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Inclusion of sorghum distillers dried grains with solubles and multi-enzyme supplementation on immune response and nutrient utilization in broilers

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Abstract
The experiment was conducted on 420 straight run commercial day old broiler chicks from 0-42 days. The chicks were randomly distributed into seven dietary treatments groups with three replicates of 20 birds each. The birds in control group A were offered basal diet adequate in all nutrients as per BIS, 2007. The birds in dietary treatment groups B, C and D were offered diet containing sorghum distillers dried grains with solubles (sDDGS) at 5, 10 and 15% levels and birds in groups E, F and G were offered diet having sDDGS at 5, 10, and 15% levels with multi-enzyme Ronozyme Max Act (GT) @ 400g/ton of feed, respectively. The antibody titers against ND (log_2 values) and ELISA antibody titers against IBD revealed non-significant differences in all treatment groups at 3rd and 6th weeks. There was a non-significant difference for dry matter intake, dry matter excreted, nitrogen intake, nitrogen excreted and DMM in all treatment groups. The nitrogen retention was significantly (P<0.05) reduced in treatment groups D (15% sDDGS) as compared to control group. However, the inclusion of sDDGS at 15% level with enzyme supplementation improved nitrogen retention in broilers and statistically non-significant as compared to control group. Thus, it is concluded that the inclusion of sorghum distiller's dried grains with soluble up to 10% without multi-enzyme and 15% level with supplementation of multi-enzyme in broiler diets was found to be beneficial in terms of immune response and nutrient utilization in broiler chicken.

Keywords: Sorghum DDGS, multi-enzyme, immune response, nutrient utilization, broilers

1. Introduction
The objective of poultry feeding is to convert low quality feed resources like cereal grains, oil cakes and other by-products of agriculture and industry in to high quality food like meat and eggs. By far, the major ingredients are corn and soybean meal, which together often account for 70-80% of the total component of poultry feed. The commonly used feed ingredients (maize, sorghum, soybean meal, etc) in poultry diets contain different anti-nutritional factors (ANFs) like phytate, trypsin inhibitors, tannin, etc. There are surprisingly only few feed ingredients are used in poultry diets today. Therefore, the partial replacement of these ingredients with alternate feed ingredients like millets, cottonseed meal, canola meal, guar meal, distiller's dried grains with soluble (DDGS), sunflower meal, etc. containing low phytate and highly digestible nutrient composition can be promising. However, the potential of these alternate feed ingredients need to be explored based on their price, availability, ANFs and nutritive value [1]. Distillers Dried grains with solubles (DDGS) are by-products of the spirit industry and of bio-ethanol production. Dried grains of cereal distillers are rich in protein, exogenous amino acids, B-group vitamins, biotin and mineral compounds, including phosphorus [2, 3].
While most of the information gathered to date has focused on corn DDGS, scanty information is available in literature regarding sorghum distillers dried grains with solubles (sDDGS). The production of sorghum accompanied by the rapid increase in demand for ethanol production and will result in increased availability of sDDGS. Therefore, research work needs to be conducted to determine its impact on broiler performance, immune response and nutrient utilization. The sDDGS is slightly higher in crude protein, significantly higher in ADF and ash, and lower in crude fat and lysine compared to corn DDGS. Although, the level of methionine and threonine are similar, tryptophan level is substantially higher and lysine and arginine are lower, resulting in a significantly lower lysine to crude protein ratio compared to

corn DDGS [4]. These differences in nutrient composition suggest that the energy value and protein quality in sDDGS would be less than corn DDGS in chickens. The estimated the MEn (kcal/kg) of bronze and yellow sorghum DDGS was 2,677 and 2,866, respectively [4]. These sorghum DDGS values are similar, but slightly lower than the MEn value reported 2827 kcal/kg reported earlier [5] and for corn DDGS 2,906 kcal/kg [6]. The sDDGS contain 28.6% CP, 10.3% ether extract, 5.2% ash, 26.0% total non starch polysaccharides, 5.3% free sugars, 6.2% starch, 0.17% P, 0.72% Ca, 0.46% Lys, and 0.40% Methionine [7]. However, the sDDGS was reported to contain approximately 20% non starch polysaccharides, appreciable level of poorly digested protein and a considerable amount of starch not fermented during the process of ethanol production [8]. In general, the DDGS are richer in fibre, protein and fat than the cereal source [9] and also contain significant amounts of non-starch polysaccharides (NSPs). Therefore, the use of most appropriate enzyme to hydrolyze these compounds may increase the nutritional value of DDGS and promote its inclusion at higher levels in broiler diets [10]. In view of the above facts, the present experiment was undertaken to study the effect of feeding sDDGS and enzyme supplementation on nutritive value of sDDGS, immune response and nutrient utilization in broilers.

2. Materials and Methods

2.1 Experimental design and management

The experiment was conducted on 420 commercial day-old Vencobb-400 straight broiler chicks from 0-42 days. The birds were randomly distributed into seven dietary treatments groups with three replicates of 20 birds each. The birds in control group (A) offered corn-soya basal diet with adequate in all nutrients as per BIS-2007 [11]. The birds in dietary treatment groups B, C and D were offered diets containing sDDGS at 5, 10 and 15% levels with replacing soybean meal and birds in groups E, F and G were offered diets sDDGS at 5, 10, and 15% level with multi-enzyme (Ronozyme Max Act (GT), DSM, Mumbai) at recommended dose 400 g/ton of feed, respectively. The experimental design used for housing the broilers is presented in Table 1.

Table 1: The details of different dietary treatments using sDDGS with or without enzyme

Treatment groups	Treatment groups	No. of birds/ replicate	No. of replicates	No. of birds
A	Control diet (Basal diet)	20	3	60
B	Basal diet + 5% sDDGS	20	3	60
C	Basal diet + 10% sDDGS	20	3	60
D	Basal diet + 15% sDDGS	20	3	60
E	Basal diet + 5% sDDGS + multi-enzyme*	20	3	60
F	Basal diet + 10% sDDGS + multi-enzyme	20	3	60
G	Basal diet + 15% sDDGS + multi-enzyme	20	3	60
Total number of birds				420

*Multi-enzyme Ronozyme Max Act (GT) @ 400g/ton of feed

The standard and uniform managemental practices were followed for all treatment groups throughout the experimental period. The birds were offered *ad-lib* fresh and clean drinking water throughout the experiment. The immunization against Ranikhet Disease (RD) B1 strain and Infectious Bursal Disease (IBD, standard strain) was carried out on 7th and 14th day through intra-ocular, respectively, followed by IBD and ND booster doses on 21st day and 28th day through drinking water, respectively.

2.2 Procurement of feed ingredients and feed preparation

The good quality feed ingredients were procured from local market for preparation of experimental diets. The sDDGS was procured from Grainotch Industries Ltd., Aurangabad – 431109 Maharashtra, India. The metabolizable energy of sDDGS was 2866 kcal/kg [4] and same was considered for the feed formulation. The multi-enzyme Ronozyme Max Act (GT) was supplied by DSM, Nutritional Products India Pvt. Ltd., Mumbai- 400 098, Maharashtra, India. The Ronozyme Max Act (GT) was containing the enzyme activities of Phytase 3250000FYT, alpha amylase 20000KNU, Endo β-glucanase 39000FBG, Protease 31875000PROT and Xylanase 70000FXU.

The rations were formulated for pre-starter, starter and finisher phases and all the diets were isocaloric and iso-nitrogenous and also the lysine and methionine levels were balanced [11]. The inclusion of various levels of sDDGS with partial replacement of soybean meal and energy sources in different dietary treatments. The ingredients and nutrient composition of different dietary treatments A, B, C and D has been presented in Table 2.

Table 2: Ingredient and nutrient composition of various dietary rations containing sDDGS at different levels

Feed Ingredients	Pre-Starter				Starter				Finisher			
	A (Control)	B (5%)	C (10%)	D (15%)	A (Control)	B (5%)	C (10%)	D (15%)	A (Control)	B (5%)	C (10%)	D (15%)
Maize	52.00	50.38	48.76	47.14	53.22	51.60	49.98	48.36	57.76	56.14	54.52	52.90
Soybean Meal	40.71	37.34	33.97	30.60	38.25	34.88	31.51	28.14	32.90	29.53	26.16	22.79
Sorghum DDGS	0	5.00	10.00	15.00	0.00	5.00	10.00	15.00	0	5.00	10.00	15.00
Vegetable Oil	3.15	3.07	2.99	2.91	4.45	4.37	4.29	4.21	5.30	5.22	5.14	5.06
Dicalcium Phosphate (DCP)	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80	1.80
Limestone Powder (LSP)	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20
Salt (NaCl)	0.27	0.27	0.27	0.27	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Trace Mineral mixture*	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Vitamin Premix**	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
DL-Methionine	0.20	0.20	0.20	0.20	0.18	0.18	0.18	0.18	0.15	0.15	0.15	0.15

L-Lysine	0.05	0.12	0.19	0.26	0.03	0.10	0.17	0.24	0.02	0.09	0.16	0.23
Choline Chloride 60%	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12
Toxin Binder	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Coccidiostat	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Sodium Bicarbonate	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
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 on dry matter basis												
ME (kcal/kg)	3002.54	3003.49	3004.44	3005.39	3101.37	3102.32	3103.27	3104.22	3201.56	3202.51	3203.46	3204.41
Crude Protein (%)	23.00	23.00	23.00	23.00	22.00	22.00	22.00	22.00	20.00	20.00	20.00	20.00
Ether Extract (%)	5.52	5.81	6.09	6.37	6.84	7.12	7.41	7.69	7.82	8.10	8.39	8.67
Crude Fiber (%)	4.21	4.19	4.17	4.16	4.08	4.07	4.05	4.04	3.87	3.86	3.84	3.82
Calcium (%)	1.01	1.01	1.01	1.01	1.00	1.00	1.00	1.00	0.98	0.98	0.98	0.98
Total Phosphorus (%)	0.70	0.71	0.72	0.73	0.69	0.70	0.71	0.72	0.67	0.68	0.69	0.70
Available Phosphorus (%)	0.42	0.42	0.43	0.43	0.42	0.42	0.42	0.42	0.42	0.42	0.42	0.42
Total Lysine (%)	1.31	1.31	1.31	1.31	1.23	1.23	1.23	1.23	1.08	1.08	1.08	1.09
Total Methionine (%)	0.55	0.55	0.54	0.54	0.51	0.51	0.51	0.51	0.46	0.46	0.45	0.45

The diets E, F and G were prepared by adding multi-enzyme Ronozyme Max Act (GT) to diets B, C and D at recommended level of 400 g/ton of feed, respectively.

*Trace Mineral Mixture: - Each kg contains: Copper-15g, Iodine-2g, Iron-90g, Manganese-100g, Selenium-0.3g and Zinc-80g.

** Vitamin Premix: -Each 500g contains: Vit. A12.50MIU, Vit. D3-2.50 MIU, Vit. E-12g, Vit. K-1.50g, Thiamine (B1)-1.50g, Riboflavin (B2)-5g, Pyridoxine (B6)-2g, Cyanocobalamin (B12)- 0.015g, Niacin-15g, Cal D Pantothenate-10g and Folic acid-0.50g.

2.3 Chemical analysis of sDDGS

The sDDGS was procured from M/s. Grainotch Industries Ltd., Aurangabad-431109, Maharashtra, India. The chemical analysis of sDDGS was carried out as per AOAC (2000) [12].

2.4 Immune Response

The birds under the experimental trials were assessed for the antibody titer against the New Castle Disease Virus (NCDV) and Infectious Bursal Disease (IBD). Two birds from each replicate and a total of six birds from each treatment group were randomly selected for the blood collection at the end of 3rd and 6th week of age. The blood samples were collected from wing vein from each bird. The serum was separated by centrifugation at 3000 RPM for 20 minutes and decanted into clean, sterile plastic vials and stored under deep freeze at –18° to –20 °C. These serum samples were used for Haemagglutination Inhibition (HI) test to detect the antibody titer against New Castle Disease Virus by Beta procedure [13]. This was performed to evaluate, the humoral immune response of bird to viral antigen (NCDV Lasota strain) by beta procedure (constant virus diluted serum), utilizing 8HA unit of Lasota virus. The HA unit of NCDV (Lasota strain) were determined by the standard procedure by using micro HA- HI plate [13]. The serum was also used to specific antibody titers against Infectious Bursal Disease Virus (IBDV) as quantified by enzyme-linked immunosorbent assay (ELISA) kit at the end of 3rd and 6th week of age.

2.5 Nutrient Utilization

A metabolic trial was conducted for three consecutive days at the end of 6th week of experiment. For metabolic trial two birds from each replicate and thus, six birds from each treatment group were taken randomly and caged individually giving pre-experimental period of two days. During metabolic trial, record of feed offered, leftover was maintained on daily basis. A representative sample of feed offered, leftover on everyday were collected for dry matter determination. All the excreta from each group will collected on polythene sheet over a period of 24 hours. A representative sample (1/10) of excreta was taken for nitrogen estimation in 10% H_2SO_4 solution to avoid the nitrogen loss. The excreta collected from each groups was oven dried at 80 ^{0}C for 72 hours till the constant weight for dry matter determination. The dried samples of three consecutive days were pulled and then thoroughly mixed, powdered and used for nitrogen analysis as per AOAC, (2000) [12].

2.6 Statistical Analysis

Data generated on various parameters was subjected to statistical analysis by using Complete Randomized Design and the treatment means were compared by critical differences [14].

3. Results and Discussion

The antibody titers against ND (log_2 values) and Infectious Bursal Disease Virus (IBDV) as quantified by enzyme-linked immunosorbent assay (ELISA) kit at the end of 3rd and 6th week of age were presented in Table 3. At the end of experiment the nutrient utilization in broilers fed different levels of sDDGS with or without enzyme was presented in Table 4.

3.1 Chemical composition of sDDGS

The chemical analysis of sDDGS was carried out as per AOAC (2000). Chemical analysis of sDDGS on DM basis revealed that moisture 8.20%, crude protein 33.23%, crude fat 9.09%, total ash 2.94%, crude fibre 5.04%, total phosphorus 0.98% and calcium 0.37%. Some studies have showed reported that crude protein level in sDDGS was 29.04 and 28.60%, respectively [15, 7]. Furthermore, the drying process can have crucial influence not only on variability of nutrients but also on concentration and availability of nutrients in different samples.

3.2 Immune response at 3rd and 6th weeks

The analysis of variance for the antibody titers against ND (log_2 values) revealed non-significant differences in all treatment groups at 3rd and 6th weeks of age. The antibody titers against ND (log_2 values) were numerically lower in treatment groups D receiving diet at 15% sDDGS without enzyme whereas, it was increased by the supplementation of multi-enzyme at 3rd and 6th week of age. Similar observations have also reported that 10% cDDGS with Galzym supplementation in the diets resulted in non-significant difference in the ND titer compared to control [16]. However, antibody titer against Newcastle disease was non-significant by the inclusion of cDDGS at 5, 10 and 15% in broiler diet with or without enzyme at 3rd and 6th week of age [17].

The ELISA antibody titers against IBD have reported non-significant differences in all treatment groups at 3rd and 6th weeks of age in broilers. The IBD titers were marginally improved in treatment groups receiving diet sDDGS with multi-enzyme as compared to their non-enzyme supplemented counterparts at 3rd and 6th weeks of age. The present results are in agreement with the inclusion of corn DDGS has the beneficial effects on immune functions for broilers to some degree [18]. It was also observed that DDGS inclusion in broiler diets led to improve the physiological and immunological responses [19]. The study noted that corn DDGS appeared to promote secretary immunoglobulin (slgA) secretion and enhance anti-inflammatory status in broilers [20].

Table 3: Antibody titers against ND (log_2 values) and IBD ELISA at 3rd and 6th weeks in broilers fed different levels of sDDGS with or without enzyme

Treatment groups	3rd week		6th week	
	ND titers	IBD titers	ND titers	IBD titers
A	2.00±0.52	361.00±49.95	5.17±0.31	1464.50±93.81
B	2.17±0.40	376.83±75.42	4.67±0.49	1082.17±184.43
C	2.17±0.40	264.67±26.43	5.33±0.33	1286.33±120.19
D	1.67±0.42	220.00±31.79	4.17±0.60	1050.00±130.80
E	2.67±0.42	378.67±98.71	5.50±0.22	1321.50±175.95
F	2.67±0.33	323.17±72.88	5.00±0.52	1333.83±112.30
G	1.83±0.48	235.33±20.22	4.50±0.43	1084.00±158.73
CD	NS	NS	NS	NS
CV%	48.407	47.786	21.614	28.436

NS- Non-significant, CD-Critical difference, CV-coefficient of variance

There is scanty information available in the literature regarding immune response of broilers fed diets containing DDGS. It the present experiment the immune response slightly reduced at 15% sDDGS inclusion level which was elevated numerically by multi-enzyme supplementation in accordance with certain immune response and nutrient utilization in broilers.

3.3 Nutrient Utilization

There was a non-significant difference for dry matter intake, dry matter excreted, nitrogen intake, nitrogen excreted and Dry matter metabolizability (DMM) in all treatment groups. The nitrogen retention was significantly ($P<0.05$) reduced in treatment groups D (15% sDDGS) as compared to control group. However, the inclusion of sDDGS at 15% level with enzyme supplementation improved nitrogen retention in broilers; however, the difference was statistically non-significant as compared to control group.

The findings are in accordance with the feed additives such as enzymes (xylanase+phytase), probiotic, and chitosan can increase nutrient digestibility and retention of nitrogen and calcium with a high level of corn DDGS [21]. Whereas, the researcher reported that Distiller Dried Grain (DDG) fed at 0, 10, 20, 30 and 40% inclusion levels in broiler diets, the nutrient retention was significantly affected by dietary DDG. Protein and fat retention decreased with increase in level of dietary DDG [22]. However, the feeding DDGS did not influence N-corrected metabolisable energy (AMEn), total tract dry matter (DMR) and nitrogen retention (NR). However, enzyme supplementation improved AMEn, AMEn intake, DMR and NR [23]. Similarly, the inclusion of wheat DDGS with enzyme supplementation in broiler diet improved dietary apparent metabolizable energy, dry matter retention, nitrogen retention and fat digestibility compared to non-supplemented diets [24].

Table 4: Nutrient utilization in broilers fed different levels of sDDGS with or without enzyme

Particulars	Treatment groups							CD	CV%
	A	B	C	D	E	F	G		
Dry matter intake (g/bird/day)	118.28 ±12.60	116.00 ±5.66	117.57 ±6.33	114.00 ±4.84	116.10 ±4.43	110.76 ±5.39	112.80 ±5.95	NS	14.800
Dry matter excreted (g/bird/day	31.32 ±3.32	32.94 ±2.43	33.31 ±1.85	34.36 ±1.48	32.16 ±1.30	31.08 ±1.46	32.32 ±1.97	NS	15.646
Nitrogen intake (g/bird/day)	3.66 ±0.39	3.63 ±0.18	3.87 ±0.21	3.69 ±0.16	3.70 ±0.14	3.42 ±0.17	3.64 ±0.19	NS	14.681
Nitrogen excreted (g/bird/day)	1.30 ±0.14	1.40 ±0.06	1.51 ±0.11	1.49 ±0.05	1.35 ±0.05	1.23 ±0.06	1.38 ±0.06	NS	14.619
Nitrogen retention (%)	64.26^{a} ±0.69	61.35^{ab} ±1.01	61.09^{ab} ±1.55	59.37^{b} ±1.37	63.44^{a} ±0.88	63.91^{a} ±1.13	61.79^{ab} ±0.91	3.192*	4.380
DMM (%)	73.41 ±1.33	71.73 ±0.94	71.68 ±0.50	69.71 ±1.30	72.27 ±0.65	71.91 ±0.35	71.40 ±0.48	NS	2.994

Means bearing different superscripts within a row differ significantly. * $P<0.05$, NS-Non-Significant, CD-Critical difference, CV-coefficient of variance

4. Conclusion

Thus, it is concluded that the inclusion of sorghum distiller's dried grains with soluble up to 10% without multi-enzyme and 15% level with supplementation of multi-enzyme in broiler diets was found to be beneficial in terms of immune response and nutrient utilization in broiler chicken.

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