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Effect of feeding rice based brewer's dried grain on immune response and haemto-biochemical parameters of broiler chickens

Vinay Singh[1] . Chandra Deo[2] . Sunil Doley[3] . A.B. Mandal[2] . S.K. Bhanja[2] . B.K. Kandpal[1]

[1]ICAR Research complex for NEH Region, Tripura Centre, Lembucherra, West Tripura

[2]ICAR-Central Avian Research Institute, Izatnagar, Uttar Pradesh

[3]ICAR Research complex for NEH Region, Umiam, Meghalaya

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ABSTRACT

An experiment was conducted to study the response of feeding sun dried rice based brewer's dried grain (RBDG) on immune response and haemto-biochemical parameters of broiler chickens. For this, a feeding trial of 175 day old broiler chicks was conducted for six weeks with completely randomized design (CRD) divided into five treatments with 35 chicks with five replicates per treatment. Five experimental diets as per ICAR (2013) were prepared by incorporating rice brewer's dried grain (RBDG) at inclusion level of (0, 5, 10, 15 and 20%) respectively by replacing maize and soybean meal from basal diet so that all the test diets becomes iso-caloric and iso-nitrogenous. The results revealed that the cellular immune response and humoral immune response and immune organs weight such as bursa, spleen and thymus did not differed significantly due to inclusion of different levels of rice based brewer's dried grain. The various blood biochemical parameters namely glucose, serum total protein, albumin, globulin, albumin-globulin ratio and haemoglobin did not differ significantly. However total serum cholesterol and triglycerides were significantly ($P<0.01$) reduced as increasing graded levels of dietary rice based brewer's dried grain. Significantly lower serum cholesterol and triglycerides were observed at 15 and 20% inclusion levels of rice based brewer's dried grain than those recorded in other dietary treatments. Thus, it is concluded that rice based brewer's dried grain (RBDG) can be safely incorporated in broiler diet at the inclusion level of 20% for better immune response and haemto-biochemical parameters.

1. Introduction

Major conventional feed ingredients used in poultry diets are maize and soybean meal, but the ever increasing cost of conventional feedstuffs for livestock and poultry has resulted increase cost of production. Soybean meal (SBM) is the major protein source used in poultry diet due to high protein content, better amino acid profile, easily minimizable anti nutritional factors, low level of crude fibre (CF) and acid insoluble ash (AIA) as compared to other plant protein sources. Poultry production may continue to be ulcerative if costly conventional feedstuffs are not replaced with cheaper and available feedstuffs. It is therefore a recent trend among the animal nutritionist to use non-conventional feedstuffs in order to cut down on the cost of production which constitutes 60-70% of the total cost of production. In this situation, brewery by-products like brewer's dried grains (BDGs) are worthy of consideration as potential non-conventional feeds. BDG is available in large quantities throughout the year, but its main application has been limited to animal feeding. Brewery dried grain (BDG) which contains 21-29% crude protein on dry matter and costing lower than rice bran and coconut oil cakes.

Corresponding author: vinvet1@gmail.com

Brewer's grains are also called wet brewer's grains, dried brewer's grains and brewer's spent grain. Brewer's grains are the residue left after the processing of germinated and dried cereal grains (malt) for the production of beer and other malt products (malt extracts and malt vinegar). Though barley is the main grain used for brewing, beers also made from rice, maize, sorghum, wheat and millet. Remaining product is a concentrate of proteins and fibre that is suitable for animal feeding, particularly for ruminants (Crawshaw, 2004). The nutritional value of brewer's grains varies depending on the grain used (barley, wheat, rice, or corn), the extent of the fermentation, and the type of fermentation process used. Brewer's grains are primarily fed to dairy cattle but also have some nutritional value for poultry birds. The major problem limiting the use of dried brewer's grains in poultry rations is related to the grains high fiber content. Brewer's dried grain is an excellent source of quality by-pass protein and digestible fiber, with good amino acid profile and it has high mineral and water soluble vitamin content. Brewer's dried grain is relatively cheap, readily available with little or no competition between humans, farm animals and industries. The use of brewery waste as feedstuff can help to improve farmer's economic returns and environment. From the numerous studies it was found that brewers dried grain is potential non-conventional feedstuffs which need to be evaluated the feeding value of rice based brewers dried grain in diets of poultry birds. Considering the above-mentioned, the present study was designed to study the response of feeding sun dried rice based brewer's dried grain (RBDG) on immune response and haemto-biochemical parameters of broiler chickens.

2. Materials and Methods

Experimental design

A six week biological experiment was undertaken in a completely randomized design (CRD) with five dietary treatments (D1 to D5) containing different levels of rice based brewer's dried grain (0, 5, 10, 15 and 20%) respectively. Each treatment was fed to 5 replicated groups of 7 color broiler chicks each, accommodating 35 broiler chicks per treatments.

Experimental diets

A basal diet was prepared based on maize and soybean meal (solvent extracted) to meet the energy and protein requirement as per (ICAR, 2013) standard for coloured broiler chicks. From this basal diet, five test diets (D1-D5) were formulated by incorporating the rice brewer's dried grain (RBDG) at 0, 5, 10, 15 and 20% respectively in basal diet replacing maize and soybean meal so that all the experimental diets become iso-caloric and iso-nitrogenous. Each diet was prepared for starting (0-3wk) and finishing phase (4-6wk) of age and offered to five replicated groups of 7 chicks each. The ingredients and nutrients composition of different experimental diets are presented in Table 1.

Experiment protocal

The experimental birds were housed group wise in randomly allotted cabins or tiers of the electrically heated battery brooders with the provision of wire-mesh floor, feeder and waterer, located in the well ventilated room with 24 hours light and uniform and standard management practice. Weighed amount of each test diet used during starting period (0-3 wks) and finishing period (4-6 wks) and fresh water was of fed daily to all the birds during the study period.

The CMI response was assessed by cutaneous basophilic hypersensitivity test *in vivo* by using PHA-P as per Corrier and Deloach, 1990. At 25th day, ten birds from each treatment were selected and the toe thickness of both left and right foot at 3rd and 4th inter digital spaces were measured by micrometer. Just after measurements 0.1 mg of PHA-P suspended in 0.1 ml of phosphate buffer saline (PBS) and 0.1 ml of PBS was injected into right and left foot (as control), respectively. The foot web index was calculated as the difference between the swelling in the right minus left foot before and after 24 hour of injection and expressed as millimeter.

The antibody response to the SRBC was studied in 10 birds per treatment at 29 day of post-hatch, wherein 1ml suspension of 1% SRBC was injected I/V to the birds. After 5 day of SRBC immunization 2 ml blood was collected from wing vein and the antibody titer was recorded by haemagglutination (HA) titer (Van der Zijpp, 1983 and Siegel and Gross, 1980).

At the end of 42 days of experimental period, 10 birds from each dietary group were selected randomly and slaughtered after 12 hrs of fasting with *ad lib* drinking water for evaluation of development of immune organs like bursa, thymus and spleen. The immune organs weight was calculated on the % live weight (pre-slaughter) basis.

At the end of feeding experiment, blood samples from 10 birds/ treatment were randomly collected into sterile glass test tube without addition of anticoagulant. Test tubes containing the blood were kept in slanted position at room temperature for half an hour to facilitate separation of

Table 1: Ingredients and nutrients compositions of experimental diets containing different levels of RBDG during experimental period (0-6wks) of age

Ingredients %	Broiler Starter (0-3wks) of age					Broiler Finisher (4-6wks) of age				
	D1	D2	D3	D4	D5	D1	D2	D3	D4	D5
Maize	60.8	58.88	56.94	54.4	52.08	60.34	58.07	56.14	54.59	52.57
RBDG	0	5	10	15	20	0	5	10	15	20
Oil	0	0	0	0	0	2	2	2	1.3	1.3
SBM	30.74	27.66	24.47	21.99	19.2	29.2	26.4	23.3	20.4	17.5
FM	5	5	5	5	5	5	5	5	5	5
Limestone	0.9	0.9	1	1	1	1	1	1	1	0.9
DCP	1.2	1.2	1.2	1.2	1.3	1.2	1.2	1.2	1.3	1.3
Methionine	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.1
Lysine HCL	0	0	0.03	0.05	0.06	0	0.07	0.1	0.15	0.17
Salt	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36
TM Mix*	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Vitamin premix**	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Toxin binder	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100	100	100	100	100	100
Analyzed (%DM)										
Crude protein	21.65	21.6	21.64	21.72	21.65	20.05	20.1	20.08	20.06	20.02
Calcium	1.1	1.08	1.06	1.09	1.1	0.85	0.87	0.82	0.86	0.83
Total phosphorus	0.79	0.78	0.8	0.81	0.82	0.69	0.7	0.71	0.68	0.71
Calculated										
ME (Kcal/kg)	2950	2950	2950	2950	2950	3050	3050	3050	3050	3050
E:P ratio	136.57	136.57	136.35	135.78	136.57	152.5	152.5	152.5	152.5	152.5
AP%	0.45	0.45	0.45	0.45	0.45	0.38	0.38	0.38	0.38	0.38
Lysine%	1.07	1.07	1.07	1.07	1.07	0.98	0.98	0.98	0.98	0.98
Methionine%	0.52	0.52	0.51	0.5	0.49	0.4	0.4	0.4	0.4	0.4
Feed Cost/Kg	25.77	24.69	23.61	22.64	21.63	25.96	25.01	23.97	22.78	21.77

*Trace mineral premix supplied mg/kg diet: Mg 300, Mn 55, I 0.4, Fe 56, Zn 30 and Cu 4.2. **The vitamin premix supplied per kg diet: Vit. A 8250 IU, Vit. D_3 2400IU, Vit.E 15mg, Vit. K 1mg, Vit. B_1 4mg, Vit.B_2 6mg, Vit. B_{12} 8mg, Niacin 12mg, Pyridoxin 1.6mg, Choline chloride 500mg.

serum. Serum was separated by centrifugation at 3000 rpm for 10 minutes and serum was decanted into plastic vials, and then stored at -20^{0}c for estimation of glucose (Trinder, 1969), serum total protein (Doumas, 1975), serum albumen (Doumas, 1971), serum cholesterol (Wybenga and Pileggi, 1970) & triglycerides (Fossati and Lorenzo, 1982) by using commercial diagnostic kits. For the estimation of hemoglobin (Hb) same birds were also used to collect the blood samples in dry sterilized vials containing anticoagulant, EDTA (Ethylene Diamine Tetra Acetic Acid) @ 1mg/ml) and estimation of hemoglobin was done as per method of Sahli's haemoglobinometer.

The data obtained through the experiment subjected to statistical analysis in a completely randomized design as per standard procedures of Snedecor and Cochran, (1989) and significant means differences were tested as per Duncan's multiple range test (Duncan, 1955).

3. Results and Discussion

Immune Response

The results revealed the mean cellular immune response (Foot pad index) and hum oral immune response (HA titre to SRBC) did not differed significantly due to inclusion of different levels of rice based brewer's dried grain (Table 2). The mean cellular immune response (Foot pad index) varied from minimum 0.72 with 0 and 5 % inclusion levels of rice based brewer's dried grain to maximum 0.89 with 20 % inclusion levels of rice based brewer's dried grain. The mean humoral immune response (HA titre to SRBC) varied from minimum 6.63 with 0 % inclusion level of rice based brewer's dried grain to maximum 7.30 with 20 % inclusion level of rice based brewer's dried grain.

Our results are in agreements with work reported by Gupta *et al.* (2017) who reported that no significant (P>0.05) difference in humoral and cell mediated immunity by feeding 10% inclusion level of RDDGS. In contrary to our finding Barekatain *et al.* (2013) found that incorporation of 20% level of sorghum DDGS in the diets significantly (P<0.01) improved the IgA and IgG titre in broiler. Better humoral immunity in RDDGS diets may be associated with type and composition of amino acids particularly higher level of methionine present in DDGS. Dinani *et al.* (2018) reported that the humoral immunity of birds was significantly (P <0.05) better at 12.5% and 15% rice DDGS level as compare to control diet. However cell mediated immunity did not show any significant difference as compare to control group. The mean immune organ weight such as bursa, spleen and thymus at 42days of age did not differed significantly due to inclusion of different levels of RBDG (Table 3). The mean spleen weight varied from minimum 0.14 with 0 and 5 % inclusion level of rice based brewer's dried grain to maximum 0.16 with 20% inclusion level of rice based brewer's dried grain (RBDG). Present results get support from work reported by Dinani *et al.* (2018) who observed non-significant difference on various immune organ weights due to incorporation of different levels (0, 7.5, 10, 12.5 and 15%) of rice DDGS in broiler diets. Anyanwu *et al.* (2008) reported that there was no specific trend in carcass traits particularly internal organ weights in broilers fed increasing levels of BDG compare to ground corn.

Table 2: Cellular and humoral immunity of broiler chicks as influenced by feeding different levels of RBDG

Diets	RBDG (%)	Foot web index (mm)	HA titre (log2)
D1	0	0.72	6.63
D2	5	0.72	6.94
D3	10	0.76	7.21
D4	15	0.78	7.00
D5	20	0.89	7.30
Pooled SEM		0.05	0.17
P value		NS	NS

NS- Non- significant

Table 3: Immune organs weight of broiler chicks as influenced by feeding different levels of RBDG

Diets	RBDG (%)	Immune organ weight (% of live weight)		
		Bursa	Thymus	Spleen
D1	0	0.16	0.32	0.14
D2	5	0.14	0.29	0.14
D3	10	0.13	0.28	0.15
D4	15	0.13	0.29	0.15
D5	20	0.17	0.33	0.16
Pooled SEM		0.01	0.01	0.01
P value		NS	NS	NS

NS- Non- significant

Haemato-biochemical parameters

The various blood biochemical parameters namely glucose, serum total protein, albumin, globulin, albumin-globulin ratio and haemoglobin did not differ significantly due to replacing maize and soybean meal with rice based brewer's dried grain (Table 4). However, serum total cholesterol and triglyceride differed significantly due to different dietary treatments. The total protein and Hb increased as increasing graded levels of dietary RBDG but change was not up to significant level. The serum total cholesterol and triglycerides were significantly ($P<0.05$) reduced as increasing graded levels of dietary rice based brewer's dried grain. Significantly lower serum cholesterol and triglycerides were observed at 15 and 20 % inclusion levels of RBDG than those recorded in other dietary treatments. The serum cholesterol and triglycerides observed at 10, 15 and 20% inclusion levels of RBDG did not differ significantly to each other. Similarly diet containing 0, 5 and 10% rice based brewer's dried grain remained statistically similar for the total cholesterol and triglycerides contents.

Present results get support from earlier observation reported by Youssef *et al.* (2013) who reported that different levels of DDGS (0, 5, 10 and 15%) on haemato-biochemical parameters did not affected significantly. However, DDGS inclusion in the diet significantly ($P<0.05$) decreased glucose concentration in serum. Anjola *et al.* (2016) conducted a study to investigate the effects of brewers spent grains (BSG) on growth performance and serum biochemistry characteristics of broiler chickens replacing soybean meal by 0%, 36%, 57%, 76% and 100% BSG. It was found that blood haematology and serum chemistry indices did not follow a particular trend. Cholesterol concentration reduced with increasing level of BSG in the diet. This result is also in agreement with present finding. Dinani *et al* (2018) who also reported that the various serum biochemical parameters such as glucose, protein, albumin, globulin, albumin-globulin ratio, SGOT, SGPT and ALT did not show any significant differences due to feeding of different levels (0, 7.5, 10, 12.5 and 15%) of rice DDGS in broiler diets. Author also found that increasing the rice DDGS level in broiler diets significantly decreased serum total cholesterol and triglyceride at higher inclusion level of rice DDGS in broiler diets.

Table 4: Haemato-biochemical parameters of broiler chicks as influenced by feeding different levels of RBDG

Diets	RBDG (%)	Glucose (mg/dl)	Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A:G ratio	Cholesterol (mg/dl)	Triglyceride (mg/dl)	Hb (g/dl)
D1	0	208.60	4.74	1.71	3.03	0.56	174.40^{b}	123.02^{b}	10.35
D2	5	205.82	4.88	1.72	3.16	0.55	175.90^{b}	122.45^{b}	10.71
D3	10	200.44	4.87	1.87	2.99	0.63	168.50^{ab}	117.42^{ab}	11.02
D4	15	203.16	4.90	1.84	3.06	0.60	156.70^{a}	109.06^{a}	11.27
D5	20	194.46	5.07	1.98	3.08	0.64	151.60^{a}	106.91^{a}	11.36
Pooled SEM		2.69	0.09	0.05	0.06	0.01	2.83	2.04	0.14
P value		NS	NS	NS	NS	NS	P<0.05	P<0.05	NS

Values bearing different superscripts within column differ significantly ($P<0.05$), NS- Non-significant

4. Conclusion

In conclusions, based on the present findings rice based brewer's dried grain (RBDG) can be safely incorporated in broiler diet at the inclusion level of 20% for better immune response and haemto-biochemical parameters.

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