDDGS) up to 15% and soybean meal based diets without or with protease, xylanase and multienzymes in broiler chickens did not have any adverse effect on haematological and serological parameters. Protease supplementation has beneficial effect on serum biochemistry in DDGS diet.

**Keywords:** Broiler, Enzyme, Haematology, Rice DDGS, Serology

Chicken is one of the highly accepted animal proteins worldwide. Feed accounts nearly 65 to 75% of total recurring expenditure of broiler production. Feed cost is primarily driven by the cost of protein. Substitution of expensive proteinic ingredient like soybean meal with low cost protein ingredient like distillers dried grains with solubles (DDGS) will potentially reduce the cost of broiler production (Gupta *et al.,* 2017). The DDGS is the co-product of bioethanol production. The DDGS availability is increasing now days due to higher demand for ethanol. Government of India has given guideline to gradually increase the inclusion level of ethanol up to 20% by 2025 in petroleum products (AFVP, 2014). Maize, wheat, barley, sorghum and rice are the common ingredients used for ethanol production.

Haematology dealing study of blood plays a leading role in growth and nutritional physiology. The blood and serum metabolites provide useful information on nutritional status and clinical investigation of an individual, hence WHO recommended the use of blood parameters for medical and nutritional assessments (WHO, 1963). Blood and serological parameters are indicators of the health status of the birds and influenced by type of feed and their nutrient composition. Type and level of crude fiber in feed, their amino acid composition and type of incriminating factors present in the feed play pivotal role affecting blood and serum parameters (Chessson, 2001).

Enzyme supplementations in poultry diets are nutritionally, economically and environmentally justified. However,

limited information is available on use of substrate specific enzyme and their effect on serum and blood biochemicals that are specific for broiler corn-soya diet and soybean meal partially replaced with rice DDGS.

No research is available regarding effect of feeding rice based DDGS without or with different enzymes on haematology and serum biochemistry of broiler chickens and however few researchers (Ghazalah *et al*., 2011; Youssef *et al*., 2013; Choi *et al.,* 2014; Hack *et al.,* 2015; Gupta *et al.,* 2017) are available on Maize, wheat, barley, sorghum based DDGS feeding on blood and serum parameters. In view of the above, a study was conducted for *in vitro* and *in vivo* investigation regarding feeding rice based DDGS without or with different enzymes on haematology and serum biochemistry of broiler chickens.

## MATERIALS AND METHODS

### Ethical approval

All the procedures carried out on the birds were approved by the Institutional Animal Ethics Committee (IAEC) and committee for the purpose of control and supervision of experiments on animals (CPCSEA) of ICAR-Central Avian Research Institute, Izatnagar, Utter Pradesh-243122. The IAEC/CPCSEA number is 452/01/ab/CPCSEA.

### Experimental design and diets

The experiment was conducted as per 3×4 factorial completely randomized design (CRD). A total of 384 broiler chicks 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 rice DDGS were taken (12.5 and 15%). Protease, xylanase and multienzymes supplementation under different treatments were done. Experimental layout for feeding different level of rDDGS with or without enzymes is presented in Table 1.

Ingredients and nutrient composition (%) of pre-starter, starter and finisher diets with or without enzymes for different level of rDDGS are presented respectively in Table 2, 3 and 4. Corn-soya meal (CS) based basal diets to meet standard for broiler chickens were formulated (ICAR, 2013).

The three commercial enzyme preparations (protease, xylanase and multienzymes) were analyzed for different enzyme activities as per standard method (Sastry *et al*., 1999). 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.

### Haematological parameter

For the analysis of haematological parameters 2 ml blood samples were collected from 8 birds (4 males and 4 females) per treatment at random in sterile heparin vials at 42 days of age of birds. All the blood samples were analyzed by automatic Abacus junior vet 5 haematoanalyzer. The parameters studied were total leukocyte count (TLC), differential leukocyte count (DLC), Hb (%), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), RBC distribution width (RDWc), platelet count, mean platelet volume (MPV) and platelet distribution width (PDWc).

### Serological parameter

For evaluation of serum biochemistry of broiler chicken at 42 days of age, the blood samples were collected in the same manner as for the haematological analysis but in vials without any anticoagulant. The various serological parameters studied were serum glucose (Trinder, 1969), total protein (Doumas *et al.*, 1971), albumin (Gustafsson *et al.,* 1978), globulin, albumin: globulin ratio (A:G), cholesterol (Wybenga and Pileggi, 1970), triglyceride (Fossati and Lorenzo,1982), alkaline phosphatase (ALP) (Kind and King, 1954), serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) (Reitman and Frankel, 1957).

### Statistical analysis

Data subjected to test of significance as per 3×4 factorial completely randomized design (CRD) were analyzed for mean, standard errors and analysis of variance (Snedecor and Cochran, 2004) using statistical package for social

sciences (SPSS) 16.0 version and comparison of means were done using Tukey test (Tukey, 1949).

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

| Exp Experimental design | | | 3×4 factorial CRD | | |
|---|---|---|---|---|---|
| **Treat-ment** | **Rice DDGS %** | **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 |
| **Total** | | **48** | | **384** | |

## RESULTS AND DISCUSSION

### Enzyme activity analysis

Protease activity was estimated 600,000 ± 849 units per g. Xylanase activity was estimated 150,000 ± 683 units per g. Multienzymes activity were estimated Cellulase 15,000, Xylanase18, 500±328, Beta glucanase 12,500±128, Amylase 1500±46, Pectinase 150±16, Protease 5000±136, Lipase 15± 3.8 and Beta mannanase 400±31.

### Haematological parameter

The results pertaining to effect of feeding different levels of rDDGS with or without enzymes on haematological parameters of the broiler chickens are presented in Table 5. Blood profile were studied in terms of total erythrocyte count (TEC), total leukocyte count (TLC), differential leukocyte count (DLC), platelet count, Hb %, PCV, MCV, MCH, MCHC, MPV, heterophils and leukocyte ratio, RBC distribution width (RDWc) and platelet distribution width (PDWc). The results revealed that no significant ($P>0.05$) difference was observed in blood profile between control and other different dietary treatments by incorporating different levels of rDDGS (0,12.5 and 15%), enzymes (xylanase, protease and multienzymes) and their interaction. All the blood parameters were found within normal physiological range as measured by Abacus junior vet 5 haematoanalyzer.

Gupta *et al.* (2017) reported inclusion of 5, 7.5 and 10% level of rDDGS significantly ($P<0.01$) enhance the PCV and Hb values than 0% inclusion level. Ghazalah *et al*. (2011) reported that DDGS level at 75% substitution for SBM significantly increased Hb % in layers, but DDGS up to 15% insignificantly affected the haematological parameters in broiler (Youssef *et al*., 2013). Thus, our results are in agreement with Ghazalah *et al*. (2011) and Youssef *et al*. (2013), but disagreement with Gupta *et al.* (2017).

### Serological parameter

Effect of different levels of rDDGS feeding with or without enzymes to broilers on serological parameters are tabulated in Tables 6. Feeding different levels of rDDGS (0, 12.5 and 15%) on serum glucose, total protein, albumin, globulin, serum enzymes SGOT, SGPT and alkaline phosphatase (ALP) did not exhibit any significant ($P>0.05$) difference as compared to with or without enzymes groups and control. Serum cholesterol was significantly ($P<0.01$) decreased in 15% rDDGS level as compared to 0 and 12.5% rDDGS levels. Serum triglycerides was significantly ($P<0.01$) decreased in 12.5 and 15% rDDGS levels as compared to 0 % rDDGS level.

Supplementation of enzymes (xylanase, protease and multienzymes) on serological parameters did not exhibit any significant ($P>0.05$) difference on serum cholesterol, triglycerides, albumin (A): globulin (G) ratio, serum enzymes (SGOT, SGPT and ALP) activity. Serum glucose was significantly ($P<0.05$) increased in multienzumes groups as compared to xylanase and without enzyme groups, but no significant ($P>0.05$) difference was found in multienzumes and protease groups. Serum total protein and albumin were significantly ($P<0.01$) increased in protease groups as compared to xylanase, multienzymes and without enzyme groups, but A : G ratio did not exhibit any significant ($P>0.05$) difference as compared to with or without enzymes groups.

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

| Ingredients | D1 | D2 | D3 | D4 | D5 | D6 | D7 | D8 | D9 | D10 | D11 | D12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 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** | **100.01** | **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 | 22.01 | 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 (₹/ 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. $D_3$, 2400 IU; Vit. E,15 and Vit. K, 1mg. Vitamin B complex supplied per kg diet: Vit. $B_1$, 5 mg; Vit. $B_2$, 6 mg; Vit. $B_6$ 5 mg; Vit. $B_{12}$, 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 3:** Ingredients and nutrient composition (%) of starter diets with or without enzymes for different level of rDDGS

| Ingredients | D1 | D2 | D3 | D4 | D5 | D6 | D7 | D8 | D9 | D10 | D11 | D12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 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 | 100.00 | 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 | 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 (₹/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. $D_3$, 2400 IU; Vit. E,15 and Vit. K, 1mg. Vitamin B complex supplied per kg diet: Vit. $B_1$, 4 mg; Vit. $B_2$, 6 mg; Vit. $B_6$ 5 mg; Vit. $B_{12}$, 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 4:** Ingredients and nutrient composition (%) of finisher diets for with or without enzymes different level of rDDGS

| **Ingredients** | **D1** | **D2** | **D3** | **D4** | **D5** | **D6** | **D7** | **D8** | **D9** | **D10** | **D11** | **D12** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 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 | 100.00 | 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 | 19.53 | 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 (₹/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. $D_1$, 2400 IU; Vit. E,15 and Vit. K, 0.8 mg. Vitamin B complex supplied per kg diet: Vit. $B_1$, 4 mg; Vit. $B_2$, 6 mg; Vit. $B_6$ 5 mg; Vit. $B_{12}$, 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.

Interaction of rDDGS and enzymes did not show any significant (P>0.05) difference in glucose, total protein, serum enzymes SGOT, SGPT and ALP activity. Serum albumen significantly (P<0.05) increased in 0% rDDGS with protease and 12.5% rDDGS with protease groups as compared to other dietary treatments and control. Serum globulin level significantly (P<0.05) increased in 15% rDDGS with protease as compared to control and other dietary treatments (T4, T5, T6, T10 and T12). Serum A:G ratio significantly (P<0.01) increased in 0% rDDGS

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

| Treatment | rDDGS % | Enzyme | TLC | Neutro | Lymph | Mono | Neutro % | Lymph % | Mono % | H:L | TRBC | Hb | PCV | MCV | MCH | MCHC | RDWc | PLT | PLT% | MPV | PDWc |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **T1** | **0** | - | 16.9 | 4.2 | 9.8 | 1.4 | 24.9 | 57.5 | 8.4 | 0.44 | 2.2 | 11.8 | 25.4 | 114.3 | 53.3 | 46.5 | 15.5 | 25.8 | 0.26 | 9.2 | 31.8 |
| T2 | **0** | X | 16.8 | 4.4 | 9.5 | 1.5 | 26.2 | 56.5 | 8.9 | 0.47 | 2.0 | 11.3 | 23.4 | 115.0 | 53.5 | 48.5 | 15.0 | 26.8 | 0.27 | 8.8 | 31.3 |
| **T3** | **0** | P | 16.8 | 4.3 | 9.7 | 1.4 | 25.5 | 57.9 | 8.3 | 0.45 | 2.4 | 11.2 | 26.5 | 114.0 | 47.0 | 41.3 | 16.0 | 29.8 | 0.30 | 9.0 | 31.0 |
| **T4** | **0** | M | 18.6 | 4.8 | 10.7 | 1.4 | 26.1 | 57.4 | 7.7 | 0.46 | 2.3 | 9.9 | 26.4 | 115.8 | 52.5 | 45.0 | 17.0 | 26.8 | 0.27 | 8.7 | 30.3 |
| **T5** | **12.5** | - | 15.9 | 4.1 | 8.9 | 1.5 | 25.5 | 55.9 | 9.5 | 0.46 | 2.3 | 11.2 | 25.0 | 110.0 | 54.0 | 50.8 | 15.0 | 30.0 | 0.31 | 9.0 | 30.8 |
| **T6** | **12.5** | X | 16.6 | 4.4 | 9.3 | 1.4 | 26.7 | 56.5 | 8.5 | 0.48 | 2.3 | 10.6 | 25.2 | 109.0 | 46.0 | 42.3 | 15.5 | 26.8 | 0.27 | 8.3 | 30.0 |
| **T7** | **12.5** | P | 17.1 | 4.1 | 10.0 | 1.4 | 24.2 | 58.3 | 8.2 | 0.42 | 2.2 | 11.0 | 24.5 | 113.5 | 50.5 | 44.5 | 15.8 | 26.3 | 0.27 | 8.6 | 29.0 |
| **T8** | **12.5** | M | 16.0 | 4.2 | 9.0 | 1.4 | 26.3 | 56.6 | 8.8 | 0.47 | 2.2 | 10.9 | 25.4 | 114.0 | 49.0 | 43.0 | 15.5 | 26.8 | 0.27 | 8.3 | 30.5 |
| **T9** | **15** | - | 15.4 | 4.0 | 8.8 | 1.4 | 25.7 | 57.6 | 9.1 | 0.45 | 2.2 | 11.2 | 24.3 | 112.0 | 51.8 | 46.3 | 15.5 | 28.8 | 0.29 | 9.1 | 30.0 |
| **T10** | **15** | X | 15.4 | 3.8 | 8.7 | 1.4 | 24.7 | 56.9 | 8.9 | 0.44 | 2.1 | 10.4 | 24.0 | 113.8 | 50.3 | 44.3 | 17.0 | 26.8 | 0.27 | 8.9 | 30.8 |
| **T11** | **15** | P | 17.7 | 4.7 | 9.9 | 1.4 | 26.3 | 57.0 | 8.0 | 0.47 | 2.2 | 11.4 | 24.5 | 112.8 | 53.0 | 47.0 | 15.0 | 27.3 | 0.28 | 8.7 | 31.0 |
| T12 | **15** | M | 15.6 | 4.0 | 8.8 | 1.5 | 25.6 | 56.6 | 9.4 | 0.46 | 2.2 | 11.7 | 25.5 | 116.0 | 53.3 | 46.0 | 13.5 | 29.0 | 0.30 | 8.3 | 30.0 |
| | Pooled SEM | | 0.29 | 0.11 | 0.17 | 0.01 | 0.38 | 0.41 | 0.16 | 0.01 | 0.03 | 0.13 | 0.30 | 0.54 | 0.67 | 0.62 | 0.26 | 0.44 | 0.00 | 0.08 | 0.24 |
| | rDDGS | | | | | | | | | | | | | | | | | | | | |
| | 0 | | 17.3 | 4.4 | 9.9 | 1.4 | 25.7 | 57.3 | 8.3 | 0.45 | 2.2 | 11.0 | 25.4 | 114.8 | 51.6 | 45.3 | 15.9 | 27.3 | 0.28 | 8.9 | 31.1 |
| | 12.5 | | 16.4 | 4.2 | 9.3 | 1.4 | 25.7 | 56.8 | 8.7 | 0.45 | 2.2 | 10.9 | 25.0 | 111.6 | 49.9 | 45.1 | 15.4 | 27.4 | 0.28 | 8.5 | 30.1 |
| | 15 | | 16.0 | 4.1 | 9.1 | 1.4 | 25.6 | 57.0 | 8.8 | 0.45 | 2.2 | 11.2 | 24.6 | 113.6 | 52.1 | 45.9 | 15.3 | 27.9 | 0.28 | 8.7 | 30.4 |
| | Enzyme | | | | | | | | | | | | | | | | | | | | |
| | - | | 16.1 | 4.1 | 9.2 | 1.4 | 25.4 | 57.0 | 9.0 | 0.45 | 2.2 | 11.4 | 24.9 | 112.1 | 53.0 | 47.8 | 15.3 | 28.2 | 0.29 | 9.1 | 30.8 |
| | X | | 16.2 | 4.2 | 9.2 | 1.4 | 25.8 | 56.6 | 8.8 | 0.46 | 2.2 | 10.8 | 24.2 | 112.6 | 49.9 | 45.0 | 15.8 | 26.8 | 0.27 | 8.7 | 30.7 |
| | P | | 17.2 | 4.4 | 9.9 | 1.4 | 25.3 | 57.7 | 8.1 | 0.44 | 2.3 | 11.2 | 25.2 | 113.4 | 50.2 | 44.3 | 15.6 | 27.8 | 0.28 | 8.8 | 30.3 |
| | M | | 16.7 | 4.4 | 9.5 | 1.4 | 26.0 | 56.9 | 8.6 | 0.46 | 2.2 | 10.8 | 25.8 | 115.3 | 51.6 | 44.7 | 15.3 | 27.5 | 0.28 | 8.4 | 30.3 |
| | Significance | | | | | | | | | | | | | | | | | | | | |
| | rDDGS | | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| | Enzyme | | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |
| | Interaction | | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS |

**NS:** Non-significant (P>0.05) Units: WBC (× 104 ul), RBC (×106ul), Hb (g/dl), MCV (fl), MCH(pg), MCHC(g/dl) and PLT(×103).

**Table 6:** Effect of feeding different level of rDDGS with or without enzymes on serological parameters

| Treatment | rDDGS% | Enzyme | Glucose | Protein | Albumin | Globulin | A:G | Cholesterol | Triglyceride | SGOT | SGPT | ALP |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| T1 | 0 | - | 192 | 3.89 | 1.53$^{a}$ | 2.35$^{ab}$ | 0.66$^{ab}$ | 128$^{cd}$ | 111$^{d}$ | 172 | 9.80 | 49 |
| T2 | 0 | X | 198 | 4.63 | 1.58$^{a}$ | 2.61$^{bc}$ | 0.61$^{a}$ | 125$^{bcd}$ | 103$^{cd}$ | 171 | 10.08 | 46 |
| T3 | 0 | P | 196 | 4.22 | 2.01$^{b}$ | 2.63$^{bc}$ | 0.79$^{c}$ | 127$^{cd}$ | 100$^{bcd}$ | 173 | 9.88 | 51 |
| T4 | 0 | M | 202 | 4.15 | 1.61$^{a}$ | 2.54$^{ab}$ | 0.64$^{a}$ | 122$^{abcd}$ | 107$^{d}$ | 173 | 9.98 | 50 |
| T5 | 12.5 | - | 191 | 4.19 | 1.62$^{a}$ | 2.56$^{ab}$ | 0.63$^{a}$ | 123$^{abcd}$ | 90$^{ab}$ | 175 | 9.27 | 49 |
| T6 | 12.5 | X | 192 | 4.64 | 1.71$^{a}$ | 2.24$^{a}$ | 0.78$^{bc}$ | 128$^{d}$ | 95$^{abc}$ | 174 | 9.58 | 48 |
| T7 | 12.5 | P | 195 | 3.96 | 2.02$^{b}$ | 2.62$^{bc}$ | 0.77$^{bc}$ | 130$^{d}$ | 94$^{abc}$ | 172 | 11.45 | 58 |
| T8 | 12.5 | M | 202 | 4.18 | 1.57$^{a}$ | 2.60$^{bc}$ | 0.60$^{a}$ | 122$^{abcd}$ | 89$^{ab}$ | 174 | 10.45 | 52 |
| T9 | 15 | - | 195 | 4.22 | 1.58$^{a}$ | 2.63$^{bc}$ | 0.60$^{a}$ | 113$^{a}$ | 85$^{a}$ | 175 | 8.53 | 61 |
| T10 | 15 | X | 190 | 4.65 | 1.61$^{a}$ | 2.52$^{a}$ | 0.64$^{a}$ | 118$^{ab}$ | 99$^{bcd}$ | 174 | 10.25 | 55 |
| T11 | 15 | P | 198 | 4.13 | 1.71$^{a}$ | 2.93$^{c}$ | 0.58$^{a}$ | 119$^{abc}$ | 99$^{bcd}$ | 173 | 11.68 | 58 |
| T12 | 15 | M | 194 | 4.20 | 1.71$^{a}$ | 2.48$^{ab}$ | 0.70$^{abc}$ | 125$^{bcd}$ | 94$^{abc}$ | 172 | 9.45 | 47 |
| | | Pooled SEM | 0.89 | 0.04 | 0.02 | 0.03 | 0.01 | 0.92 | 1.30 | 0.39 | 0.22 | 1.14 |
| | | rDDGS | | | | | | | | | | |
| | | 0 | 197 | 4.22 | 1.69 | 2.53 | 0.67 | 125$^{b}$ | 105$^{b}$ | 172 | 9.94 | 49 |
| | | 12.5 | 195 | 4.24 | 1.73 | 2.51 | 0.70 | 126$^{b}$ | 92$^{a}$ | 173 | 10.19 | 52 |
| | | 15 | 194 | 4.30 | 1.66 | 2.64 | 0.63 | 118$^{a}$ | 94$^{a}$ | 173 | 9.98 | 55 |
| | | Enzyme | | | | | | | | | | |
| | | - | 192$^{a}$ | 4.09$^{a}$ | 1.58$^{a}$ | 2.51$^{a}$ | 0.63 | 121 | 95 | 174 | 9.20 | 53 |
| | | X | 193$^{a}$ | 4.10$^{a}$ | 1.63$^{a}$ | 2.46$^{a}$ | 0.67 | 124 | 99 | 173 | 9.97 | 50 |
| | | P | 196$^{ab}$ | 4.63$^{b}$ | 1.91$^{b}$ | 2.72$^{b}$ | 0.72 | 125 | 98 | 172 | 11.01 | 56 |
| | | M | 199$^{b}$ | 4.17$^{a}$ | 1.63$^{a}$ | 2.54$^{a}$ | 0.65 | 123 | 97 | 173 | 9.96 | 50 |
| | | Significance | | | | | | | | | | |
| | | rDDGS | NS | NS | NS | NS | NS | P<0.01 | P<0.01 | NS | NS | NS |
| | | Enzyme | P<0.05 | P<0.01 | P<0.01 | P<0.05 | NS | NS | NS | NS | NS | NS |
| | | Interaction | NS | NS | P<0.05 | P<0.05 | P<0.01 | P<0.05 | P<0.05 | NS | NS | NS |

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

with protease as compared to control and other dietary treatments (T2, T4, T5, T8, T9, T10 and T11). Serum cholesterol level significantly (P<0.05) decreased in 15% rDDGS without enzyme group as compared to control and other dietary treatments (T2, T3, T5, T6 and T12). Serum cholesterol level was significantly (P<0.05) increased in 12.5% rDDGS with xylanase and 12.5% rDDGS with protease groups as compared to dietary treatments (T9, T10 and T11). Serum triglycerides level was significantly (P<0.05) decreased in 15% rDDGS without enzyme as compared to control and other dietary treatments (T2, T3, T6, T7 and T12). Serum triglycerides level was significantly (P<0.05) increased in 12.5% rDDGS with xylanase and 12.5% rDDGS with protease groups as compared to T9,T10 and T11 groups.

Choi *et al.* (2014) reported dietary inclusion of rice DDGS up to 25% did not affect the plasma content of total protein, glucose, cholesterol and triglyceride in juvenile red sea bream (*Pagrus major*) fish. Hack *et al.* (2015) reported that increasing corn DDGS level significantly (P<0.01) increased serum triglycerides, cholesterol and LDL for hens fed diet contained 22% DDGS in the diet. Gupta *et al.* (2017) reported 10% rice DDGS had significantly (P<0.01) higher effect on serum albumin, total serum protein, serum A:G ratio, serum glucose value and significantly (P<0.01) lowering effect on serum lipid profile (triglycerides, cholesterol, LDL and VLDL). Our results are in agreement with Choi *et al.*(2014), but disagreement with Hack *et al.* (2015) and Gupta *et al.* (2017).

## CONCLUSION

Thus, it may be concluded that exogenous enzyme supplementation in terms of protease, xylanase and multienzymes in rice distillers dried grains with solubles (rDDGS) up to 15% and soybean based diets did not have any adverse effect on haematological and serological parameters. However, protease supplementation has beneficial effect on serum biochemistry in DDGS diet.

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## CONFLICT OF INTEREST

Authors declare no conflict of interest.

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