International Journal of Science, Environment and Technology, Vol. 5, No 5, 2016, 2972 – 2981

ISSN 2278-3687 (O)
2277-663X (P)

EFFICACY OF FIBRE DEGRADING AND PROTEOLYTIC ENZYMES SUPPLEMENTD HIGH FIBRE DIETS ON PERFORMANCE OF BROILERS

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Abstract: A feeding trial of 6 weeks duration was conducted on 240 male broiler chicks procured and allotted randomly into eight treatments. Each treatment was divided into six replicates, with five chicks per replicate and raised in electrically heated battery brooders to evaluate the effect of exogenous enzymes on performance of broilers, fed with high and low fibre diets. Eight test diets were prepared by supplementing enzyme preparations to the reference diets *i.e.*, T_1 (low fibre) and T_2 (high fibre) diets. T_3 and T_4 were low and high fibre diets supplemented with fibre degrading enzymes. Similarly, T_5 and T_6 are low and high fibre diets supplemented with fibre degrading enzymes along with protease enzyme. T_7 and T_8 are low and high fibre diets supplemented with protease enzyme. Feed and water were provided *ad libitum* throughout the experimental period. High fibre diets (T_4, T_6 and T_8) supplemented with enzymes recorded significantly ($P<0.05$) improved body weight gains during finisher (5-6 week) and overall periods (0-6 weeks) with respect to their control diet (T_2). Supplementation of protease enzyme to high fibre diet (T_8) resulted in significantly ($P<0.05$) improved body weight gain during starter, finisher and overall periods compared to their corresponding control diet (T_2). Humoral immune response to NDV in terms of increased antibody production was found to be significant ($P<0.05$) at 42 days of age on enzyme supplementation to high fibre diets and antibody production was significantly ($P<0.05$) different among high or low fibre diets. Economic calculation revealed that the returns over feed cost on high fibre enzyme supplemented diets were better over the unsupplemented diets and also low fibre enzyme supplemented diets. Supplementation of fibre degrading enzymes (cellulase-420 IU / Kg, xylanase-4025 IU / Kg and pectinase-53 IU / Kg) and proteolytic enzyme (protease-5000 U / Kg) to high fibre diets could result in better weight gains of broilers.

Keywords: Broilers, High fibre diets, Fibre degrading enzymes, Proteolytic enzymes.

Introduction

Birds are capable of utilizing only 75-80 per cent of dietary nutrients and remaining portion is excreted undigested or unabsorbed. Additions of exogenous enzymes in the diet aid the birds to increase the digestion of undigested portion of the feed. Young birds have limited ability to

*Received Aug 17, 2016 * Published Oct 2, 2016 * www.ijset.net*

produce the types and amount of enzymes necessary to utilize a high carbohydrate and vegetable protein diet (Sklan, 2002) thus affecting nutrient digestibility (Noy and Sklan, 1994). Exogenous enzymes have been used to enhance the feeding value of rye, barley and wheat based diets, because these feedstuffs are high in soluble NSP that induce viscosity, but it is assumed that feedstuffs like corn and SBM induce less viscosity.

Use of unconventional agro-industrial by products in poultry diets is restricted due to high level of crude fibre (CF) or presence of some deleterious factors. The principle components of dietary fibre are the structural carbohydrates which are cellulose, hemicellulose, ß-glucans, arabinoxylans and pectins. Inadequate or non-availability of certain enzymes in the digestive tract of chicken reduces their utilization. Enzymes added in the diet break the polymeric chain of fibrous material more effectively, thereby reducing the gut viscosity and improve their nutritive value (Smith and Annison, 1996). Improvement in weight gain, efficiency of feed utilization and reduced sticky droppings in broilers by supplementing the diets with enzymes have been documented (Raghavan, 1990). The maximum limit of dietary fibre in broiler diet is 4% (BIS, 1992). However, the use of by-products in broiler diets increases the fibre level thereby restricting the growth.

Thus, the present investigation was carried out to evaluate the exogenous enzymes (cellulase, xylanase, pectinase, and protease) in high fibre diets on the performance, carcass traits like, dressing yield and giblet weights (Liver, Heart and Gizzard), immune response and economics of broilers.

Materials and Methods

To conduct the proposed study, 0 day-old commercial male broiler chicks (VenCobb) were procured, wing banded, individually weighed and distributed randomly in to eight groups, each with six replications, each replicate consisting of five chicks. The chicks were reared in battery brooders with optimum brooding conditions. The treatments were as follows: T_1-Low fibre diet – Reference diet; T_2- High fibre diet – Reference diet; T_3-Low fibre diet + fibre degrading enzymes; T_4-High fibre diet + fibre degrading enzymes; T_5-Low fibre diet + fibre degrading enzymes + Protease; T_6- High fibre diet + fibre degrading enzymes + Protease; T_7-Low fibre diet + Protease enzyme; T_8- High fibre diet + Protease enzyme. The enzymes used for making experimental diets were procured from Professor R.C. Kuhad, Department of Microbiology, University of Delhi, south campus, Bentio Jaurez road, New Delhi- 110021. The experimental activity of enzymes are: fibre degrading enzymes- (a) cellulose is dose is 0.525 mg or 420 I per kg and activity is 0.8 U/kg and level of inclusion is 420 IU/kg; (b)

Xylanase dose is 0.758 mg or 2000 U/kg, enzyme activity is 6.25 U / mg and level of inclusion is 4025 IU/kg; (c) Pectinase dose is 0.013 ml or 10 U per kg, enzyme activity is 737.46 U/ml and level of inclusion is 53 IU/kg. Proteolytic enzyme protease dose is 25 mg or 5000 U/kg, enzyme activity is 2 Lakhs / gm and level of inclusion is 5000 U / kg.

The experimental diets were formulated by supplementing various levels of metabolizable energy (ME), crude protein (CP) and crude fibre (CF) and other nutrients. The composition of the experimental diets, both broiler starter low fibre and high fibre (0-4 weeks) and broiler finisher low fibre and high fibre (5-6 weeks) are standard. The control diets containing low fibre starter and finisher rations were formulated with maize and soyabean meal similarly diets for high fibre starter and finisher rations were formulated with maize, soyabean meal and sunflower cake. The broiler starter low fibre diet (0-4 weeks) was formulated to contain 22.5% CP, 2950 kcal/kg of ME and 3.6% CF. Similarly starter high fibre diet was formulated to contain 21% CP, 2800 kcal/kg of ME and 7.2% CF. The broiler finisher low fibre diets (5-6 weeks) were formulated in a similar manner with around 19.5% CP, 3100 kcal/kg of ME and 3.4% CF. Similarly, finisher high fibre diets were formulated to contain 18% CP, 2950 kcal/kg of ME and 7.2% CF. Each experimental diet was allotted to six replicates of chicks and offered from 0-6 weeks of age. Chicks were housed in a battery brooder with a floor space of 0.5 sft / bird for brooding period and later given 1.0 sft / bird. During the experiment, light was provided continuously (24 hours) for first 2 weeks. Feed and water were offered *ad libitum* throughout the experimental period. All the birds were raised under similar managemental conditions. During the experimental period, birds were immunized against Newcastle Disease (ND) at 7^{th} (primary) and 28^{th} (booster) day of age with LaSota vaccine (Indovax) and Infectious Bursal Disease with IBD (Intermediate-Georgia strain) vaccine at 14^{th} day of age.

Body weight gain was calculated on individual bird basis and feed intake was recorded on replicate-wise at weekly intervals. The feed conversion ratio (FCR) was calculated as feed intake per unit body weight gain from 0-6 weeks of age at weekly intervals. Mortality was recorded throughout the experimental period. Blood samples were collected at 28^{th} and 42^{nd} day to get serum samples to study the immune response to ND vaccine. At the end of 6^{th} week serum samples were collected to study the immune response to sheep red blood cell antigen (SRBC). At the end of experimental period (6 weeks) six birds from each dietary group were sacrificed to evaluate the effect of feeding high fibre diets by incorporation of exogenous enzymes on carcass parameters such as dressing yield, relative length of small

intestine in centimeters, abdominal fat weight and relative weights of liver, heart, gizzard (Giblets) and lymphoid organs such as thymus, bursa and spleen weights, which were expressed as percentage of live body weight. Moisture content of litter was expressed on percentage basis. Six blood samples were collected from each dietary group at 28th and 42nd day of age, and antibodies specific for NDV were detected in sera of chicks by heamagglutination inhibition (HI) test and were expressed as $\log_2$ titers (Allan *et al*., 1978). On day 38th six birds from each dietary group were inoculated intravenously with 0.1ml of 0.5% suspension of sheep red blood cell (SRBC) in normal saline. Birds were bled 42nd day of age to evaluate the response to SRBC inoculation by measuring the antibody titers $(\log)_2$ using micro haemagglution procedure of Wegman and Simithies (1966).

The data were analyzed using one way ANOVA of Statistical Package for Social Sciences (SPSS) 10th version and comparison of means tested using Duncan's multiple range test and significance was considered at $P<0.05$.

Results and Discussion

A) ***Performance of Broilers***

The performance of commercial male broiler chicks on supplementation of exogenous fibre degrading enzymes and protease enzyme in high and low fibre diets are presented in Table 1.

a) Body weight gain: In the broiler growth trial the supplementation of enzymes to high fibre diets significantly ($P<0.05$) increased weekly body weight gain during 5th week. Supplementation of exogenous enzymes to high fibre diets improved body weight gain significantly ($P<0.05$) during starter and finisher phases and overall growth period also. Further, supplementation of exogenous enzymes to low fibre diets did not influence in improved body weight gains. These observations concur with findings of Berwal *et al*. (2008) who reported that a significant ($P<0.05$) increase in body weight gain with increase in crude fibre level with supplementation of enzymes in broiler diets during 0-4 and 0-6 weeks periods.

b) Feed Intake: The weekly feed intake figures on supplementation of exogenous enzymes to the both high and low fibre diets were comparable with unsupplemented control diets. The cumulative feed intake figures on enzyme supplemented to high fibre diets were slightly higher than control during 0-6 weeks. However, during early period of life (0-4 weeks), significantly ($P<0.05$) reduced feed intake was noticed with enzyme supplementation in 5% crude fibre diet which improved numerically in the post 4 week phase. Feed intake values of low fibre diets supplemented with enzymes were numerically better than control diet. These

results are in agreement with those of Hanumanth *et al.* (2003) have reported that supplementation of cellulase, xylanase, amylase, phytase and protease in corn soya diets of broilers resulted in increased feed intake values from 1-42 d age.

c) Feed Conversion Ratio: The effect of enzyme supplementation to high and low fibre diets on FCR in overall period was found to be insignificant in this study. The significantly ($P<0.05$) improved weekly feed-to-gain ratio was observed on supplementation of enzymes to high fibre diets in 5^{th} week. These observations concur with Ravneet *et al*. (2007) who reported that the supplementation of enzyme at all crude fibre levels (6%; 10%) improved ($P<0.05$) FCR during finisher and overall growth periods.

d) Livability: Supplementation of enzymes to the high fibre or low fibre diets did not influenced the livability of broilers in both finisher phase and during overall period in the present study. These results are in concur with the results of Hanumantha *et al*. (2003). However, Irish *et al*. (1995) have reported that in broilers mortality was unaffected by enzyme supplementation.

B) Carcass Parameters

The carcass characteristics of commercial male broiler chicks on supplementation of exogenous fibre degrading enzymes and protease enzyme in high and low fibre diets are presented in Table 2. Lower dressing percentage was observed in birds fed on unsupplemented diet compared to those of diets with enzyme supplementation. These results showed that enzymes treated high fibre diets improved ($P<0.05$) the dressing percentage of broilers. Improved dressing percentage of broilers in this study in high fibre diets supplemented with enzymes may be due to improved weight gain over the enzyme unsupplemented control diet. These results are in agreement with those of Khan *et al*. (2006) who found that enzyme supplementation to fibrous diet improved the growth rate thereby increasing the dressing percentage. However in low fibre diet supplemented with enzymes could result in lower dressing yield in the present study. These results are in agreement with those of Hunumanth *et al*. (*loc.cit*) who reported that the supplementation of enzymes in corn soya diets of broilers did not improve the dressing yield.

Proventriculus weight was not affected by enzyme supplementation of broiler chicks in the present study. Viveros *et al.* (1993) reported that addition of enzyme to barley-based diets also produced an effect on digestive tract of the bird, reducing the relative weight of upper digestive tract (mainly proventriculus and gizzard) and the size of small intestine and colon of chicks. Abdominal fat weight was not affected by enzyme supplementation to broiler diets in this

present study. These results are in agreement with the Hanumantha *et al.* (*loc.cit*). Visceral organ weights (liver, heart and gizzard) and intestinal length was unaffected in the present study with the enzyme supplementation to the broiler chicks. These results are in agreement with those of Hanumantha *et al*. (*loc.cit*) and Khan *et al.* (*loc.cit*).

C) Immunity Parameters

The immunity parameters and litter moisture content of commercial male broiler chicks on supplementation of exogenous fibre degrading enzymes and protease enzyme in high and low fibre diets are presented in Table 3.

a) Lymphoid organs: Lymphoid organ weights (thymus, spleen and bursa) were unaffected in this present study with the supplementation of feed enzymes to both high or low fibre broiler starter and finisher diets.

b) Immune response to NDV: Increased antibody titers were observed at 42 day of age in this experiment due to the supplementation of enzymes to the high fibre diets in broiler rations. These results concur with those of Liu *et al*. (2008). Gao *et al*. (2004) reported that supplementation of diets with nonstarch polysaccharide-degrading enzyme preparations significantly increased the anti-NDV titers of broilers.

c) Immune response to Sheep RBC: Increased antibody titers were observed at 42 day of age due to sheep RBC inoculation and improved broiler performance was noted which is due to supplementation of enzymes either to the high fibre or low fibre broiler diets. Ameer *et al.* (2005) observed improved performance of broilers, which is due to increased antibody titers against the sheep RBC. Increased antibody production could result in improving the broiler performance on enzyme supplementation.

D) Moisture Content of Litter

Moisture content of litter was increased in the present experiment, which might be due to the supplementation of enzymes to high fibre broiler diets. These findings are in agreement with those of Berwal *et al*. (*loc.cit*), who suggested that the excreta moisture was higher at higher crude fibre levels and enzyme supplemented diets were having lesser moisture in excreta than their corresponding unsupplemented diets.

E) Economics (Return over feed cost)

The economics of commercial male broiler chicks on supplementation of exogenous fibre degrading enzymes and protease enzyme in high and low fibre diets is presented in Table 4. The economics of broilers at 0-6 weeks period on enzymes supplementation to high fibre diets was more encouraging where most of the treated groups generated more profit than that

of control group in this present study. The returns over feed cost per bird in the low fibre and enzyme supplemented diets were Rs. 31.70, 33.22, 31.11 and 29.31 and the gain or loss of income per bird was Rs. 4.46, 1.52, -0.59 and -2.39 for treatments T_1 T_3, T_5, and T_7 respectively. In low fibre diets body weights obtained were less accordingly the less returns were obtained.

The returns over feed cost per bird fed in the high fibre diet and enzyme supplemented diets were Rs. 27.24, 33.67, 31.62 and the gain or loss of income per bird was Rs. -4.46, 6.43, 4.38 and 6.58 for treatments T_2 T_4, T_6, and T_8 respectively. These results are in agreement with those of Khan *et al*. (*loc.cit*) who reported that the enzyme supplementation is more feasible and economical to obtain maximum profitability from broiler production.

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Table 1: Effect of exogenous enzyme supplementation on performance of broiler chicks (0-6 weeks) *

Treatment	Body weight gain (g)			Feed intake (g)			Feed conversion ration			Livability		
	0-4 wks	5-6 wks	0-6 wks	0-4 wks	5-6 wks	0-6 wks	0-4 wks	5-6 wks	0-6 wks	0-4 wks	5-6 wks	0-6 wks
T_1	598^{bc}	724^{a}	1322^{ab}	892	1333	2225	1.49	1.85	1.68	100^{a}	100	100
T_2	568^{c}	602^{b}	1170^{c}	898	1261	2159	1.59	2.10	1.85	100^{a}	93.33	86.67
T_3	587^{bc}	674^{a}	1261^{bc}	848	1232	2080	1.44	1.83	1.64	86.67^{b}	96.67	83.33
T_4	585^{bc}	709^{a}	1294^{b}	863	1316	2179	1.48	1.86	1.69	96.67^{a}	93.33	90.00
T_5	634^{b}	726^{a}	1360^{ab}	900	1313	2213	1.42	1.81	1.62	96.67^{a}	96.67	93.33
T_6	595^{bc}	689^{a}	1284^{b}	892	1226	2118	1.49	1.79	1.65	100^{a}	83.33	83.33
T_7	564^{c}	695^{a}	1259^{bc}	838	1204	2042	1.48	1.73	1.62	100^{a}	93.33	93.33
T_8	692^{a}	713^{a}	1405^{a}	1023	1332	2355	1.48	1.86	1.68	100^{a}	93.33	93.33

***Values bearing different superscripts within a column are significantly (P < 0.05) different.**

Table 2: Effect of exogenous enzyme supplementation on dressing yield and visceral organs (% live body weight)*

Treatment	Dressing yield (%)	Abdominal fat (%)	Liver (%)	Heart (%)	Gizzard (%)	Proventriculus (%)	Length of S.I cm / 100g bird
T_1	73.49^{abc}	1.12	2.18	0.43	2.12	0.47	10.74
T_2	69.48^{c}	0.95	2.43	0.46	2.33	0.47	12.15
T_3	72.48^{bc}	1.03	2.79	0.49	2.42	0.44	13.18
T_4	73.11^{abc}	1.06	2.35	0.45	2.12	0.40	12.00
T_5	73.01^{abc}	1.17	2.38	0.48	2.25	0.44	12.00
T_6	74.40^{ab}	1.29	2.19	0.52	2.19	0.38	11.57
T_7	76.71^{a}	1.73	2.36	0.44	2.16	0.44	11.61
T_8	74.42^{ab}	1.27	2.13	0.47	2.11	0.41	11.21

***Values bearing different superscripts within a column are significantly (P < 0.05) different.**

Table 3: Effect of enzyme supplementation on lymphoid organs (% live body weight), immune response to NDV and Sheep RBC (SRBC) and Moisture content of litter*.

Treatment	Lymphoid Organs (% Live Wt)			NDV titer values (Log_2)		SRBC titer values (Log_2)	Moisture Content of Litter (%)
	Thymus (%)	28th day	42nd day	28th day	42nd day		
T_1	0.33	0.10	0.09	3.83	5.67^{abcd}	4.00^{b}	43.34^{c}
T_2	0.31	0.13	0.13	3.83	5.00^{cd}	6.14^{a}	41.87^{c}
T_3	0.42	0.11	0.11	4.33	4.67^{d}	4.00^{b}	44.50^{bc}
T_4	0.43	0.15	0.11	4.33	5.17^{bcd}	5.00^{ab}	51.26^{ab}
T_5	0.44	0.14	0.11	4.67	6.17^{abc}	5.40^{ab}	54.36^{a}
T_6	0.45	0.12	0.13	4.00	6.33^{ab}	4.86^{ab}	44.48^{bc}
T_7	0.42	0.13	0.11	3.83	6.67^{a}	5.17^{ab}	46.41^{bc}
T_8	0.46	0.09	0.09	4.67	6.17^{abc}	6.22^{a}	57.07^{a}

***Values bearing different superscripts within a column are significantly ($P < 0.05$) different.**

Table 4: Influence of feeding exogenous enzymes in high and low fibre diets, returns over feed cost and gain / loss over control diet of broilers at market age (0-6 wk)

Treatment	Cum feed intake (Kg / bird)	Cost for cum feed intake (Rs)	Body Wt at 6 wks (g)	Sale Amount (Rs / bird)	Returns over feed cost (Rs / bird)	Gain / Loss over control (Rs / bird)
T_1	2225	44.85	1367	76.55	31.70	-
T_2	2159	40.74	1214	67.98	27.24	-4.46
T_3	1971	39.91	1306	73.13	33.22	1.52
T_4	2179	41.31	1339	74.98	33.67	6.43
T_5	2213	47.57	1405	78.68	31.11	-0.59
T_6	2118	42.80	1329	74.42	31.62	4.38
T_7	2042	43.71	1304	73.02	29.31	-2.39
T_8	2355	47.38	1450	81.20	33.82	6.58

- The sale price of broilers was taken as Rs 56 / - per Kg live weight.
- The cost of fibre degrading enzyme (cellulase + xylanase + pectinase) was taken as Rs. 0.09 / - per kg diet.
- The cost of fibre degrading enzyme + protease was taken as Rs. 1.34 / - per kg diet.
- The cost of protease enzyme was taken as Rs. 1.25 / - per kg diet.