International Journal of Advanced Biochemistry Research

ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2025): 5.29
IJABR 2025; 9(10): 542-549
www.biochemjournal.com
Received: 08-07-2025
Accepted: 11-08-2025

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Effect of rice based distiller dried grain solubles (RDDGS) with or without enzyme supplementation on egg quality parameters of commercial layer chicken

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DOI: https://www.doi.org/10.33545/26174693.2025.v9.i10h.6103

Abstract
A study was conducted in 240 commercial layer chickens (BV-300) at 26 weeks age were randomly allotted to 10 treatments with 6 replicates and 4 layers in each replicate. The birds were raised in cage system under uniform management. A standard layer ration (CP 18%, ME 2600 kcal/kg diet) was offered to birds. The test compound, RDDGS was supplemented at different levels (0, 5, 10, 15 and 20%) with or without Cocktail enzyme supplementation @ 250 gm/ton of feed. Birds were aclamatised to respective treatment diets for a week (26th week) before the commencement of the actual data collection. The feed and water should be provided *adlib* during the entire experimental period (4 periods of each 28 days from 27 to 42 weeks). Parameters such as egg quality parameters (Haugh unit, shell thickness, shell breaking strength, albumen index, yolk colour, yolk index) were estimated at 4 weeks interval. The results revealed that the overall egg weight and egg mass was significantly ($p<0.05$) higher in 15% RDDGS with enzyme supplemented diets. There was no significant difference in Haugh unit and Albumen Index values in first (27-30 wks), second (31-34 wks), third (35-38 wks) and overall periods (27-42 wks). Whereas fourth (39-42 wks) period was significantly ($p<0.05$) influenced by Haugh unit and Albumen Index values. It was observed that in period-P3 (35-38 wks), period-P4 (39-42 wks) and over all period (27-42 wks) there was significant ($p<0.05$) difference in yolk index among different levels of treatment fed groups. Yolk colour was significantly higher ($p<0.05$) in RDDGS with enzyme (15, 10 and 5%) supplemented groups compared to other treatments throughout the period. The data on egg shell weight, shell thickness and shell percentage declared that there was statistically significant ($p<0.05$) difference was observed between control and various experimental groups. Between 27-42 weeks of age the mortality was within the limitation.

Keywords: Rice based distiller dried grain soluble, egg weight, Haugh unit, yolk colour and shell thickness

Introduction

Poultry is one of the fastest growing segments of the agriculture sector with annual growth rate of 8 to 10 percent. The major constituent in the poultry production is Feed, which accounts for 70% of total recurring expenditure. Feed costs are primarily driven by the cost of protein sources. The major protein source used in poultry diet is Soybean meal (SBM). There are two ways for decreasing the cost of production; one by decreasing feed cost, which seems to be impossible due to rising costs of feed ingredients and another is to explore new feed ingredient, which is cheaper, locally available and comparable with respect to its nutrient contents as compared to traditionally used feed ingredients. The byproduct of the processing of rice alcohol industry which is produced from the distillation of fermented rice is RDDGS. In processing, rice is cooked at 131 °C and 2.6 kg/m^2 pressure and yeast is added to the cooked rice for fermentation (Huang *et al.*, 1999) [6]. Then the alcohol is distilled from fermentation liquor and then leftover is known as Rice Distillers Dried Grains with Soluble (RDDGS). RDDGS also has yeast enzyme (probiotic factor) which increases the level of production. It helps to enrich the egg yolk and the color of the skin i.e., xanthophylls. Because of its high energy and protein content, RDDGS becomes an attractive substitute of expensive source of energy (corn) and protein (soybean meal) ingredients of poultry feed. Further, the exogenous enzyme supplementation in poultry diets is nutritionally, economically and environmentally justified. Feed enzymes increase nutrient digestibility, reduce water content and viscosity of the excreta, and accelerate the rate of passage of digesta through the gastrointestinal tract (Lazaro *et al.*, 2004) [9].

Materials and Methods

The experiment was conducted at the Poultry Experimental Station, Livestock Farm Complex (LFC), College of Veterinary Science, Rajendranagar, Hyderabad. The laboratory analysis of the collected biological samples was done at Department of Poultry Science, College of Veterinary Science, Rajendranagar, Hyderabad and ICAR-Directorate of Poultry Research, Rajendranagar, Hyderabad.

Experimental Design and Diets

The experiment was conducted during July to November 2019. A total of 240, 22 weeks old BV-300 White Leghorn layer birds were distributed randomly in to 10 dietary treatments, each treatment consists of 6 replicates and each replicate having 4 birds. The treatment diets were formulated to be iso-caloric and iso-nitrogenous (Crude Protein 18%, Metabolisable energy E 2600 kcal/kg diet) to meet the ME and CP requirement of laying hens according to the breeder guidelines (BV-300) requirements (Table 1).

Table 1: Schedule of Test Diets for Layers

Treatment	Experimental Diets
T_1	BD + 0% RDDGS(BD)
T_2	BD + with enzyme(BD + E)
T_3	BD + 5% of RDDGS without enzyme
T_4	BD + 10% of RDDGS without enzyme
T_5	BD + 15% of RDDGS without enzyme
T_6	BD + 20% of RDDGS without enzyme
T_7	BD + 5% of RDDGS with enzyme
T_8	BD + 10% of RDDGS with enzyme
T_9	BD + 15% of RDDGS with enzyme
T_{10}	BD + 20% of RDDGS with enzyme

Egg quality parameters were evaluated at 4 weeks interval. It includes internal and external egg quality parameters. Internal egg quality includes albumen quality (haugh unit score, albumen index), yolk quality (yolk colour, yolk index). External egg quality includes (viz., shell weight (g), shell thickness (mm), shell strength (N), shell percentage) which were measured during the four laying periods. During the last three consecutive days of each period (28days), 1 egg per replicate was randomly collected. A total of 180 eggs/period i.e. during 30th, 34th, 38th and 42nd week of age to assess the egg quality parameters and a total of 720 eggs were utilized for measuring different egg quality traits of experimental birds. The data on mortality of layers were calculated based on the mortalities recorded as and when they occurred. The data were analysed using General Linear Model procedure of Statistical Package for Social Sciences (SPSS) 15th version and comparison of means was done using Duncan's multiple range test (Duncan, 1955) [4] and significance was considered at $p<0.05$.

Table 2: Ingredient and nutrient (%) composition of commercial layer diets (27-42 weeks), Experiment-1I

	Control	Control + E	5% RDDGS + NE	10% RDDGS + NE	15% RDDGS + NE	20% RDDGS + NE	5% RDDGS + E	10% RDDGS + E	15% RDDGS + E	20% RDDGS + E
Maize	57.7	57.7	54.8	51	47.7	44.35	54.8	51	47.7	44.35
SBM	27	27	21.9	17	11.6	6.6	21.9	17	11.6	6.6
RDDGS	0	0	5	10	15	20	5	10	15	20
DORB	0.5	0.5	3.5	7	10.9	14.1	3.5	7	10.9	14.1
Enzyme (E)	0	0.025	0	0	0	0	0.025	0.025	0.025	0.025
Stone Grit	12.03	12.03	12.06	12.1	12.15	12.16	12.06	12.1	12.15	12.16
DCP	2.01	2.01	2.01	2	2.01	2.01	2.01	2	2.01	2.01
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Soda.bicarb	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
DL-Methionine	0.13	0.13	0.1	0.06	0.02	0.01	0.1	0.06	0.02	0.01
Lysine HCL	0.01	0.01	0.1	0.19	0.29	0.38	0.1	0.19	0.29	0.38
TMM	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
AB2D3K	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
B Complex	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025
Coccistat	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Antibiotic	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Choline chloride	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Toxin Binder	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Tylosine	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
TOTAL	100.3	100.3	100.4	100.3	100.6	100.6	100.4	100.3	100.6	100.6

*E-enzyme, *NE-no enzyme

Nutrient levels, on dry matter basis

	Control	Control + E	5% RDDGS + NE	10% RDDGS + NE	15% RDDGS + NE	20% RDDGS + NE	5% RDDGS + E	10% RDDGS + E	15% RDDGS + E	20% RDDGS + E
ME (K cal/kg)	2601	2601	2608	2599	2602	2601	2608	2599	2602	2601
CP (%)	18.06	18.06	18.03	18.09	18.00	18.00	18.03	18.09	18.00	18.00
Ca (%)	3.80	3.80	3.80	3.80	3.80	3.80	3.80	3.80	3.80	3.80
Av.P. (%)	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Lysine (%)	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88	0.88
Methionine (%)	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
NaCl (%)	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36

*E-enzyme, *NE-no enzyme

Vitamin premix provided per kg diet: Vitamin A 200000 IU, Vitamin D3 3000 IU, Vitamin E 10 mg, Vitamin K 2 mg, Riboflavin 25 mg, Vitamin B1 1 mg, Vitamin B6 2 mg, Vitamin B12 40 mg, and Niacin 15 mg.

*Trace mineral provided per kg diet: Manganese 120 mg, Zinc 80 mg, Iron 25 mg, Copper 10 mg, Iodine 1 mg and Selenium 0.1 mg.

Results and Discussion

Egg Weight (g)

The data on egg weight (g) in commercial White Leghorn layers was significantly ($p<0.05$) influenced by different levels of RDDGS in feed with or without enzyme supplementation is presented in Table 3. The egg weight (g) was higher in RDDGS with enzyme supplemented diets compared to control diets. The same trend continued from 27-42 weeks of age in commercial layers. The results are in agreement with the findings of Masa'deh (2011) [11]; Romero *et al.* (2012) [13] and Gupta *et al.* (2019) [5] Enzyme supplementation resulted in significant ($p<0.01$) improvement of egg mass and revealed that egg weight and mass were decreased as DDGS increased in the diet during phase I of production, meanwhile, it was not affected by DDGS levels during phase II. On contrary to Swiatkiwicz and Koreleski (2006) [14] revealed no differences in egg weight, they also realized that daily egg weight was negatively influenced by the inclusion of DDGS in laying hen diets up to 20%.

Table 3: Effect of dietary inclusion of Rice based distillers dried grain soluble (RDDGS) with or without Enzyme Supplementation on Egg weight (g) in White Leghorn layers during 27-42 weeks of age

Treatment	RDDGS% in diet	Enzyme @ 250 g/ton of feed	Age in weeks				Mean
			P1(27-30)	P2 (31-34)	P3 (35-38)	P4 (39-42)	27-42 wks
T_1	0	-E	55.01	54.37	55.53^{ab}	55.71^{ab}	55.16^{abc}
T_2	0	+ E	54.92	55.37	55.64^{ab}	55.58^{abc}	55.38^{ab}
T_3	5	-E	54.76	55.18	55.87^{ab}	55.87^{ab}	55.42^{ab}
T_4	10	-E	55.78	55.22	53.87^{c}	54.15^{c}	54.75^{bc}
T_5	15	-E	54.77	54.88	55.18^{abc}	55.09^{bc}	54.98^{abc}
T_6	20	-E	53.89	53.63	54.80^{abc}	55.23^{bc}	54.36^{c}
T_7	5	+ E	54.83	55.08	56.38^{a}	56.38^{ab}	55.67^{a}
T_8	10	+ E	54.99	54.58	54.68^{bc}	55.28^{bc}	54.88^{abc}
T_9	15	+ E	55.17	55.07	56.27^{ab}	56.13^{ab}	55.68^{a}
T_{10}	20	+ E	54.53	55.02	56.36^{ab}	56.82^{a}	55.66^{a}
		N	6	6	6	6	6
		P Value	0.610	0.413	0.013	0.014	0.012
		SEM	0.165	0.160	0.179	0.163	0.095

Mean bearing atleast one common superscript in a column differ significantly ($p<0.05$)
P-value: Probability value, SEM: Standard Error Mean, N: Number of replicates,
P1-period 1, P2-period 2, P3-period 3 and P4-period 4
+ E; with enzyme,-E; without enzyme

Egg Quality Parameters

Albumen quality parameters

The data on Haugh unit (HU) and albumen index in layers as influenced by different levels of RDDGS in fed with or without enzyme supplementation is presented in Table 4 and 5. There was no significant difference in Haugh unit values during first (27-30 wks), second (31-34 wks), third (35-38 wks) and overall periods (27-42 wks). Where as in fourth (39-42 wks) period there was a significant ($p<0.05$) difference in values of Haugh unit in different dietary treatments. There was significant ($p<0.05$) difference in fourth (39-42 wks) period there was significant in values of Albumen Index in different dietary treatments. The highest albumen index value was observed in 20% RDDGS with enzyme group compared to others treatment groups. The results are in agreement with the findings of Jung and Batal (2009) [8]; Masa'deh *et al.*, (2011) [11] who reported no significant differences in Haugh unit, between hens fed basal diet that contained different inclusion levels of DDGS. Where as in fourth (39-42 wks) period there was a significant ($p<0.05$) difference in Haugh unit and albumen index values of different treatments. Similar results observed with Pineda *et al.* (2008) [12], who recommended that laying hens could be fed on high level of DDGS (69%) without adverse effects on egg production or Haugh units. Deniz *et al.* (2013) [2] and Jiang *et al.* (2013) [7] revealed that 15% CDDGS can be added to the diet of laying hens without compromising the Haugh units. There were no statistical differences in Haugh units among the levels of DDGS for the entire egg production period. The higher the Haugh units value, the better the egg albumen quality. The benefit of feeding DDGS to improve Haugh units in laying hens were not substantiated by Lumpkins *et al.* (2005) [10]; Swiatkiewicz and Koreleski (2006) [14] and Pineda *et al.* (2008) [12] did not record any improvement in interior egg quality when DDGS was fed to laying hens.

Table 4: Effect of dietary inclusion of Rice based distillers dried grain soluble (RDDGS) with or without Enzyme Supplementation on Haugh Unit in White Leghorn layers during 27-42 weeks of age

Treatment	RDDGS% in diet	Enzyme @ 250 g/ton of feed	Age in weeks				Mean
			P1(27-30)	P2 (31-34)	P3 (35-38)	P4 (39-42)	27-42 wks
T_1	0	-E	98.95	97.11	96.83	95.19^{abc}	97.02
T_2	0	+ E	99.84	98.17	96.56	92.47^{c}	96.76
T_3	5	-E	98.22	99.67	95.67	93.01^{bc}	96.64
T_4	10	-E	98.00	97.83	96.78	97.88^{a}	97.62
T_5	15	-E	99.95	96.33	98.50	97.23^{a}	98.00
T_6	20	-E	97.11	97.50	94.72	96.71^{ab}	96.51
T_7	5	+ E	99.83	97.39	98.00	97.68^{a}	98.22
T_8	10	+ E	98.44	96.22	93.17	94.47^{abc}	95.57
T_9	15	+ E	97.06	99.45	96.89	92.99^{bc}	96.59
T_{10}	20	+ E	99.56	98.89	94.06	91.56^{c}	96.02
		N	6	6	6	6	6
		P Value	0.198	0.285	0.112	0.001	0.087
		SEM	0.374	0.389	0.433	0.454	0.225

Mean bearing atleast one common superscript in a column differ significantly ($p<0.05$)
P-value: Probability value, SEM: Standard Error Mean, N: Number of replicates,
P1-period 1, P2-period 2, P3-period 3 and P4-period 4.
+ E; with enzyme,-E; without enzyme

Table 5: Effect of dietary inclusion of Rice based distillers dried grain soluble (RDDGS) with or without Enzyme Supplementation on Albumen index White Leghorn layers during 27-42 weeks of age

Treatment	RDDGS% in diet	Enzyme @ 250 g/ton of feed	Age in weeks				Mean
			P1(27-30)	P2 (31-34)	P3 (35-38)	P4 (39-42)	27-42 wks
T_1	0	-E	0.073	0.069	0.071	0.076^{abc}	0.072
T_2	0	+ E	0.075	0.065	0.071	0.071^{bc}	0.071
T_3	5	-E	0.071	0.070	0.072	0.076^{abc}	0.074
T_4	10	-E	0.068	0.068	0.066	0.069^{c}	0.065
T_5	15	-E	0.075	0.072	0.068	0.076^{abc}	0.072
T_6	20	-E	0.070	0.068	0.067	0.076^{abc}	0.070
T_7	5	+ E	0.069	0.070	0.080	0.082^{ab}	0.074
T_8	10	+ E	0.064	0.068	0.061	0.072^{bc}	0.069
T_9	15	+ E	0.074	0.073	0.066	0.083^{ab}	0.074
T_{10}	20	+ E	0.071	0.069	0.065	0.087^{a}	0.073
		N	6	6	6	6	6
		P Value	0.001	0.000	0.001	0.001	0.000
		SEM	0.454	0.578	0.198	0.033	0.179

Mean bearing at least one common superscript in a column differ significantly ($p<0.05$)
P-value: Probability value, SEM: Standard Error Mean, N: Number of replicates,
P1-period 1, P2-period 2, P3-period 3 and P4-period 4
+ E; with enzyme,-E; without enzyme

Yolk quality parameters

The present study shows the data on yolk index values in Commercial layers as influenced by different levels of RDDGS in feed with or without enzyme supplementation is presented in Table 6. In period-P3 (35-38 wks), period-P4 (39-42 wks) and over all period (27-42 wks) there was significant ($p<0.05$) difference in yolk index among different levels of treatment fed groups. The overall period yolk index was better in RDDGS with or without enzyme supplemented groups compared to the control group. Overall yolk colour was significantly higher ($p<0.05$) in RDDGS with enzyme (15, 10 and 5%) supplemented groups compared to other treatments, where as the lowest value was observed in the birds fed control. A linear increase was observed in the colour of the yolk of birds as the level of RDDGS was increased in the diet which is shown in Table 7.

There was no significant difference in yolk index values during period-P1 (27-30 wks) and period-P2 (31-34 wks) fed with different levels of RDDGS with enzyme, RDDGS without enzyme and control fed groups. Similar findings were reported by Abd El-Hack and Mahgoub (2015) [1], who found that the best yolk index was obtained from hens fed the basal diets that contained 5 and 10% DDGS compared with those fed 15% DDGS**.** The results are in agreement with the findings of Masa'deh (2011) [11] and Abd El-Hack and Mahgoub (2015) [1] the egg yolk color was linearly increased ($p<0.01$) as dietary level of DDGS increased throughout the experiment. Deniz *et al.* (2013) [2] revealed that 15% CDDGS can be added to the diet of laying hens without compromising the egg yolk color. The RDDGS levels had significant effect on yolk colour but no regular trend was observed ($p<0.05$). The enzyme supplementation significantly increased the yolk colour. The yolk colour improvement may be due to the presence of carotenoid pigments in DDGS fed group compared to control (Gupta *et al.* 2019) [5].

Table 6: Effect of dietary inclusion of Rice based distillers dried grain soluble (RDDGS) with or without Enzyme Supplementation on Yolk index White Leghorn layers during 27-42 weeks of age

Treatment	RDDGS% in diet	Enzyme @ 250 g/ton of feed	Age in weeks				Mean
			P1(27-30)	P2 (31-34)	P3 (35-38)	P4 (39-42)	27-42 wks
T_1	0	-E	0.469	0.473	0.483^{abc}	0.464^{cd}	0.472^{c}
T_2	0	+ E	0.472	0.473	0.479^{abcd}	0.469^{cd}	0.473^{c}
T_3	5	-E	0.487	0.493	0.503^{ab}	0.470^{cd}	0.476^{c}
T_4	10	-E	0.480	0.485	0.479^{abcd}	0.479^{abc}	0.477b^{c}
T_5	15	-E	0.478	0.485	0.505^{a}	0.475^{bcd}	0.478^{bc}
T_6	20	-E	0.484	0.490	0.467^{cd}	0.453^{c}	0.467^{c}
T_7	5	+ E	0.470	0.485	0.479^{abcd}	0.497^{ab}	0.495^{a}
T_8	10	+ E	0.471	0.481	0.477^{bcd}	0.483^{abc}	0.482^{abc}
T_9	15	+ E	0.478	0.476	0.483^{abc}	0.502^{a}	0.493^{ab}
T_{10}	20	+ E	0.485	0.476	0.455^{d}	0.465^{cd}	0.476^{c}
		N	6	6	6	6	6
		P Value	0.002	0.002	0.003	0.002	0.001
		SEM	0.765	0.615	0.003	0.001	0.005

Mean bearing at least one common superscript in a column differ significantly ($p<0.05$)
P-value: Probability value, SEM: Standard Error Mean, N: Number of replicates,
P1-period 1, P2-period 2, P3-period 3 and P4-period 4
+ E; with enzyme,-E; without enzyme

Table 7: Effect of dietary inclusion of Rice based distillers dried grain soluble (RDDGS) with or without Enzyme Supplementation on Yolk colour White Leghorn layers during 27-42 weeks of age

Treatment	RDDGS% in diet	Enzyme @ 250 g/ton of feed	Age in weeks				Mean
			P1(27-30)	P2 (31-34)	P3 (35-38)	P4 (39-42)	27-42 wks
T_1	0	-E	3.889	5.167^{ab}	4.611	5.389	4.764^{ab}
T_2	0	+ E	4.222	5.167^{ab}	4.778	5.000	4.792^{ab}
T_3	5	-E	4.833	5.056^{abc}	5.000	4.333	4.806^{ab}
T_4	10	-E	4.389	4.556^{bc}	4.556	4.333	4.458^{b}
T_5	15	-E	4.778	5.056^{abc}	4.500	4.333	4.667^{ab}
T_6	20	-E	4.389	4.722^{abc}	4.833	4.167	4.528^{b}
T_7	5	+ E	4.778	5.334^{a}	4.833	5.000	4.986^{a}
T_8	10	+ E	4.445	5.056^{abc}	4.667	4.833	4.750^{ab}
T_9	15	+ E	4.389	4.500^{c}	4.778	4.167	4.458^{b}
T_{10}	20	+ E	4.444	4.556^{bc}	4.389	4.667	4.514^{b}
		N	6	6	6	6	6
		P Value	0.235	0.018	0.391	0.125	0.048
		SEM	0.08	0.067	0.056	0.108	0.041

Mean bearing at least one common superscript in a column differ significantly ($p<0.05$)
P-value: Probability value, SEM: Standard Error Mean, N: Number of replicates,
P1-period 1, P2-period 2, P3-period 3 and P4-period 4
+ E; with enzyme,-E; without enzyme

Shell Quality Parameters

There was no significant difference in shell weight, shell thickness, shell percentage and shell breaking strength between control and various experimental groups (Table 8 to 11). Similar findings were observed by Jung and Batal (2009) [8], who reported no significant differences in eggshell thickness or shell breaking strength between hens fed basal diets that contained different inclusion levels of DDGS. On the contrary, Deniz *et al.* (2013) [2] and Abd El-Hack and Mahgoub (2015) [1] revealed that 15% CDDGS can be added to the diet of laying hens without compromising the exterior (eggshell thickness and shell breaking strength) egg quality**.** The RDDGS levels had significant effect on shell thickness but no regular trend was observed ($p<0.05$). The enzyme supplementation significantly increased the shell thickness (Gupta *et al.* 2019) [5].

Table 8: Effect of dietary inclusion of Rice based distillers dried grain soluble (RDDGS) with or without Enzyme Supplementation on Egg shell weight White Leghorn layers during 27-42 weeks of age

Treatment	RDDGS% in diet	Enzyme @ 250 g/ton of feed	Age in weeks				Mean
			P1(27-30)	P2 (31-34)	P3 (35-38)	P4 (39-42)	27-42 wks
T_1	0	-E	5.432	5.427	5.692^{a}	5.040^{f}	5.398^{cd}
T_2	0	+ E	5.352	5.541	5.741^{a}	5.352^{def}	5.497^{bcd}
T_3	5	-E	5.339	5.150	5.924^{a}	5.991^{ab}	5.601^{ab}
T_4	10	-E	5.490	5.401	5.839^{a}	6.278^{a}	5.752^{a}
T_5	15	-E	5.421	5.350	5.829^{a}	5.565^{cd}	5.541^{bc}
T_6	20	-E	5.441	5.366	5.792^{a}	5.246^{ef}	5.461^{bcd}
T_7	5	+ E	5.339	5.150	5.852^{a}	5.991^{ab}	5.603^{ab}
T_8	10	+ E	5.490	5.401	5.855^{a}	6.278^{a}	5.486^{bcd}
T_9	15	+ E	5.421	5.350	5.184^{b}	5.565^{cde}	5.328^{d}
T_{10}	20	+ E	5.441	5.366	5.643^{a}	5.246^{ef}	5.432^{bcd}
		N	6	6	6	6	6
		P Value	0.503	0.146	0.002	0.001	0.001
		SEM	0.031	0.033	0.041	0.054	0.021

Mean bearing atleast one common superscript in a column differ significantly ($p<0.05$)
P-value: Probability value, SEM: Standard Error Mean, N: Number of replicates,
P1-period 1, P2-period 2, P3-period 3 and P4-period 4
+ E; with enzyme,-E; without enzyme

Table 9: Effect of dietary inclusion of Rice based distillers dried grain soluble (RDDGS) with or without Enzyme Supplementationon Egg shell thickness White Leghorn layers during 27-42 weeks of age

Treatment	RDDGS% in diet	Enzyme @ 250 g/ton of feed	Age in weeks				Mean
			P1(27-30)	P2 (31-34)	P3 (35-38)	P4 (39-42)	27-42 wks
T_1	0	-E	0.413	0.416	0.416	0.436^{abcd}	0.420
T_2	0	+ E	0.401	0.408	0.382	0.446^{ab}	0.409
T_3	5	-E	0.392	0.412	0.434	0.441^{abc}	0.420
T_4	10	-E	0.378	0.381	0.414	0.457^{a}	0.408
T_5	15	-E	0.411	0.407	0.414	0.415^{de}	0.412
T_6	20	-E	0.411	0.404	0.418	0.402^{e}	0.409
T_7	5	+ E	0.379	0.404	0.423	0.451^{ab}	0.414
T_8	10	+ E	0.403	0.406	0.402	0.452^{ab}	0.415
T_9	15	+ E	0.403	0.399	0.386	0.433^{bcd}	0.405
T_{10}	20	+ E	0.406	0.398	0.403	0.422^{cde}	0.408
		N	6	6	6	6	6
		P Value	0.114	0.172	0.425	0.001	0.376
		SEM	0.003	0.002	0.075	0.003	0.018

Mean bearing atleast one common superscript in a column differ significantly ($p<0.05$)
P-value: Probability value, SEM: Standard Error Mean, N: Number of replicates,
P1-period 1, P2-period 2, P3-period 3 and P4-period 4
+ E; with enzyme,-E; without enzyme

Table 10: Effect of dietary inclusion of Rice based distillers dried grain soluble (RDDGS) with or without Enzyme Supplementationon Egg shell percentage White Leghorn layers during 27-42 weeks of age

Treatment	RDDGS% in diet	Enzyme @ 250 g/ton of feed	Age in weeks				Mean
			P1(27-30)	P2 (31-34)	P3 (35-38)	P4 (39-42)	27-42 wks
T_1	0	-E	9.870	9.989	10.26^{ab}	9.888	10.00^{ab}
T_2	0	+ E	9.771	10.02	10.34^{ab}	10.27	10.10^{ab}
T_3	5	-E	9.766	9.337	10.62^{ab}	10.56	10.07^{ab}
T_4	10	-E	9.876	9.794	10.86^{a}	10.03	10.14^{ab}
T_5	15	-E	9.961	9.775	10.52^{ab}	10.48	10.18^{a}
T_6	20	-E	10.11	10.01	10.57^{ab}	10.32	10.26^{a}
T_7	5	+ E	10.00	9.869	10.39^{ab}	10.21	10.12^{ab}
T_8	10	+ E	9.731	9.632	10.71^{a}	10.22	10.07^{ab}
T_9	15	+ E	9.639	9.515	9.22^{c}	9.94	9.57^{8c}
T_{10}	20	+ E	9.479	9.366	10.01^{b}	10.50	9.839^{bc}
		N	6	6	6	6	6
		P Value	0.621	0.100	0.001	0.156	0.001
		SEM	0.064	0.065	0.082	0.062	0.036

Mean bearing atleast one common superscript in a column differ significantly ($p<0.05$)
P-value: Probability value, SEM: Standard Error Mean, N: Number of replicates,
P1-period 1, P2-period 2, P3-period 3 and P4-period 4
+ E; with enzyme,-E; without enzyme

Table 11: Effect of dietary inclusion of Rice based distillers dried grain soluble (RDDGS) with or without Enzyme Supplementation on Egg shell breaking strength White Leghorn layers during 27-42 weeks of age

Treatment	RDDGS% in diet	Enzyme @ 250 g/ton of feed	Age in weeks				Mean
			P1(27-30)	P2 (31-34)	P3 (35-38)	P4 (39-42)	27-42 wks
T_1	0	-E	26.44	28.72	28.06	22.25[c]	26.37
T_2	0	+ E	25.63	31.76	27.14	25.30[bc]	27.46
T_3	5	-E	24.69	28.25	33.63	33.82[a]	30.10
T_4	10	-E	29.53	28.14	29.73	35.78[a]	30.79
T_5	15	-E	23.01	30.90	28.84	26.31[b]	27.27
T_6	20	-E	29.16	28.20	30.61	27.61[b]	28.90
T_7	5	+ E	28.38	28.15	29.22	29.16[b]	28.73
T_8	10	+ E	23.54	34.17	32.18	26.03[bc]	28.98
T_9	15	+ E	32.65	29.70	23.47	26.43[b]	28.06
T_{10}	20	+ E	24.20	26.41	30.58	25.72[bc]	26.73
		N	6	6	6	6	6
		P Value	0.133	0.614	0.092	0.001	0.184
		SEM	0.808	0.784	0.701	0.627	0.386

Mean bearing atleast one common superscript in a column differ significantly ($p<0.05$)
P-value: Probability value, SEM: Standard Error Mean, N: Number of replicates,
P1-period 1, P2-period 2, P3-period 3 and P4-period 4
+ E; with enzyme,-E; without enzyme

Mortality

Between 27-42 weeks of age the mortality was within the limitation. During first (27-30 wks) second (31-34 wks) and fourth (39-42 wks) periods there was no mortality of birds but where as in third period (35-38 wks) only four birds died in 20% RDDGS without enzymeand control groups (Table 13). The results are in agreement with the findings of (Dinani *et al.*, 2018) [3] observed that the mortality was well within normal limits during the entire period of experiment in all treatment groups.

Table 13: Effect of dietary inclusion of Rice based distillers dried grain soluble (RDDGS) with or without Enzyme Supplementation on mortality in White Leghorn layers during 27-42 weeks of age

Treatment	RDDGS% in diet	Enzyme @ 250 g/ton of feed	Age in weeks				Mean
			P1(27-30)	P2 (31-34)	P3 (35-38)	P4 (39-42)	27-42 wks
T_1	0	-E	-	-	2	-	0.5
T_2	0	+ E	-	-	-	-	-
T_3	5	-E	-	-	-	-	-
T_4	10	-E	-	-	-	-	-
T_5	15	-E	-	-	-	-	-
T_6	20	-E	-	-	2	-	0.5
T_7	5	+ E	-	-	-	-	-
T_8	10	+ E	-	-	-	-	-
T_9	15	+ E	-	-	-	-	-
T_{10}	20	+ E	-	-	-	-	-

Mean bearing at least one common superscript in a column differ significantly ($p<0.05$)
P-value: Probability value, SEM: Standard Error Mean, N: Number of replicates,
P1-period 1, P2-period 2, P3-period 3 and P4-period 4
+ E; with enzyme,-E; without enzyme

Conclusion

Based on the overall results, it can be concluded that 15% RDDGS with enzyme in layer diets without affecting the egg quality parameters.

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