



Effects of Feeding Graded Levels of Distillers Dried Grains with Soluble (DDGS) With or Without Supplementation of Multi-enzymes on Blood Bio-Chemical Constituents of Indigenous Chicken

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ABSTRACT

An experiment was conducted using distillers dried grains with soluble (DDGS) at 0, 10 and 20% levels with or without enzymes supplementation in the diets of indigenous chicken to study the effect on different blood bio-chemicals parameters. A total of one hundred and eighty (21 days old) indigenous chicks were divided into six groups viz. T₁, T₂, T₃, T₄, T₅ and T₆ containing 30 chicks in each group. The birds of T₁ (control) and T₂ groups were offered the standard chick, grower and layer feeds as per BIS, 2007 without and with multi-enzymes (Xzyme), respectively. The birds of T₃, T₄, T₅ and T₆ groups were fed the rations containing 10% DDGS without and with enzymes and 20% DDGS without and with enzymes, respectively. The feeding trial was conducted for a period of 13 fortnights. The average levels of different blood parameters, estimated at the end of the experiment, viz. haemoglobin, glucose, serum protein, lipid, cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), calcium and phosphorus ranged from 9.96±0.20 to 10.91±0.24 g/dl, 185.08±0.54 to 188.67±1.86 mg/dl, 3.05±0.17 to 3.32±0.09 g/dl, 72.45±0.29 to 72.96±0.49 mg/dl, 130.91±0.59 to 133.71±2.07 mg/dl, 71.66±0.44 to 72.28±0.40 U/L, 11.42±0.32 to 11.93±0.12 U/L, 10.16±0.44 to 10.81±0.29 mg/dl and 4.93±0.11 to 5.94±0.14 mg/dl, respectively. Non-significant (P>0.05) differences were observed with respect to average values of different blood parameters among the treatment groups except for blood inorganic phosphorus. Serum concentration of inorganic phosphorus was significantly (P<0.05) lower in T₁ as compared to other groups. It can be concluded that the incorporation of DDGS up to 20% level in the diets of indigenous chicken did not have any adverse effect on the blood bio-chemical constituents of experimental birds.

Key words: Blood bio-chemical, Distillers dried grains with soluble (DDGS), Enzyme, Indigenous chicken

INTRODUCTION

The maize and soybean meal are the major conventional sources of energy and protein, respectively, in poultry feeds. These ingredients are becoming scarce day by day due to their increased demand in livestock and poultry sector. Further, due to its use for production of biofuel ethanol, availability of maize for use in poultry sector may be challenging. So, utilization of non-conventional feed resources in the poultry feeding is indispensable. The replacement of costlier traditional ingredients with cheaper non-conventional ingredients is probably the most viable proposition to address this situation. Distillers dried grains with soluble (DDGS), a co-product of ethanol production process, has been identified as one of the promising non-conventional feed resource that can be used in the poultry ration as energy and protein source. In the recent years there is

an escalation of DDGS production and its quality has also seen a positive change when derived from new generation ethanol plants (Panda *et al.*, 2016). It is a good source of energy, protein, exogenous amino acids, B-group vitamins and minerals, including phosphorus (Koreleski and Swiatkiewicz, 2006; Thaker and Widyaratne, 2007; Min *et al.*, 2008). The DDGS is also better source of fibre, protein and fat than cereal grains (Koreleski and Swiatkiewicz, 2008). However, DDGS contains considerable amount of non-starch polysaccharides (NSP) (Ward *et al.*, 2008), which restrict its extensive use in poultry ration. In this regard, exogenous enzymes are able to offer nutritional benefits by hydrolyzing NSP (Cost *et al.*, 2008). The use of appropriate enzymes to hydrolyze these compounds can increase the nutritional value of DDGS and enables greater inclusion in poultry diets (Juanpere

at al., 2005). In view of the above facts, the present study was undertaken to investigate the effect of dietary incorporation of distillers dried grains with solubles (DDGS) at different levels with or without multi-enzyme supplementation on the blood bio-chemical constituents of indigenous chicken.

MATERIALS AND METHODS

All procedures followed in this experiment was approved by Institutional Animal Ethics Committee, Assam Agricultural University, Khanapara, Guwahati (770/ac/CPCSEA/FVSc/U/IAEC/17-18/479 dated: 09.08.2017). Following a completely randomized design, a total of one hundred and eighty numbers of 21 days old indigenous chicks were randomly distributed into six groups *viz.* T₁, T₂, T₃, T₄, T₅ and T₆ containing 30 chicks with 3 replicates of 10 chicks in each group. The birds were reared under deep litter system of management throughout the experimental period following standard and uniform managemental practices and in similar environmental condition (temperature 17-28°C, humidity 80-92% and rainfall 0-14 mm). The birds of T₁ group (control) were offered the standard chick, grower and layer feeds as per BIS, 2007 (Table 1). The birds of T₂ group were fed with the same standard chick, grower and layer feeds as per BIS, 2007 with supplementation of multi-enzyme (Xzyme). Maize DDGS was incorporated at 10% level in the rations of T₃ and T₄ groups, whereas, the rations of T₄ group was additionally supplemented with Xzyme. In the same way, the birds of T₅ and T₆ groups were fed with rations containing 20% DDGS without and with enzymes, respectively. The feeding trial was conducted for a period of 182 days (13 fortnights) using chick feeds for first 3 fortnights (0-42 days), grower feeds for next 7 fortnights (43-140 days) and layer feeds for last 3 fortnights (141-182 days). Weighed quantities of feeds were offered thrice daily at 6.30 AM, 12.30 PM and 5.00 PM to the birds of different treatment groups ensuring *ad libitum* feeding throughout the study period. During the last week of the experiment, blood was collected from four numbers of birds from each experimental group at 9.30 AM, after three hours of

post feeding. Blood samples were collected aseptically from brachial vein of birds in two different tubes, one part in clean sterilized tube containing EDTA for haemoglobin analysis and another part in clean sterilized glass tubes and kept in slanted position at room temperature for serum collection. The samples were then centrifuged at 3000 rpm for 5 minutes, serum harvested and transferred to 2 ml Eppendorf tubes which were then stored at -20°C for estimation of other blood biochemical parameters.

The blood haemoglobin and glucose as well as other blood bio-chemical constituents (serum total protein, cholesterol, lipids, AST, ALT, calcium and inorganic phosphorous) were analyzed by using commercial kits following particular protocols as per the manufacturers' guidelines. The statistical analyses of the experimental data were carried out according to the method described by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Total feed consumption per bird for the last fortnight (13th) and entire period of experiment were 109.25 and 11653, 110 and 11665, 108.75 and 11664, 111.50 and 11669, 110.25 and 11739, and 111.25 and 11748 g for groups T₁, T₂, T₃, T₄, T₅ and T₆, respectively. Feed intakes (g/bird) were found to be comparable among the birds. Other research workers (Thacker and Widyaratne, 2007; Youssef *et al.* 2013; Hassan and Aqil, 2015) also reported no significant difference in feed consumption among different treatment groups with DDGS inclusion levels up to 10-15%. In this experiment, maximum and minimum total consumption was recorded in T₆ and T₁ groups, respectively. Similar trend was also observed by Pineda *et al.* (2008) who reported that the higher levels of incorporation of DDGS in the layer ration increased feed intake.

No significant ($P>0.05$) difference was observed in Hb. levels of experimental birds among various treatment groups (Table 2). However, a non-significant ($P>0.05$) increase in blood Hb. levels in birds fed DDGS supplemented with multi-enzyme was observed, which might be due to the positive effects of enzymes as well

Table 1: Composition of chick, grower and layer rations

Ingredients (%)	Rations																	
	Chick						Grower						Layer					
	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆
Maize	40.40	40.40	34.00	34.00	29.32	29.32	40.40	40.40	32.26	32.26	29.32	29.32	43.18	43.01	34.21	34.21	32.27	32.27
Soybean meal	15.50	15.50	10.50	10.50	5.00	5.00	15.50	15.50	10.00	10.00	5.00	5.00	25.00	25.10	19.85	19.85	14.30	14.20
Rice polish	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	7.00	7.00	8.00	8.00
Distillers dried grains with soluble	00	00	10.00	10.00	20.00	20.00	00	00	10.00	10.00	20.00	20.00	00	00	10.00	10.00	20.00	20.00
Di-calcium phosphate	1.30	1.30	1.20	1.20	1.00	1.00	1.30	1.30	1.20	1.20	1.00	1.00	1.30	1.30	1.20	1.20	1.00	1.00
Limestone powder	1.70	1.70	2.00	2.00	2.00	2.00	1.70	1.70	2.00	2.00	2.00	2.00	7.00	7.00	7.20	7.20	7.30	7.30
Methionine	0.10	0.10	0.07	0.07	0.08	0.08	0.10	0.10	0.07	0.07	0.08	0.08	0.10	0.10	0.07	0.07	0.08	0.08
Lysine	0.00	0.00	0.05	0.05	0.18	0.18	0.00	0.00	0.05	0.05	0.18	0.18	0.00	0.00	0.05	0.05	0.18	0.18
Salt	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27
Minpremix ¹	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Toxin binder	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
BrokenRice	7.05	7.05	10.00	10.00	10.00	10.0	7.05	7.05	10.00	10.00	10.00	10.00	6.00	6.50	10.0	10.0	6.00	6.00
De-oiled rice bran	28.53	28.53	26.76	26.76	27.00	27.0	28.53	28.53	29.00	29.00	27.00	27.00	12.00	11.50	10.00	10.00	10.45	10.50
Enzymes	00	0.05	00	0.05	00	0.05	00	0.05	00	0.05	00	0.05	00	0.05	00	0.05	0.00	0.05
Crude Protein (%)	19.25	19.37	19.33	19.41	19.22	19.33	15.49	15.53	15.54	15.61	15.45	15.53	17.55	17.65	17.51	17.53	17.47	17.48
ME ² (kcal/kg)	2798	2798	2793	2793	2800	2800	2527	2527	2503	2503	2539	2539	2580	2580	2600	2600	2604	2604

¹Mineral mixture contains (per 1.2 kg): Calcium- 255 g, Phosphorous- 127.5 g, Magnesium- 6 g, Manganese- 1.5 g, Iron- 1.5 g, Iodine- 325 mg, Copper- 4.2 g, zinc-9.6 g, Cobalt- 150 mg, Sulphur- 7.2 g, Potassium- 100 mg, Sodium- 6mg, Selenium- 10 mg, Vitamin A- 700000 IU, Vitamin D₃ - 70000 IU, Vitamin E- 250 mg, Nicotinamide- 1000 mg & Chromium- 78 mg.; Composition of multi-enzyme (Xzyme): Each kg of Xzyme premix contains: Lactic Acid Bacillus-30,000 million spores, Saccharomyces Cervisiae- 100 billion CFU, Amylase- 29,000 IU, Betaglucanase-4,05,000 IU, Phytase- 44,500 IU, Lipase- 31,000 IU, Protease- 7,40,000 IU, Cellulase- 5,500 IU, Pectinase -1,01,000 IU and Hemicellulase- 25,000 IU;

²(Metabolizable Energy) Calculated value

as higher iron contents of DDGS. Similarly, Gupta *et al.* (2017) reported that the inclusion of DDGS at 5, 7.5 and 10% levels enhanced the Hb level as compared to control. Further improvement in Hb level was observed when DDGS was supplemented with multi-enzyme.

The mean concentration of blood glucose were 188.67 ± 1.86 , 188.40 ± 1.96 , 187.20 ± 1.61 , 187.61 ± 1.37 , 185.08 ± 0.54 and 185.79 ± 0.30 mg/dl in T₁, T₂, T₃, T₄, T₅ and T₆ groups, respectively. The mean values for blood glucose levels in birds of different groups were comparable though some numerical differences existed among the values. Findings of Jiang *et al.* (2013) was in the same line with the present study who reported that dietary inclusion of rice DDGS up to 25% did not affect the plasma content of glucose in juvenile red seabream. However, Gupta *et al.* (2017) reported that glucose level decreased significantly in rice DDGS supplemented groups. In this study, the non-significant ($P > 0.05$) decrease in blood glucose levels in DDGS added groups might be due to lower starch and higher fibre contents in the rations of various treatment groups.

The highest (3.32 ± 0.09 g/dl) mean total serum protein was recorded in the birds of T₆ group while the lowest (3.05 ± 0.17) value for same was recorded in T₁ group (Table 2). No significant ($P > 0.05$) difference was observed in total serum protein values of experimental birds among the treatment groups. Bor-Ling *et al.* (2011) also reported that feeding of corn DDGS at different levels (0, 6, 12 and 18) did not influence the serum

protein in laying hen. Numerically increased total serum values were recorded in T₂, T₄ and T₆ groups, where enzymes were supplemented, in comparison to T₁, T₃ and T₅ groups. Osmana *et al.* (2011) conducted a feeding trial in Hubbard broiler chicks using DDGS and reported higher plasma protein values in birds fed 20% DDGS along with enzymes supplementation. In this study, the non-significant ($P > 0.05$) increase of serum total protein levels in enzyme treated groups might be due to the effect of protease present in the multi-enzyme used in the experiment.

There was no significant ($P > 0.05$) difference in blood lipid values of experimental birds among the treatment groups (Table 2). Similarly, Bor-Ling *et al.* (2011) reported that feeding of corn DDGS at different levels (0, 6, 12 and 18) did not influence the serum triglycerides of laying hens. Findings of Jiang *et al.* (2013) are also in the same line with the present study, who reported that dietary inclusion of rice DDGS up to 25% did not affect the plasma content of triglycerides in juvenile red seabream. No significant ($P > 0.05$) difference was observed in blood cholesterol values of experimental birds among different treatment groups (Table 2). Jiang *et al.* (2013) also reported that dietary inclusion of rice DDGS up to 25% did not affect the plasma content of cholesterol in juvenile red seabream. Activity of AST and ALT were similar among the groups (Table 2). The present findings corroborate well with that of Ghazalah *et al.* (2011), who reported substitution

Table 2. Mean ($\pm$ SE) blood parameters in experimental birds of different treatment groups

Parameters	Treatments groups						P value
	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	
Haemoglobin (g/dl)	9.96 ± 0.20	10.40 ± 0.16	10.65 ± 0.27	10.74 ± 0.36	10.85 ± 0.22	10.91 ± 0.24	0.118446
Glucose (mg/dl)	188.67 ± 1.86	188.40 ± 1.96	187.20 ± 1.61	187.61 ± 1.37	185.08 ± 0.54	185.79 ± 0.30	0.439116
Total serum protein (g/dl)	3.05 ± 0.17	3.20 ± 0.13	3.18 ± 0.09	3.25 ± 0.10	3.21 ± 0.10	3.32 ± 0.09	0.714567
Total lipids (mg/dl)	72.51 ± 0.08	72.45 ± 0.29	72.96 ± 0.49	72.84 ± 0.19	72.78 ± 0.30	72.64 ± 0.09	0.771293
Cholesterol (mg/dl)	133.71 ± 2.07	131.85 ± 2.28	132.61 ± 1.84	131.71 ± 1.04	131.90 ± 0.97	130.91 ± 0.59	0.869824
AST (U/L)	72.28 ± 0.40	72.09 ± 0.04	72.00 ± 0.12	71.66 ± 0.44	72.11 ± 0.25	71.97 ± 0.43	0.831994
ALT (U/L)	11.79 ± 0.33	11.75 ± 0.10	11.93 ± 0.12	11.42 ± 0.32	11.69 ± 0.17	11.67 ± 0.28	0.776553
Serum calcium (mg/dl)	10.59 ± 0.36	10.81 ± 0.29	10.37 ± 0.31	10.53 ± 0.41	10.16 ± 0.44	10.63 ± 0.34	0.851216
Serum phosphorous (mg/dl)	$4.93^a \pm 0.11$	$5.62^b \pm 0.11$	$5.56^b \pm 0.22$	$5.75^b \pm 0.17$	$5.69^b \pm 0.18$	$5.94^b \pm 0.14$	0.009138

Means bearing the same superscript within the row do not differ significantly ($P < 0.01$)

of soybean meals with DDGS at four levels (0, 25, 50 or 75%) and supplementation of Avizyme 1500 at two levels (0 or 0.075%) did not cause any change in the activity of these enzymes.

The mean blood ALT levels of experimental birds of different treatment groups ranged from 11.42 ± 0.32 to 11.93 ± 0.12 U/L. There was no significant ($P > 0.05$) difference in blood ALT values of experimental birds among various treatment groups. The results of the present study was in agreement with the findings of Ghazalah *et al.* (2011), who reported from a feeding trial in Bovans Brown layers by substituting soybean meals with DDGS at four levels of 0 or 25 or 50 or 75% and adding Avizyme 1500 at two levels (0 or 0.075%) that there was no significant ($P > 0.05$) difference among the groups for ALT levels.

The mean serum concentration of calcium of experimental birds of different treatment groups ranged from 10.16 ± 0.44 to 10.81 ± 0.29 mg/dl. No significant ($P > 0.05$) difference was observed in average serum calcium values of experimental birds among the treatment groups. The overall values of blood calcium in all the treatment groups were found to be on higher side which might be due to the fact that the birds of all the groups were in egg laying stage at the time of collection of blood. The results of the present study were in agreement with the findings of Ghazalah *et al.* (2011), who reported that there was no significant difference among the birds for calcium levels in Bovans Brown layers. Hack *et al.* (2015) also noticed that serum calcium was not altered when hens were fed with diets containing DDGS.

The serum inorganic phosphorus values were found to be maximum (5.94 ± 0.14 mg/dl) in T_6 and minimum (4.93 ± 0.11 mg/dl) in T_1 groups (Table 2). Statistically significant ($P < 0.05$) difference was observed in serum phosphorus levels between T_1 (control group) and all other treatment groups. There was increased levels of serum phosphorus in all the DDGS as well enzyme supplemented groups in comparison to control group, which corroborated well with the findings of Ali *et al.* (2006), who reported that the addition of enzyme preparation to laying hen diet increased plasma

phosphorous level. Dorra *et al.* (2013) also observed significant differences in plasma phosphorous level with different inclusion level of corn DDGS. In the present study the significant difference in blood inorganic phosphorus levels between T_1 and rest of the treatment groups might be due to presence of higher amount of available phosphorus in DDGS and supplementation of phytase in the diet which are in concurrence with the observations of earlier researchers.

CONCLUSION

Based upon the results of this experiment, it is evident that the incorporation of DDGS up to 20% level in the diets of indigenous chicken did not have any adverse effect on the blood bio-chemical constituents of experimental birds. Therefore, it can be concluded that DDGS can be used as a cheap source of protein and energy in the rations of indigenous chicken for economical and profitable poultry production.

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