

Original Research Article

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## Efficacy of Enzymes on the Performance of Broilers Fed with Ration Containing High Level of Non-starch Polysaccharide

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### ABSTRACT

Five weeks feeding trail with 80 day old broiler chicks was conducted to find out the effect of two supplemental enzymes with ration containing high level of non starch polysaccharides (NSP). Day old chicks (Cobb) of both sexes were distributed randomly into four experimental groups with two replicates of ten birds in each replicate. A maize-soyabean meal diet formulated to have 7% total NSP served as control (T1). Ration T2 was formulated to have 10 % total NSP. Ration T2 was fortified with enzyme mix-A (pectinase 15000 U, hemicellulase 2250 U, cellulase 2250 U, xylanase 4000 U and beta-glucanase 250 U/kg feed) and enzyme mix-B (pectinase 15000 U, hemicellulase 4500 U and cellulase 4500 U/kg diet) to form ration T3 and T4. Birds fed high NSP diet without enzyme (T2) had slightly lower body weight gain and feed to gain ratio ( $1302.9 \pm 46.61g$  and  $2.07$ ) compared to birds fed ration T1 ( $1360.50 \pm 32.85g$  and  $1.91$ ). Supplementation of enzymes showed relatively better body weight gain and feed to gain ratio in T2 ( $1328.2 \pm 33.21g$  and  $1.94$ ) and T3 ( $1354.4 \pm 46.80g$  and  $1.91$ ) which is comparable to that of ration T1. Excreta moisture and AME content were not altered significantly by higher level of NSP in the ration and with or without enzyme supplementation. Supplementation of enzymes showed a tendency for better AME, ileal protein digestibility, reduction in ileal NSP recovery and length and weight of various segments of intestine when compared to un-supplemented group (T2). The results of this study indicated that broiler ration containing 10% NSP did not reduce the growth of birds significantly. However, 10% NSP containing ration can be supplemented with enzymes mixture at the rate of pectinase 15000 U, hemicellulase 4500 U and cellulase 4500 U/kg diet to compensate the feed to gain ratio in birds to that of maize soya diet.

#### Keywords

Insoluble NSP, enzyme, Body weight, Viscosity, NSP digestibility

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### Introduction

The anti-nutritive effect of soluble non-starch polysaccharide (NSP) of cereals such as wheat, barely and rye have been documented by many authors (Choct and Annison, 1990).

Major anti-nutritional effects of NSP are reported to be associated with viscous property of soluble NSP in the gut. Enzyme supplementation is reported to hydrolyse these polymeric structure of soluble NSP and reduced the anti-nutritive property thereby

improved the nutritive value. On the other hand, insoluble NSP makes up the bulk in the diet, but they have minimum or no effect on nutrient utilization in monogastric animals (Smits and Annison, 1996 and Hetland and Svihus, 2001). It is clearly evident that the efficacy of feed enzymes depends on their substrate specificity, activity and stability. Inclusion of some of the agro-industrial by-products such as sunflower meal in poultry feeds is limited due to high level of insoluble NSP content. Increasing interest in application of feed enzymes resulted in the development of specific enzyme preparations designed for feeds of different compositions. With the above points in view, the present study was conducted to find out the effect of ration containing higher level of NSP to study the efficacy of two enzyme mixtures supplemented with such diet on the performance of broilers.

### **Materials and Methods**

Two enzyme combinations were evaluated in a broiler feeding trial for a period of five weeks. With the approval of CPCSEA, 80 numbers of day-old straight run broiler chicks (Cobb) were wing banded, weighed and distributed randomly into four experimental groups with two replicates of 10 birds in each replicate. Birds were maintained under uniform management conditions in battery type cages with *ad libitum* supplies of feed and potable water. Maize-soya bean meal based ration containing 7 % total NSP with 2800 kcal/kg metabolizable energy (ME) served as a control (T1). Ration T2 was formulated by replacing maize and soyabean meal with pearl millet and sunflower meal to have 10 % total NSP, 2600 kcal/kg ME and isonitrogenous to that of T1. Individual carbohydrases were obtained from Biocon India Pvt Ltd. and Novozymes South Asia Pvt. Ltd. Two combinations of enzyme mixes (A and B) were formulated. With the enzyme

preparations, ration T3 was prepared by supplementing enzyme mix-A to ration T2 to have cellulase 2250, hemicellulase 2250, pectinase 15000, xylanase 4000 and beta-glucanase 250 U/kg diet. Ration T4 formulated by supplementing T2 diet with enzyme mix-B to have pectinase 15000, hemicellulase 2250 and cellulase 2250 U/kg diet. The ingredient composition and calculated nutrient composition of the experimental diet are presented in Table 1.

At the end of feeding period, six birds from each treatment were randomly selected and placed in individual cages for apparent metabolizable energy (AME) assay. Feed and water were provided *ad libitum*. The entire trial lasted for seven days, wherein first 4 days served as adaptation period and the last three days served as collection period. The representative samples of feed and droppings were collected for nutrient analysis. After the balance trial, birds were fed with experimental rations containing titanium dioxide as indicator at the rate of 5g/kg diet for three days. All the birds were then slaughtered, sufficient quantities of the ileal (from Meckel's diverticulum to ileo-caecal junction) contents were collected in screw capped tubes and immediately placed in freezer for the analysis of volatile fatty acids and viscosity. Length of small intestine, caecum and colo-rectum were recorded. Empty weight of gizzard, liver, spleen, pancreas, small intestine, caecum, colo-rectum and length of small intestine, caecum and colo-rectum were also recorded.

Experimental rations were subjected to analysis of crude protein, crude fibre (AOAC, 1980) and non-starch polysaccharides (Englyst and Cummings, 1988 and Scott, 1979). The energy content of the feed and droppings were measured using an adiabatic bomb calorimeter.

Samples of excreta from each replicate were dried in forced draft oven at 60°C for 48 hours to determine excreta dry matter. Titanium dioxide in feed samples and ileal contents were analysed by UV-VIS spectrophotometer (Lambda 25, Perkin Elmer) as suggested by Short *et al.*, (1996).

The content of ileum were collected, centrifuged at 5000 rpm and the supernatant was separated and stored for the determination of relative viscosities using Oswald U-tube viscometer, comparing with distilled water as suggested by Choct and Annison (1992). Volatile fatty acid contents of the ileal supernatant samples were determined by external standard method using gas liquid chromatography (Netel, Omega QC+) fitted with flame ionization detector, as described by Playne (1985).

The data collected from the feeding trial were subjected to one way analysis of variance (Snedecor and Cochran, 1980). Data on body weight of birds were subjected to sex-correction before applying statistical analysis.

## Results and Discussion

The effect of enzyme supplementation on weekly body weights in broilers from 0-5 weeks of age is given in Table 2. No significant difference was observed between control (7% NSP) and T2 (10% NSP). However, increasing NSP in the diet decreases the apparent metabolizable energy (AME), increased feed intake, without affecting the performance significantly. Addition of either enzyme mix-A or enzyme mix-B to the ration containing 10 % NSP did not show any significant improvement in body weight of birds compared to 10 % NSP without enzymes (T2). Similar trend was observed in cumulative weight gain among the experimental groups.

Increasing NSP in the diet (T2) resulted in poor feed to gain ratio (2.06) compared to T1 (1.91). Birds fed ration T3 showed 5.8% improvement in feed to gain ratio (1.94), while ration T4 had improvement in feed to gain ratio to 7.3% comparable to that of T1 (Table 2). Reports exist for and against enzyme supplementation to ration containing sunflower meal that contributed major part of NSP. Sorensen (1996) mentioned that increasing sunflower inclusion from 5 to 15% in maize-soya ration supplemented with enzymes did not cause any drop in egg production. In contrast, addition of commercial enzymes did not have significant improvement in broilers fed with ration containing sunflower meal (Kocher *et al.*, 2000 and Meeusen and Vallet, 2001). Schang and Azcona (1998) formulated the diet to which enzyme was added, the values for energy and protein observed for sunflower meal were increased by 7%. Birds fed such ration did not show any decrease in performance, and compensated for nutrient reduction due to supplemental enzymes. In this study, addition of sunflower meal and pearl millet increased NSP in the diet. Though there is no significant reduction in the growth performance, the feed to gain ratio was decreased due to increased level of NSP. Among the enzymes supplemented groups, enzyme mix-B showed relatively better performance than enzyme mix A, in terms of feed to gain ratio and maintained body weight comparable to that of typical maize-soya diet having 7% total NSP.

The excreta moisture content (Table 2) did not differ significantly among various dietary treatments. The excreta moisture content of different groups ranged between 62.90±0.94 to 65.37±1.58 %. Higher level of NSP with or without supplementation of enzyme did not have any significant influence over the moisture content of excreta. Excreta moisture content and vent pasting are predominantly

affected by soluble NSP in the diet (Rotter *et al.*, 1989 and Choct *et al.*, 1996) whereas, Kocher *et al.*, (2000) reported no significant difference with regard to excreta moisture content of birds fed semi-purified sunflower meal based diet with or without enzyme supplementation. This finding is in support with the findings of the present study as the total contributed by the sunflower meal and pearl millet is predominately of insoluble NSP.

Increased dietary NSP reduced the AME of the diet (Table 2). No significant difference

existed between T1 and T2 diets. Birds fed T3 and T4, had increase in AME of 1.8% and 1.4% respectively. Schang and Azcona (1998) observed that addition of multi-enzyme were found to compensate about 7% of AME when supplemented with diet containing sunflower meal in layers where as Kocher *et al.*, (2000) recorded no improvement in AME due to enzyme addition with sunflower meal in broiler diet. The results of the present study indicated that the supplemental enzymes able to hydrolyse the polysaccharides thereby improved AME of 10% NSP diet than un-supplemented control.

**Table.1** Per cent ingredient and calculated nutrient composition of the broiler starter ration

| <b>Exp. Rations</b>                    | <b>T1</b> | <b>T2</b>     |
|--|-----------|---------------|
| <b>Total NSP (%)</b>                   | 7         | <b>10</b>     |
| <b>Energy (kcal/kg)</b>                | 2800      | <b>2600</b>   |
| <b>Ingredients</b>                     | <b>%</b>  |               |
| <b>Maize</b>                           | 50.5      | <b>26.5</b>   |
| <b>Pearl millet</b>                    | 7.0       | <b>20.0</b>   |
| <b>Soyabean meal</b>                   | 33.0      | <b>21.0</b>   |
| <b>Sunflower meal</b>                  | -         | <b>23.0</b>   |
| <b>Fish meal</b>                       | 7.0       | <b>7.0</b>    |
| <b>Sunflower oil</b>                   | -         | -             |
| <b>Dicalcium phosphate</b>             | 0.5       | <b>0.5</b>    |
| <b>Mineral mixture*</b>                | 2.0       | <b>2.0</b>    |
| <b>Calculated nutrient composition</b> |           |               |
| <b>ME (kcal/kg)</b>                    | 2850.5    | <b>2623.5</b> |
| <b>Crude Protein</b>                   | 23.3      | <b>23.4</b>   |
| <b>Crude fibre</b>                     | 3.53      | <b>7.92</b>   |
| <b>Total NSP</b>                       | 7.00      | <b>10.85</b>  |
| <b>Insoluble NSP</b>                   | 5.98      | <b>7.76</b>   |
| <b>Soluble NSP</b>                     | 1.57      | <b>3.08</b>   |

Supplied per kg of diet – Calcium – 6.4 g, Phosphorus - 1.2g, Manganese - 55 mg, Iodine -2 mg, Zinc - 52 mg, Copper - 2 mg, Iron - 20 mg.

Vitamin mixture was added 10g/100 kg of feed: Each gm contained Vit.A-82000 IU, D3- 12000 IU, K- 10 mg and B2-50 mg.

Coccidiostat (Diclazuril) was added at the rate of 10g/100kg of feed

Toxin binder (Sodium alumino silicate) was added at the rate of 10g/100kg of feed

Vit B complex was added at the rate of 25g/100kg feed supplied thiamine 1mg, pyridoxine 2 mg, cyanocobalamine 15 mcg, niacin 15 mg, calcium pantothenate 10 mg and folic acid 1 mg.

**Table.2** Effect of dietary NSP with or without enzyme supplementation on body weight (g), weight gain (g), feed to gain ratio, excreta moisture, apparent metabolizable energy, ileal protein digestibility, ileal NSP recovery, ileal volatile fatty acids and ileal viscosity in broilers

| Ration                                     | T1             | T2             | T3             | T4             |
|--|----------------|----------------|----------------|----------------|
| NSP (%)                                    | 7              | 10             | 10             | 10             |
| Parameters                                 | Control        | No enzyme      | Enzyme mix-A   | Enzyme mix-B   |
| Body wt (g) at 5 wks <sup>NS</sup>         | 1406.2 ± 32.58 | 1348.7 ± 43.53 | 1371.3 ± 35.19 | 1399.5 ± 42.27 |
| Weight gain (g) <sup>NS</sup>              | 1360.5 ± 32.85 | 1302.9 ± 46.61 | 1328.2 ± 33.21 | 1354.4 ± 46.80 |
| Feed/gain ratio                            | 1.91           | 2.06           | 1.94           | 1.91           |
| Excreta moisture <sup>NS</sup>             | 65.37 ± 1.58   | 64.30 ± 1.95   | 63.47 ± 0.85   | 62.90 ± 0.94   |
| AME (kcal/kg diet) <sup>NS</sup>           | 2840.3 ± 24.67 | 2672.3 ± 34.05 | 2717.2 ± 43.64 | 2708.0 ± 57.73 |
| Ileal protein digestibility (%)            | 81.09 ± 1.23   | 78.23 ± 1.20   | 79.20 ± 1.31   | 80.43 ± 1.10   |
| Ileal NSP recovery (g/g TiO <sub>2</sub> ) | 3.58 ± 0.46    | 5.52 ± 0.72    | 4.95 ± 0.57    | 4.89 ± 0.64    |
| Ileal volatile fatty acids <sup>NS</sup>   |                |                |                |                |
| a. Acetate                                 | 23.56 ± 2.60   | 24.28 ± 3.23   | 23.78 ± 1.83   | 24.14 ± 1.94   |
| b. Butyrate                                | 3.56 ± 0.64    | 3.23 ± 0.70    | 2.81 ± 0.61    | 2.63 ± 0.82    |
| c. Propionate                              | T              | T              | T              | T              |
| Ileal viscosity <sup>NS</sup>              | 1.32 ± 0.06    | 1.26 ± 0.05    | 1.23 ± 0.07    | 1.37 ± 0.05    |

<sup>NS</sup> – Not significant; T- Trace**Table.3** Weight and length of the digestive organs of broilers fed with ration containing 10 % NSP with or without enzyme supplementation (Mean ± SE)

| Ration   | T1                  | T2            | T3            | T4            |
|--|---------------------|---------------|---------------|---------------|
| Total NSP (%)                                  | 7                   | 10            | 10            | 10            |
| Age in weeks                                   | No enzyme (control) | No enzyme     | Enzyme mix A  | Enzyme mix B  |
| <b>Weight (g/kg live weight)<sup>NS</sup></b>  |                     |               |               |               |
| Gizzard  | 28.69 ± 1.96        | 33.24 ± 2.68  | 33.26 ± 4.29  | 36.90 ± 1.30  |
| Liver  | 34.57 ± 4.32        | 30.60 ± 1.36  | 34.90 ± 1.20  | 26.80 ± 4.61  |
| Pancreas                                       | 3.26 ± 0.15         | 3.70 ± 0.57   | 3.44 ± 0.33   | 3.68 ± 0.14   |
| Small intestine                                | 31.00 ± 0.14        | 36.31 ± 1.53  | 32.97 ± 0.63  | 34.74 ± 1.74  |
| Caecum   | 5.86 ± 0.61         | 5.95 ± 0.53   | 5.20 ± 0.28   | 4.59 ± 0.19   |
| Colo-rectum                                    | 2.83 ± 0.72         | 3.87 ± 1.33   | 4.13 ± 0.48   | 3.54 ± 0.44   |
| <b>Length (cm/kg live weight)<sup>NS</sup></b> |                     |               |               |               |
| Small intestine                                | 130.60 ± 4.40       | 150.53 ± 2.39 | 135.63 ± 8.29 | 137.13 ± 5.69 |
| Caecum   | 14.74 ± 0.26        | 17.48 ± 0.70  | 15.53 ± 0.08  | 15.60 ± 2.85  |
| Colo-rectum                                    | 7.38 ± 0.48         | 11.24 ± 0.77  | 10.28 ± 0.34  | 9.43 ± 0.28   |
| Total  | 152.72 ± 5.14       | 179.24 ± 2.61 | 161.44 ± 8.59 | 162.15 ± 8.82 |

Means of six observations: <sup>NS</sup> – Not significant



Data on ileal protein digestibility was not differing significantly between the experimental groups. Ileal recovery of NSP showed that increase in dietary NSP increased the ileal recovery of NSP and supplemental enzymes failed to exert any significant difference on the ileal NSP recovery. With regard to ileal volatile fatty acid content (Table 2), acetate was the most abundant followed by butyrate whereas, propionate was detected only at trace level irrespective of the dietary treatments. Similarly, relative viscosity of ileal content was not differing among the experimental groups and was ranged between  $1.23 \pm 0.07$  to  $1.37 \pm 0.005$ . Kocher *et al.*, (2000) reported a significant increase in ileal protein digestibility when semi-purified sunflower based diet supplemented with enzymes and suggested that disruption of the cell-wall matrix by the exogenous glycanases in the upper intestine led to easy access of the endogenous proteolytic enzymes to digest the entrapped proteins. However, Poor ileal protein digestibility in pigs while increasing the dietary fibre level has been reported by Jorgensen *et al.*, (1996a) and suggested that increased feed passage through the digestive tract and hindrance to the access of digestive enzymes to cell contents could be the possible reason for reduced protein digestibility. Data in this study suggest that increase in the dietary NSP or supplementation of enzymes did not have significant influence over the ileal protein digestibility due to higher level of insoluble NSP in the diet.

Jorgensen *et al.*, (1996 a, b) have reported that the increasing dietary NSP increased the ileal and faecal recovery of NSP in pigs and poultry. Kocher *et al.*, (2000) reported that the ileal digestibility of birds fed sunflower meal based semi-purified diet with multi enzymes did not show any influence over the ileal NSP recovery. The results of the present study are in accordance with these reports. Presence of

high level of insoluble NSP in the diet might have undergone slow surface peeling action of enzyme but fails to exert any influence over the ileal NSP recovery.

Ration containing high levels of viscous cereals (high soluble NSP) reported to increase the intestinal viscosity associated with increased microbial fermentations in the distal part of small intestine leading to increased volatile fatty acids production (Langhout *et al.*, 2000). Carre *et al.*, (1990) reported that soluble NSP undergo greater degradation than insoluble NSP and are not reported to have any viscous property (Farrell and Martin, 1998 and Hetland and Svihus, 2001). However, in the present study rations were found to contain more of insoluble NSP and hence supplementation of enzymes did not have any role on the intestinal viscosity and volatile fatty acids concentration.

The weight of gizzard, liver, pancreas, intestine and length of intestine (Table 3) were not differing significantly between the experimental groups. Significant change in the intestinal morphology with increased mitotic activity and mucosal growth was reported due to addition of guar gum (soluble NSP) in the ration of rats (Johnson *et al.*, 1984). Whereas Zanella *et al.*, (1999) observed that supplementation of enzymes with maize soya based ration did not have any significant influence over the morphology of digestive organs. The present study, increasing the NSP level from 7% to 10% did not have significant influence over gut morphology and supplemental enzymes have not shown much influence on the gut morphology.

It was concluded that broilers fed with 10 % total NSP and have predominately of insoluble NSP, indicated that the broiler birds were able to tolerate up to 10 per cent NSP in the diet without significant reduction in the

performance. Further, broilers fed with ration containing 10 per cent NSP supplemented with enzyme mixture (pectinase 15000 U, hemicellulase 4500 U and cellulase 4500 U/kg diet), have shown to counteract the nutrient dilution effect of dietary NSP resulted in an better feed to gain ratio in broilers.

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