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Effect of feeding rice based distillers dried grains with solubles and gluten meal on the hamato-serological parameters in finisher stage of broiler chickens

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Abstract
A biological experiment was conducted to evaluate the effect of feeding rice distillers dried grains with solubles (rDDGS) and rice gluten meal (RGM) combination as soybean replacement along with exogenous enzyme supplementation on the hamato-serological parameters in finisher phase of broiler chicken at 42 days of age. The experimental diets used were T1 (no rDDGS/RGM/enzyme), T2 (no rDDGS/RGM, with multienzymes), T3 (12.5% rDDGS, 15% RGM, no enzyme), T4 (12.5% rDDGS, 15% RGM, with protease enzyme), T5 (10% rDDGS, 12.5% RGM, no enzyme), and T6 (10% rDDGS, 12.5% RGM, with protease enzyme). The results revealed that no significant *(P*>0.05) differences were observed in blood profile between control and other different dietary treatments having different levels of rDDGS and RGM combinations with or without enzymes and their interaction. Serum glucose, total protein, albumen (A): globulin (G) ratio, cholesterol and triglyceride level decreased significantly while serum enzymes SGOT,SGPT and alkaline phosphatase (ALP) increased significantly in 12.5% rDDGS + 15% RGM with or without protease enzyme groups as compared to control and 10% rDDGS +12.5% RGM with or without protease enzyme groups. The study concludes that the feeding of rDDGS and RGM in combination up to the levels of 12.5% and 15%, respectively with or without enzyme supplementation do not have any adverse effects on the haematological parameters, but adversely affecting serum biochemical parameters during finisher phase of broiler chicken at 42 days.

Keywords: Rice distillers dried grains with solubles (rDDGS), rice gluten meal (RGM), protease, broiler, haematology, serum biochemistry

Introduction
The diet and plane of nutrition are prominent factors that influence the blood picture and serum biochemistry of animals, which are sensitive indicators of the state of health and reflect the intensity of metabolic processes taking place in the animals [1, 19, 24]. Similarly, it has been reported that the serum biochemical parameters are the good indicators of the physiological, nutritional, and pathological status of animals/birds and can be correlated to identify the impact of nutritional factors and additives supplied in the diet [22]. Thus, the dietary manipulations in broiler chicken are expected to reveal differential effects on the serum blood picture and serum biochemistry of broiler chicken. In this regard, the rice distillers dried grains with solubles (rDDGS) and rice gluten meal (RGM) are two potential economic feed ingredients of poultry ration produced by rice processing industries.India is one of the largest producers of rice in world producing approximately 105 million tons of rice in 2015-16 [7]. The rising cost of broiler chicken ration can be overcome by the use of 'rice DDGS and rice gluten meal'; protein rich alternative feed ingredients to replace costly soybean meal. An increase in ethanol production over the last 5-10 years, due to increasing prices of conventional oil and limited underground reserves, has led to an increased supply of DDGS that is available as livestock feed [17]. On the other hand, the RGM, a by-product of wet-milling of rice, is available in appreciable amounts at lower cost compared to soybean. RGM contains 3152 kcal ME/kg, 46.45% crude protein, 3.4% ether extract and a favorable amino acid profile with relatively higher abundance of methionine [14]. It has been also designated as a source of rumen undegradable protein with the highest known metabolizable protein value among plant proteins [11].

Further, the exogenous enzyme supplementations in poultry diets, nutritionally, economically and environmentally justified, increase nutrient digestibility, reduce water content and viscosity of the excreta, and accelerate the rate of passage of digesta through the gastrointestinal tract [15, 20]. In this study it is hypothesised that the use of rDDGS, RGM along with enzyme supplementation will have significant impact on the blood parameters and serum biochemistry of broiler chicken. Thus, to test this hypothesis the objective of this study was to evaluate the effects of feeding different combinations of rDDGS and RGM levels along with enzyme supplementation on the haematology and serum biochemical parameters of broiler chicken.

Materials and Methods

Birds, experimental diets and design

A total of 240 CARIBRO Vishal broiler chicks of same hatch and uniform weight were procured from the institutional hatchery and housed in specially designed battery brooder cages with standard watering and feeding facilities. The broiler chicken ration was formulated by using rDDGS and RGM as replacement of soybean meal in the basal diets along with enzyme supplementation [12]. The levels of rDDGS and RGM and the suitable enzymes were standardised in a preliminary trail. The rDDGS levels of 10% & 12.5%, and RGM levels of 12.5% & 15% along with either multienzymes or protease enzyme were selected to formulate the experimental diets as the pre-starter, starter and finisher diets. The feed ingredients and the nutrient composition of diets have been given in Table 1. A 2x3 factorial design resulted in six experimental diets *viz* T1 (no rDDGS/RGM/enzyme), T2 (no rDDGS/RGM, with multienzymes), T3 (12.5% rDDGS, 15% RGM, no enzyme), T4 (12.5% rDDGS, 15% RGM, with protease enzyme), T5 (10% rDDGS, 12.5% RGM, no enzyme), and T6 (10% rDDGS, 12.5% RGM, with protease enzyme). Each treatment was allocated 5 replicates of chicks, with 8 birds in each. The feeding trial was conducted for six weeks and the feed as well as drinking water were provided *ad libitum* to the birds during the entire experimental period.

Sampling and Measurement

All the procedures carried out on the birds were approved by the Institutional Animal Ethics Committee of Indian Veterinary Research Institute, Izatnagar (452/01/ab/CPCSEA). 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 leucocyte 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).

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 [23], total protein [3], albumin [9], globulin, albumin: globulin ratio (A:G), cholesterol [26], triglyceride [5], SGOT [18], SGPT [18], and ALP [13].

The data collected were analysed by two way ANOVA using GLM procedure [21] to present the results as means and standard errors by using statistical package for social sciences (SPSS) 16.0 version and the comparison of significant mean differences was done by Duncan's multiple range test [4].

Results and Discussion

The results of feeding different level of rDDGS and RGM combinations with or without enzymes on haematological parameters of the broiler chicken of 42 days of age are given in Table 2. The blood profile was 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) differences were observed in blood profile between control and other different dietary treatments having different levels of rDDGS and RGM combinations with or without enzymes and their interaction. All the blood parameters studied were well within the normal physiological range. In line with the results of the present study, no significant (P>0.05) effect of DDGS inclusion up to 15% level was found [6, 27]. Similarly, no significant (P>0.05) difference was reported in Hb and PCV of broiler chicken by incorporating RGM up to 17.5% level with or without protease supplementation [25]. However, in contrast to the results of the present study, inclusion of rDDGS level above 5% level significantly (P<0.01) enhance the PCV and Hb values of broiler chicken compared to control diet fed birds [8].

The results pertaining to feeding different level of rDDGS and RGM combinations on serological parameters on 42 days are presented in Table 3. The results revealed that 0% rDDGS +0% RGM (DR1), 12.5% rDDGS +15% RGM (DR2) and 10 % rDDGS +12.5% RGM (DR3) combination levels showed no significant (P>0.05) difference in serum globulin level. Serum glucose and albumin (A): globulin (G) ratio were significantly (P<0.01) decreased in DR2 as compared to DR1 and DR 3 combination levels. Serum protein decreased significantly (P<0.05) in DR2 as compared to DR1, but it did not show any significant (P>0.05) difference from DR3. Serum albumin level was significantly (P>0.01) decreased in DR2 followed by DR3 as compared to DR1. Serum cholesterol was significantly (P<0.01) decreased in DR2 and DR3 as compared to DR1.Serum triglyceride was significantly (P<0.01) decreased in DR2 as compared to DR1 and DR3 combination levels. The serum enzyme SGOT was significantly (P<0.01) increased in DR2 as compared to DR1 and DR3 combination levels. Serum enzymes SGPT and ALP were significantly (P<0.05) increased in DR2 level as compared to DR1.However, SGPT and ALP did not show any significant (P>0.05) difference in DR3 as compared to DR1 level. Effect of with or without enzymes on feeding different level of rDDGS and RGM combinations did not show any significant (P>0.05) difference in serological parameters except serum glucose. Serum glucose level was increased significantly (P<0.05) in enzymes supplemented groups as compared to without enzymes groups. Interaction of rDDGS and RGM combinations with or without enzymes did not show any significant (P>0.05) difference on serological parameters except on SGOT. Serum SGOT was significantly (P<0.01) increased in 12.5% rDDGS +15% RGM without enzyme combination as compared to control and other dietary treatments.

Our results were in agreement with researchers [2, 16, 14, 25]. 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 [2]. No significant (P>0.05) difference in serum biochemical parameters (serum lipid profile, glucose, total protein, albumin and globulin) up to addition of 12.5% RGM in diet of broiler chicken [16]. Similarly, no significant (P>0.05) difference in the serological variables (glucose, blood urea nitrogen, plasma proteins and non esterified fatty acids) on addition of RGM up to 21% level in the diet of growing dairy calves [14]. No significant (P>0.05) difference on feeding different levels of RGM up to 20% level with or without protease enzyme supplementation [25]. Moreover, serum biochemical parameters are indicators of the physiological, nutritional and pathological status of birds and can be correlated to identify the impact of nutritional factors and additives supplied in the diet.

Our results were in disagreement with researchers [10, 8]. Increasing corn DDGS level significantly (P<0.01) increased serum triglycerides, cholesterol and LDL for hens fed diet contained 22% DDGS in the diet [10]. Similarly, 10% rice DDGS had significantly (P<0.01) higher effect on serum albumin, total protein, A:G ratio, glucose value and significantly (P<0.01) lowering effect on serum lipid profile (triglycerides, cholesterol, LDL and VLDL) [8].However, no such study on rDDGS and RGM combination feeding were done in poultry on blood biochemical parameters.

Table 1: Ingredient and nutrient composition of broiler chicken diets

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

SBM: Soybean meal,, DORB: Deoiled rice bran, DDGS: Dried distillers grains with solubles, RGM: Rice gluten meal, LSP: Limestone powder, DCP: Di-calcium phosphate, CP: Crude protein, ME: Metabolizable energy

*Constant (0.4% salt, 0.1% trace mineral premix, 0.15% vitamin premix, 0.015% vit. B complex, 0.05% choline chloride and 0.05% Toxin binder)

1: Trace mineral premix supplied (mg/kg diet): Mg 300; Mn 55; I 0.4; Fe 56; Zn 30; Cu 4.

2: Vitamin premix supplied (per kg diet): Vitamin A 8250 IU; Vitamin D3 1200 IU; Vitamin K 1mg; Vitamin E 40 IU.

3: B complex: Vitamin B1 2 mg; Vitamin B2 4 mg; Vitamin B12 10 µg; niacin 60 mg; pantothenic acid 10 mg; choline 500 mg.

Table 2: Effect of feeding different level of rDDGS and RGM combinations haematological parameters

rDDGS (%)	RGM (%)	Enzyme	TLC	Hetero.	Lymph	Mono	Hetero. %	Lymph %	Mono %	H:L	TEC	Hb	PCV	MCV	MCH	MCHC	RDWc	PLT	PLT %	MPV	PDWc
0.0	0.0	-	16.2	3.9	9.8	1.2	24.4	59.8	6.9	0.42	2.4	12.5	28.2	118	52.2	44.0	13.2	35.5	0.36	8.7	31.2
0.0	0.0	M	13.7	3.7	8.3	1.1	27.0	60.1	7.9	0.45	2.2	11.2	26.8	120	49.7	42.0	13.1	35.5	0.36	8.9	29.0
12.5	15.0	-	15.1	3.6	9.1	1.2	24.1	59.5	7.7	0.41	2.2	10.5	26.4	119	47.7	40.0	12.0	35.2	0.35	9.0	30.0
12.5	15.0	P	15.1	4.1	8.4	1.1	27.1	55.4	7.2	0.49	2.3	11.6	26.5	113	50.0	43.7	12.0	35.7	0.36	9.1	32.0
10.0	12.5	-	13.8	3.7	7.7	1.1	26.5	56.1	7.8	0.48	2.1	10.7	24.4	114	50.5	44.0	11.8	37.5	0.38	8.4	29.7
10.0	12.5	P	16.5	4.0	9.6	1.1	24.3	58.4	6.8	0.42	2.3	11.4	26.6	116	50.0	43.0	11.3	33.5	0.34	8.9	31.0
Pooled SEM			0.31	0.07	0.38	0.09	0.29	0.02	0.45	0.77	0.18	0.01	0.03	0.25	0.37	0.75	0.78	0.68	0.28	0.52	0.01
Main effects																					
rDDGS/RGM combination (%)																					
0.0	0.0		15.3	3.9	9.2	1.2	25.5	59.5	7.2	0.43	2.3	11.8	27.7	119	50.4	42.4	12.9	35.7	0.36	8.8	30.4
12.5	15.0		14.9	3.9	8.6	1.1	25.8	58.0	7.6	0.45	2.2	11.2	26.4	116	49.5	42.4	12.3	35.3	0.35	9.0	30.6
10.0	12.5		15.1	3.8	8.6	1.1	25.4	57.2	7.3	0.45	2.2	11.1	25.5	115	50.2	43.5	11.5	35.5	0.36	8.6	30.3
Enzyme supplementation																					
Without enzyme			15.0	3.7	8.8	1.2	25.0	58.4	7.4	0.43	2.2	11.2	26.3	117	50.1	42.6	12.3	36.0	0.36	8.7	30.3
With enzyme			15.1	3.9	8.8	1.1	26.1	58.0	7.3	0.45	2.3	11.4	26.7	116	49.9	42.9	12.1	34.9	0.35	8.9	30.6
Significance																					
rDDGS/RGM combination			NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Enzyme supplementation			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
Values bearing different superscripts within the column differ significantly NS: Non-significant (P>0.05) Units: WBC (x 10^4 ul),RBC (x10^6ul),Hb (g/dl),MCV (fl),MCH(pg),MCHC(g/dl) and PLT(x10^3ul)																					

Table 3: Effect of feeding different level of rDDGS and RGM combinations on serological parameters

rDDGS (%)	RGM (%)	Enzyme	Glucose mg/dl	Protein (g/dl)	Albumin g/dl	Globulin g/dl	A:G ratio	Cholesterol mg/dl	Triglyceride mg/dl	SGOT U/ml	SGPT U/ml	ALP U/ml
0.0	0.0	-	206	4.6	1.8	2.8	0.65	132	106	186^{ab}	12	50
0.0	0.0	M	211	4.8	1.9	3.0	0.63	131	99	186^{ab}	14	61
12.5	15.0	-	186	4.4	1.7	3.0	0.55	125	90	194^{c}	17	68
12.5	15.0	P	191	4.5	1.7	3.0	0.57	121	93	187^{ab}	15	62
10.0	12.5	-	204	4.7	1.8	2.6	0.68	127	100	182^{a}	16	58
10.0	12.5	P	210	4.6	1.7	2.8	0.63	127	98	187^{b}	15	59
Pooled SEM			1.881	0.037	1.88	0.03	0.02	0.04	0.01	1.03	1.49	0.80
Main effects												
rDDGS/RGM combination (%)												
0.0	0.0		208^{b}	4.7^{b}	1.9^{c}	2.8	0.64^{b}	131^{b}	102^{b}	186^{a}	13^{a}	55^{a}
12.5	15.0		188^{a}	4.4^{a}	1.6^{a}	2.9	0.55^{a}	123^{a}	91^{a}	191^{b}	16^{b}	65^{b}
10.0	12.5		207^{b}	4.6^{ab}	1.7^{b}	2.7	0.65^{b}	126^{a}	98^{b}	185^{a}	15^{ab}	58^{ab}
Enzyme supplementation												
Without enzyme			198^{a}	4.6	1.8	2.8	0.63	128	98	188	15	59
With enzyme			203^{b}	4.6	1.8	2.9	0.61	127	97	187	15	61
Significance												
rDDGS/RGM combination			P<0.01	P<0.05	P<0.01	NS	P<0.01	P<0.01	P<0.01	P<0.01	P<0.05	P<0.05
Enzyme supplementation			P<0.05	NS	NS	NS	NS	NS	NS	NS	NS	NS
Interaction			NS	NS	NS	NS	NS	NS	NS	P<0.01	NS	NS
Values bearing different superscripts within the column differ significantly A:G ratio: Abbumin: Globulin ratio, NS: Non-significant (P>0.05) M: Multienzyme, P: Protease												

Conclusion

The study concludes that the feeding of rDDGS and RGM in combination up to the levels of 12.5% and 15%, respectively with or without enzyme supplementation do not have adverse effects on the haematological parameters, but adversely affecting serum biochemical parameters of broiler chicken at 42 days post hatch.

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Conflict of interest

Authors declare no conflict of interest.

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