



Effect of Rice Based Distillers Dried Grain Soluble (Rddgs) With or Without Enzyme Supplementation on Nutrient Retention and Antioxidant Activity Parameters of Commercial Broiler Chicken

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ABSTRACT

The objective of this study was to evaluate the Effect of Rice Based Distiller Dried Grain Solubles (RDDGS) with or without Enzyme Supplementation on Nutrient Retention and Antioxidant Activity Parameters of Commercial broiler chicken. Four hundred day old commercial broilers (*Vencobb* 400) were randomly allotted to 10 treatments each treatment containing 8 replicates and 5 chicks in each replicate. The basal diet consisted of corn and soya bean meal. The remaining experimental diets were prepared with inclusion of RDDGS at 4 levels (4, 8, 12 and 16%) with or without enzyme supplementation as given in Table 4. The composition of the experimental diets of broiler starter (23% crude protein and metabolizable energy (ME) 3000 K.cal/kg) and finisher rations (19.50% crude protein and metabolizable energy 3150 K.cal/kg). Cocktail Enzyme supplemented @ 250 gm/ton of feed. The feed and water were provided *adlib* during the entire experimental period of 42 days. Authors concluded that 16% RDDGS without enzyme supplementation was more effective among all the dietary treatments in terms of Nutrient Retention and Antioxidant Activity Parameters of Commercial broilers during the entire experimental period.

HIGHLIGHTS

- Alternatives for protein and energy sources in poultry diets minimize the cost of feed production.
- RDDGS becomes an attractive substitute of expensive source of energy and protein ingredients of poultry feed.

Keywords: *Vencobb* Broilers, Dry Matter, Crude Protein, Glutathione Peroxidase, Glutathione Reductase and Superoxide Dismutase

RDDGS is the byproduct of the processing of rice alcohol industry which is produced from the distillation of fermented rice. In processing, rice is cooked at 131°C and 2.6 kg/ m² pressure and yeast is added to the cooked rice for fermentation. Then the alcohol is distilled from fermentation liquor and then leftover is known as Rice

Distillers Dried Grains with Solubles (RDDGS). Corn,

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wheat, barley and sorghum cereals are the commonly used ingredients for fermentation during bioethanol production. In the present context, rice as substrate for bioethanol production is increasing due to its relative lower price, higher production and easy availability there by leading to increased availability of co-product rice DDGS (RDDGS). It contains all the nutrients from grain in a concentrated form except for the majority of the starch, which has been utilized in the fermentation process. It contains 65% distiller's grain and 35% its soluble. Most of the research work done is limited to corn, wheat, barley and sorghum DDGS. Very scanty information is available in literature regarding the feeding value of RDDGS in poultry. So, there is need to explore its nutritive and feeding value due to its increased availability as a low cost alternate feed ingredient. RDDGS contains a valuable amount of supplemental protein to the tune of 47% compared to 45% in soybean meal and metabolisable energy around 3500 kcal/ kg.

It is also more nutritious than the cereal grains from which it is made up of, as it contains other nutrients recovered from the fermented grains. It is also high in protein compared to rice and more in energy and less in fiber than its byproduct like rice bran or rice polishing. The final product also contains yeast residue. It does not contain any antinutritional factor, as might be the case with trypsin inhibitors in soybean. The cost of RDDGS is always less than that of the soy bean meal. The nutrient content and feeding value of RDDGS is expected to be different from the other most commonly available DDGS sources since the ingredients for rice liquor production include primarily rice grains.

MATERIALS AND METHODS

Four hundred day old commercial broilers (*Vencobb 400*) were randomly allotted to 10 treatments each treatment containing 8 replicates and 5 chicks in each replicate. The basal diet consisted of corn and soya bean meal. The remaining experimental diets were prepared with inclusion of RDDGS at 4 levels (4,8,12 and 16%) with or without enzyme supplementation as given in Table 4. The composition of the experimental diets of broiler starter (23% crude protein and metabolizable energy (ME) 3000 K.cal/kg) and finisher rations (19.50% crude protein and metabolizable energy 3150 K.cal/

kg) along with their nutrient composition. Cocktail Enzyme supplemented @ 250gm/ton of feed.

A growth trial was conducted in randomized block design, comprising of ten dietary treatments. A standard broiler ration (CP 18%, ME 2600 kcal/kg diet) was offered to birds in T₁ (without enzyme) and T₂ (with enzyme). Experimental diets from T₃ to T₆ were formulated with four levels of RDDGS without enzyme (4%, 8%, 12% and 16%). Experimental diets from T₇ to T₁₀ were formulated with four levels of RDDGS with enzyme (4%, 8%, 12% and 16%). Cocktail Enzyme supplemented @ 250gm/ton of feed. The feed and water were provided *adlib* during the entire experimental period of 42 days. Parameters such as nutrient retention (Dry Matter, Crude Protein) and antioxidant activity (Glutathione Peroxidase, Glutathione Reductase and Superoxide Dismutase) were studied by using commercial kits at the end of trial.

Estimation of DM

To estimate DM an aliquot of 1/20th of total faecal output was dried overnight in a pre-weighed Petridish in a forced-draft hot air oven at 65 ± 5°C, equilibrated at ambient temperatures for 24 hours. The dried faeces were pooled for 3 days individually for each replicate.

Estimation of CP

A weighed quantity of representative feed/faecal sample was digested using Turbotherm (Gerhardt, Germany) with suitable quantity of concentrated sulphuric acid (H₂SO₄) in presence of a catalytic digestion mixture (potassium sulphate and copper sulphate in the ratio of 9:1). After digestion, the tubes were placed for distillation along with boric acid. The ammonia liberated was titrated against standard acid (sulphuric acid) of known strength. The titre values were used for calculating nitrogen content of the sample which was multiplied by 6.25 factor to arrive at crude protein percentage expressed on DMB (Association of Official Analytical Chemists, 2012).

$$\text{CP (\%)} = \frac{14 \times 100 \times \text{normality of sulphuric acid} \times \text{titer value} \times 6.25}{\text{Sample weight}}$$

Antioxidant activity

The antioxidant enzymes such as GSHPx, GSHRx and SOD were estimated following the methods of Paglia and Valentine (1967), Carlberg and Mannervik (1985) and Madesh and Balsubramanian (1998) respectively.

Glutathione peroxidase (GSHPX) enzyme activity in serum

GSHPx activity was determined by the method proposed by Paglia and Valentine (1967) with slight modifications. Microtiter plates (96 well) were used to measure Glutathione peroxidase activity. To the 12.5 µl of serum, 250 µl of 0.1 mM PBS (pH 7.4), 12.5 µl of H₂O₂, 12.5 µl of reduced glutathione were added to wells and incubated at room temperature for 5 minutes, following which 12.5 µl of nicotinamide adenine dinucleotide phosphate (NADPH) solution was added and optical density was measured at 340 nm against the blank using ELISA reader - µQuant (BioTek instruments) for 5 minutes at 60 seconds interval and expressed as units/mg protein.

Glutathione reductase (GSHRX) enzyme activity in serum

GSHRx activity was determined according to method described by Carlberg and Mannervik (1985) with slight modifications. Microtiter plates (96 well) were used to measure Glutathione reductase activity. To the 12.5 µl of serum, 250 µl of 0.1 mM PBS (pH 7.4), 12.5 µl of oxidized glutathione, 12.5 µl of FAD, 12.5 µl of 80 mM EDTA were added and incubated at room temperature for 15 minutes. Optical density was measured at 340 nm against the blank by using ELISA reader - µQuant (BioTek instruments) for 5 times at 60 second interval after addition of 12.5 µl of NADPH solution at last and expressed as units/mg protein.

Superoxide dismutase (SOD) enzyme assay in serum

Microtiter plates (96 well) were used for assay of SOD activity. To the 100 µl of test sample, 6 µl of 1.25 mM 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) was added in duplicate for each sample. 15 µl of 100 µM pyrogallol and 29 µl of 25 mM PBS were added to make the volume to 150 µl. Pyrogallol was freshly prepared and added after the addition of all other reagents and incubated for 10 minutes at room temperature and the reaction was terminated with addition of 150 µl of

dimethyl sulfoxide (DMSO), which arrests the reaction and dissolve the MTT formazan crystals formed. Plates were shaken well and optical density recorded at 570 nm using ELISA reader - µQuant (BioTek instruments) (Madesh and Balsubramanian, 1998).

RESULTS AND DISCUSSION

Results regarding nutrient retention in terms of per cent DM and CP are shown in Table 1. Results showed that there was no significant ($P>0.05$) difference in DM and protein retention among the treatments. However, percent DM and CP retentions were higher in RDDGS with or without enzyme supplemented groups when compared to control groups. Results regarding nutrient retention showed that there was no significant ($P<0.05$) difference in per cent DM and protein retention among the treatments. The results are in agreement with the findings of Shalash *et al.* (2010) and Ghazalah *et al.* (2011) revealed that there was no significant effects due to DDGS levels on digestibility coefficient values for crude protein, crude fiber, ether extract and nitrogen free extract. On the contrary, to Dinani *et al.* (2019) who reported that 15% RDDGS inclusion level, nutrient utilization (dry matter and energy metabolizability, nitrogen retention %) were significantly ($P<0.05$) influenced by dietary treatments. Similar findings were observed by Kakasaheb Khose *et al.* (2017) who reported the inclusion of corn distillery dried grain with soluble (CDDGS) up to 10% levels in broiler diets with or without enzyme was found to be beneficial in terms of nutrient utilization in broiler chicken.

In the experiment, data obtained on different serum antioxidant status activity-influenced by different treatments are presented in Table 2. There was ($P<0.05$) significant difference in the GSHPx concentrations by inclusion of RDDGS at different levels (0, 4, 8, 12 and 16%) with or without Cocktail enzyme supplementation in the present study. Inclusion of 4% RDDGS (515.1 units/ml) without enzyme supplemented group recorded lowest GSHPx activity and all treatment groups showed better GSHPx activity. Data on serum GPx was presented in Table 2 and Fig. 1. There was a significant ($P<0.05$) difference in the GSHRx concentrations by the inclusion of RDDGS at different levels (0, 4, 8, 12 and 16%) with or without cocktail enzyme supplementation in the present study. Inclusion of 12% RDDGS (3425.5 units/ml) with

Table 1: Effect of dietary inclusion of Rice based distillers dried grain soluble (RDDGS) with or without Enzyme Supplementation on nutrient retention of commercial broilers at 42 d of age

Treatment	RDDGS % in diet	Enzyme(@250g/ton of feed	Nutrient Retention(%)	
			DM Retention	CP retention
T ₁	0	-E	82.83	60.17
T ₂	0	+E	83.12	60.82
T ₃	4	-E	86.95	63.90
T ₄	8	-E	87.74	65.23
T ₅	12	-E	83.84	64.10
T ₆	16	-E	86.77	65.17
T ₇	4	+E	87.76	67.16
T ₈	8	+E	85.80	64.93
T ₉	12	+E	85.62	65.01
T ₁₀	16	+E	83.72	62.75
N			8	8
P Value			0.615	0.102
SEM			2.320	2.485

Mean bearing atleast one common superscript in a column differ significantly (P<0.05); +E : supplementation with enzyme, - E :supplementation without enzyme.

Table 2: Effect of dietary inclusion of Rice based distillers dried grain soluble (RDDGS) with or without Enzyme Supplementation on serum antioxidant activity of commercial broiler chicken at 42 d of age

Treatment	RDDGS % in diet	Enzyme(@250g/ton of feed	At 42 nd d		
			Glutathione Peroxidase (units/ml)	Glutathione Reductase (units/ml)	Superoxide Dismutase (units/mg protein)
T ₁	0	-E	512.5 ^b	1,471.2 ^e	6.78 ^{abc}
T ₂	0	+E	563.3 ^{ab}	1,960.4 ^{cd}	8.34 ^{abc}
T ₃	4	-E	515.1 ^b	2,139.5 ^{cde}	6.74 ^{abc}
T ₄	8	-E	621.0 ^{ab}	2,316.3 ^{bcd}	8.78 ^{ab}
T ₅	12	-E	778.8 ^{ab}	3,584.8 ^a	8.36 ^{abc}
T ₆	16	-E	805.3 ^{ab}	3,267.2 ^{ab}	9.31 ^a
T ₇	4	+E	849.3 ^a	2,860.7 ^{abcd}	5.94 ^{bc}
T ₈	8	+E	769.4 ^{ab}	3,065.4 ^{abc}	6.72 ^{abc}
T ₉	12	+E	767.0 ^{ab}	3,425.5 ^a	9.29 ^a
T ₁₀	16	+E	606.8 ^{ab}	2,642.4 ^{abcd}	5.56 ^c
N			8	8	8
P Value			0.019	0.001	0.042
SEM			30.990	125.35	0.328

Mean bearing atleast one common superscript in a column differ significantly (P<0.05); +E : supplementation with enzyme, - E: supplementation without enzyme.

enzyme group significantly increased the activity of GSHRx, when compared with control. Data on serum GSHRx is presented in Table 2 and Fig. 2. Significant (P<0.05) difference was observed in SOD concentrations

by inclusion of RDDGS at different levels (0, 4, 8, 12 and 16%) with or without Cocktail enzyme supplementation in the present study. Data on serum SOD is presented in Table 2 and Fig. 3.

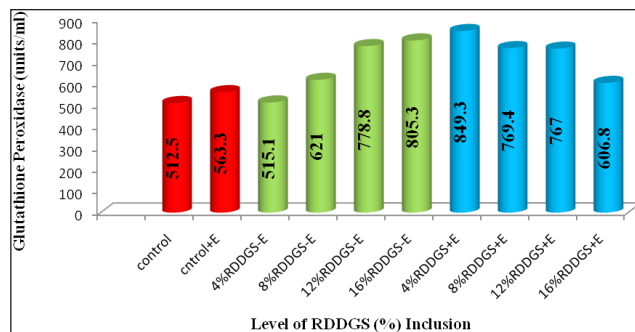


Fig. 1: Effect of dietary inclusion of Rice based distillers dried grain soluble (RDDGS) with or without Enzyme Supplementation on Glutathione Peroxidase (units/ml) of broiler chicken at 42 d of age

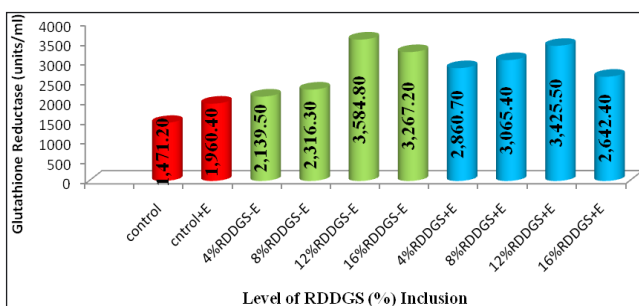


Fig. 2: Effect of dietary inclusion of Rice based distillers dried grain soluble (RDDGS) with or without Enzyme Supplementation on Glutathione Reductase (units/ml) of broiler chicken at 42 d age

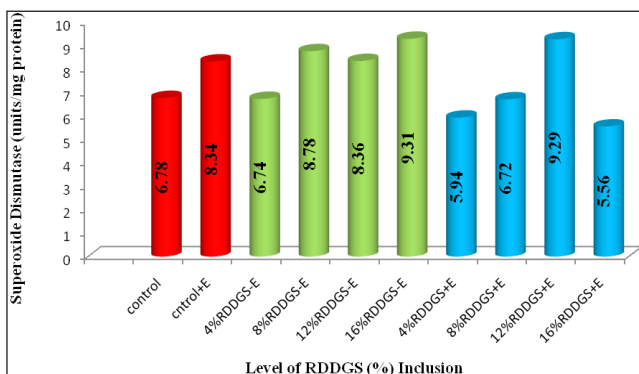


Fig. 3: Effect of dietary inclusion of Rice based distillers dried grain soluble (RDDGS) with or without Enzyme Supplementation on superoxide dismutase (units/mg protein) of broiler chicken at 42 d

In dietary treatments, inclusion of RDDGS with or without enzyme showed highest SOD enzyme activity

when compared to control. Serum antioxidant activity (GSHPx, GSHRx and SOD) of broilers was significantly influenced by the dietary supplementation of RDDGS with or without enzyme. These results hint that a higher oxidative susceptibility, which agrees with the previous studies (Loar *et al.*, 2010 and Min *et al.*, 2012). This may be attributed to the presence of polyunsaturated fatty acids, which has been shown to be more susceptible to oxidation (Giordano and Visoli, 2014), and may increase oxidative stress in a fashion. The results are in agreement with the findings of Min *et al.* (2015) who reported that the inclusion of 15% dietary CDDGS has beneficial effects on antioxidant functions [malondialdehyde (MDA), glutathione peroxidase (GSHPx) and total superoxide dismutase (T-SOD)] in broilers. Similar findings were noticed by Akbar *et al.* (2018) who concluded that 10% DDGS feeding increases serum antioxidant enzyme (SOD, GSH-Px and GR) and MDA concentrations, which indicates an increase of serum lipid oxidation.

These negative effects of DDGS feeding may be attributed to their ability to increase the Unsaturated Fatty Acid content of broilers which makes it more prone to oxidation which leads to more free radical generation. However, no literature is available citing the effect of DDGS feeding on these anti-oxidant parameters in broiler chicken. The SOD converts superoxide radicals to hydrogen peroxide, which is acted upon by CAT. Hence, an increase in SOD activity results in an increase in catalase activity. A similar trend was observed in GSHPx and Glutathione Reductase activity. Similar to our study regarding the effects of DDGS, it was observed that a significant increase in serum SOD content occurs with increasing DDGS levels (Akbar *et al.* 2017).

CONCLUSION

16% RDDGS without enzyme supplementation was more effective among all the dietary treatments in terms of antioxidant activity (GSHPx, GSHRx and SOD) at 42 d of age in commercial broilers. RDDGS with or without enzyme supplementation did not show any significant effect on Nutrient Retention (Dry Matter and Crude Protein) in broilers.

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