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# EFFECT OF MULTI ENZYME SUPPLEMENTATION ON ILEAL DIGESTIBILITIES OF DRY MATTER, PROTEIN, ETHER EXTRACT, PHYTATE PHOSPHORUS AND NON-STARCH POLYSACCHARIDES IN BROILER DIET

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## ABSTRACT

A biological trial was conducted to study the effect of multi enzyme supplementation on ileal digestibilities of dry matter (DM), crude protein (CP), ether extract (EE), phytate phosphorus (PP) and non-starch polysaccharides (NSPs) in terms of neutral detergent fibre (NDF), acid detergent fibre (ADF), hemicellulose and cellulose in broilers for a period of 0-6 weeks. The broilers used in this trial were fed diets supplemented with enzyme at 0, 250, 500, 750 and 1000 g/ton of feed with a proportionate reduction in metabolizable energy (ME-1.25, 2.5, 3.75 and 5 %), crude protein (CP-0.75, 1.5, 2.25 and 3 %), methionine + cystine (0.5, 1, 1.5 and 2 %) and available phosphorus (2.2, 4.4, 6.6 and 8.8 %). The level of individual enzyme per gram of multi enzyme supplement was cellulase 146 IU, xylanase 241 IU, pectinase 98 IU, protease 74 IU, amylase 778 IU and phytase 33 IU. The ileal digestibility was conducted on 6<sup>th</sup> week of trial. The ileal DM digestibility (%) was significantly ( $p < 0.01$ ) increased in 750 g and 1000 g enzyme supplemented groups than other treatment groups. The increase in ileal DM digestibility was significantly ( $p < 0.01$ ) higher (6.2 %) in the group fed with 750 g enzyme supplementation than control. The digestibility of ileal crude protein (%) significantly ( $p < 0.01$ ) increased in 500, 750 and 1000 g enzyme supplemented groups than control. The increase in ileal protein digestibility was 12.20 % in 500 g/ton, 9.90 % in 750 g/ton and 10.12 % in 1000 g enzyme supplemented groups than control. However a non significant difference was observed in ileal EE digestibility in all treatment groups. The ileal phytate phosphorus digestibility significantly ( $p < 0.01$ ) increased in all the enzyme supplemented groups (33.69 % in 250 g/ton, 38.75 % in 500 g/ton, 39.53% in 750 g/ton and 42.11% in 1000 g/ton) than control (31.71%). A highly significant ( $P < 0.01$ ) increase in ileal neutral detergent fibre (NDF) and hemicellulose was observed in 500 to 1000 g enzyme supplemented groups than control. Similarly significant ( $P < 0.01$ ) increase in ileal acid detergent fibre (ADF) and cellulose digestibilities (%) were observed in all enzyme supplemented groups than control. It was concluded that the multi enzyme supplementation at minimum inclusion level of 500 g per ton of feed increased the ileal digestibilities of CP, PP, NDF, ADF, hemicelluloses and cellulose by 12.20 %, 18.17 %, 13.57 %, 55.63 % 12.77 % and 52.64 % respectively than control in broiler diet than the control diet in broilers.

**Key words:** Enzyme supplementation, ileal digestibility and nutrients

## INTRODUCTION

The improvement of feed utilization is an important issue in poultry nutrition because of the need to reduce the environmental pollution from poultry and to decrease the production cost. Feedstuffs contain certain compounds like non starch polysaccharides (NSP's) and phytate phosphorus which either birds cannot digest or it may interfere with the bird's digestive system. Frequent reason for these indigestibility problems is that the birds are unable to produce the necessary enzymes to degrade these compounds (Khattak *et al.*, 2006). In recent decades, much research has been performed in the study of chicken nutrition to investigate the use of exogenous enzymes to improve nutrient utilization (Leeson and Summon, 2005; Bharathidhasan *et al.*, 2010; Truonga *et al.*, 2017) and many commercial enzyme products are currently available for use in chicken nutrition. Further, the major challenge before the animal nutritionist is to minimize the feed cost without compromising the quality or to maintain the feed cost and improve the quality of the feed to exploit the genetic potential of the bird to the maximum extent. The shortage in availability of feed ingredients for poultry production compels to utilize the newer feed or alternative nutrient resources, which also contain higher levels of anti nutritive factors (ANFs) like non starch polysaccharides (NSPs) and phytate phosphorus. These ANFs interfere with the normal digestion process of birds there by reducing the availability of nutrients, due to lack of enzymes like cellulase and hemicellulase fractions, which are required for the utilization of the NSPs. Further,

two thirds of the phosphorus in vegetable feed ingredients is present as phytate phosphorus, the utilization of which is limited due to lack of enzyme phytase in birds. The inclusion of enzyme preparations containing cellulase, hemicellulase and phytase was found to be helpful in enhancing the nutritive value of feedstuff with high NSPs (Friesen *et al.*, 1992, Bharathidhasan *et al.*, 2010) and phytate phosphorus (Simons and Vesteegh., 1990, Bharathidhasan *et al.*, 2010). The mode of action of exogenous enzyme to improve the utilization of feed is well documented, which is directly related to digestion and hence the utilization can be studied using "Ileal Digestibility" experiment. Therefore this paper deliberates on the effect of multi enzyme supplementation on ileal digestibilities of nutrients in broilers on nutrient reduced diets.

## MATERIAL AND METHODS

Five experimental broiler starter and finisher diets were formulated (BIS., 1992) containing commercial feed enzyme levels at 0, 250, 500, 750 and 1000 g per ton of feed with dose dependant reduction of metabolizable energy (ME-1.25, 2.5, 3.75 and 5 %), crude protein (CP-0.75, 1.5, 2.25 and 3 %), methionine+cytine (0.5, 1, 1.5 and 2 %) and available phosphorus (2.2, 4.4, 6.6 and 8.8 %). Each gram of feed enzyme contained the level of cellulase 146 IU, xylanase 241 IU, pectinase 98 IU, protease 74 IU, amylase 778 IU and phytase 33 IU. The ingredients and chemical composition (AOAC, 2007) of the formulated broiler diets are presented in **Table 1**. One hundred and sixty five Vencobb broiler straight

run chicks were wing banded, weighed individually and distributed randomly to five experimental diets with three replicates of eleven chicks each. The birds were housed in deep litter system from day one to six weeks and fed with weighed quantities of feed with *ad libitum* water following uniform standard managemental practices. The ileal digestibility was conducted by indirect method of digestibility using Titanium dioxide ( $\text{TiO}_2$ ) as the external marker at sixth week of feeding trial and it was mixed at the rate of 5 g/kg of feed. Sixteen birds were selected randomly from each treatment group and they were provided experimental diet for five days before measurement of ileal digestibility in order to steady state of gastro intestinal conditions. After six days four birds per day per treatment were killed by decapitation, dissected immediately and the terminal ileal contents were squeezed out into a plastic container for four subsequent days. The terminal ileum was defined as equaling the length of the caeca plus 1 to 2cm ending 1cm from the ileo caecal junction (Danicke *et al.*, 1997). Titanium dioxide was estimated according to the method of Short *et al* (1996) and the ileal digestibility was estimated for dry matter, crude protein, ether extract (EE), phytate phosphorus (Haugh and Lantzsh.,1993) and fibre fractionations (Goering and Vansoest., 1970) viz NDF, ADF, hemicellulose, cellulose. The data collected on various parameters were statistically analyzed as per the method of Snedecor and Cochran (1989).

## RESULTS AND DISCUSSION

The mean ileal digestibility values of dry matter (DM), crude protein (CP), ether

extract (EE) and phytate phosphorus are presented in **Table 2** and ileal digestibilities of NSPs viz. neutral detergent fibre (NDF), acid detergent fibre (ADF), hemicelluloses and cellulose are presented in **Table 3**.

The ileal DM digestibility (%) was significantly ( $p<0.01$ ) increased in 750 g and 1000 g enzyme supplemented groups than other treatment groups. The increase in ileal DM digestibility was highest (6.2 %) in the group fed with 750 g enzyme supplemented group than control. Similarly, Steinfeldt and Pettersson (2001) also reported that the enzyme at 300 mg/kg improved ( $p<0.02$ ) the ileal organic matter digestibility by 8.9 % compared with control. Langhout *et al.* (1997) observed that a significant increase in the digestibility of organic matter in endo-xylanase added to wheat and rye based broiler diets. The increase in dry matter digestibility might be due to the utilization of non starch polysaccharides, protein and phytate phosphorus while inclusion of feed enzymes in the diet.

The digestibility of ileal protein (%) was significantly ( $p<0.01$ ) increased in 500, 750 and 1000 g enzyme supplemented groups than control. The increase in ileal protein digestibility was 12.20 % in 500 g/ton, 9.90 % in 750 g/ton and 10.12 % in 1000 g enzyme supplemented groups than control. A significant ( $P<0.001$ ) improvement in ileal protein digestibility (Steenfeldt and Petersson, 2001) was found in earlier study in the chickens fed medium level of enzyme supplementation (200 mg/kg), increased the ileal protein digestibility by 12.4 % when compared to control. The enzyme supplementation was also increased ( $p<0.05$ ) the ileal nitrogen digestibility

from 8 to 9.1 % in enzyme supplemented diet than control (Selle *et al.*, 2009). Saki *et al.* (2005) also observed a significant effect ( $P<0.05$ ) on ileal protein digestibility while in combination of different levels of enzyme and 2900 kcal/ kg ME in broiler chicken. The results of this study was also in par with earlier observations made by Baidoo *et al.* (1998) and Zanella *et al.* (1999) who noted that the influence of enzyme increased the ileal protein digestibility. Significant ( $p<0.01$ ) increase in protein digestibility by 6.08 % was observed in the proximal ileum when 500 FTU/kg phytase included in the broilers diet than control (Truonga *et al.*, 2017). Further, Friesen *et al.* (1992) observed an increase in protein digestibility by 3 % in wheat based diet, 4 % in Bedford barley based diet, 7 % in rye based diet, 16 % in oat based diet and 20 % in scout barley based diet in the broiler chicken supplemented with crude cellulase enzyme. The increase in ileal protein digestibility is the result of the increased protein availability, which could be due to the release of trapped proteins by protease and phytase in the supplemented enzyme. Also the protease (74 IU/g) and phytase (33 IU/g) in the supplemented enzyme could have contributed to the increase in ileal protein digestibility.

There ileal EE digestibility showed only numerical increase in EE digestibility in all enzyme treated groups. Similarly Glamocic *et al.* (2011) and Selle *et al.* (2009) also reported that the exogenous enzyme had no effect on ileal EE digestibility in reduced ME diet. In contrary to the present study Steinfeldt and Petersson (2001) reported that the enzyme at 300 mg/kg increased ( $p<0.0007$ ) the ileal fat digestibility by 17

% compared with control. Also, Allen *et al.* (1997) reported that crude fat digestibility was significantly increased by 9.4 % when endoxylanase was added at a level of 100 mg/kg of animal fat based broiler diet. Multi enzyme supplementation numerically enhances the ileal digestibility of EE, but not significant in the present study might be due to the nutrients reduced diet used for broilers or the enzyme lipase was not included in the multi enzyme preparations.

The ileal phytate phosphorus digestibility was significantly ( $p<0.01$ ) increased in all the enzyme supplemented groups (33.69 % in 250 g/ton, 38.75 % in 500 g/ton, 39.53% in 750 g/ton and 42.11% in 1000 g/ton) than control (31.71%). The increase in ileal phytate phosphorus digestibility was from 5.88 % to 24.7 % in all the enzyme supplemented groups than control. Glamocic *et al.* (2011) also reported that the exogenous enzyme had improved the ileal total ash digestibility in reduced ME diet. Similarly Simons and Versteegh, (1990) reported that the addition of microbial phytase to the broilers fed with the diets containing 0.45% total phosphorus increased the availability of phosphorus from 49.8 % in the control to 56.5, 59.6, 59.6, 62.5 and 64.5% in 250, 500, 750, 1000 & 1500 Units per kg enzyme supplemented groups respectively. Edwards (1993) reported that the addition of phytase (600 U/Kg) decreased the fecal phytate phosphorus by 28 % than the control and the addition of 10 g/kg 1, 25 dihydroxy cholecalciferol further reduced the fecal phytate phosphorus content by 75 %. The result suggests that phytase enzyme present in the diet increased the utilization of phytate phosphorus. Also Carlos and

Edwards (1998) observed in laying hens that the addition 6000 U/kg of phytase to

the corn soyabean diet containing 0.33 % total phosphorus enhanced the phosphorus retention by 83 %.

**Table 2. Influence of multi enzyme supplementation on ileal digestibilities of dry matter, crude protein, ether extract and phytate phosphorus in broiler chicken.**

Enzyme g/ton of feed	Dry matter (%)	Crude Protein (%)	Ether extract* (%)	Phytate phosphorus (%)
0	60.02 ± 0.25 <sup>a</sup>	72.57 ± 0.69 <sup>a</sup>	69.58 ± 0.65	31.71 ± 0.20 <sup>a</sup>
250	61.15 ± 0.53 <sup>a</sup>	74.97 ± 0.16 <sup>a</sup>	69.78 ± 0.65	33.69 ± 0.19 <sup>b</sup>
500	61.94 ± 0.47 <sup>a</sup>	82.65 ± 0.88 <sup>b</sup>	71.16 ± 0.60	38.75 ± 0.28 <sup>c</sup>
750	63.99 ± 0.74 <sup>b</sup>	80.54 ± 0.91 <sup>b</sup>	71.21 ± 0.69	39.53 ± 0.21 <sup>d</sup>
1000	63.98 ± 0.47 <sup>b</sup>	80.74 ± 0.29 <sup>b</sup>	71.06 ± 0.56	42.11 ± 0.04 <sup>c</sup>

Mean of four observations. \*Non significant

Means bearing different superscripts in the same column differ significantly (p<0.01)

A highly significant (p<0.01) increase in ileal NDF digestibility (%) was observed in 500 g (40.74), 750 g (42.72), 1000 g (41.78)/ton of enzyme supplemented groups than control (35.21). The increase in ileal digestibility was by 13.57% in 500g/ton, 17.58% in 750g/ton and 15.73 % in 1000g/ton over that of control. The NDF digestibility was also significantly (p<0.05)

improved while supplementation exogenous enzymes in broiler diet (Glamocic *et al.*, 2011). The finding was also in close agreement with Slominski and Campbell (1990), who observed an increase in the NSPs digestibility from 2.3 to 37 % when laying birds were fed with semi purified diet containing 40 % commercial canola meal (16-22 % NSPs) with 1 % enzyme.

**Table 3. Effect of multi enzyme supplementation on ileal digestibilities of NDF, ADF, cellulose and hemicellulose in broiler chicken.**

Enzyme g/ton of feed	NDF (%)	ADF (%)	Hemi cellulose (%)	Cellulose (%)
0	35.21 ± 0.38 <sup>a</sup>	9.19 ± 0.10 <sup>a</sup>	39.82 ± 0.30 <sup>a</sup>	17.32 ± 0.41 <sup>a</sup>
250	36.02 ± 0.63 <sup>a</sup>	10.99 ± 0.25 <sup>b</sup>	40.28 ± 0.41 <sup>a</sup>	22.39 ± 0.47 <sup>b</sup>
500	40.74 ± 0.76 <sup>b</sup>	20.71 ± 0.22 <sup>c</sup>	45.65 ± 0.57 <sup>b</sup>	36.57 ± 0.62 <sup>c</sup>
750	42.72 ± 0.34 <sup>b</sup>	23.96 ± 0.26 <sup>c</sup>	47.35 ± 1.09 <sup>b</sup>	39.57 ± 0.62 <sup>d</sup>
1000	41.78 ± 0.61 <sup>b</sup>	22.37 ± 0.41 <sup>d</sup>	45.42 ± 0.75 <sup>b</sup>	39.32 ± 0.41 <sup>d</sup>

Mean of four observations. \*Non significant

Means bearing different superscripts in the same column differ significantly (p<0.01)

The ileal ADF digestibility significantly ( $p < 0.01$ ) increased by 16.38 %, 55.63 %, 61.64 % and 58.92 % at 250, 500, 750 and 1000g/ton of enzyme supplemented groups respectively over that of control. The finding is similar to Manoj Sharma and Katoch (1993) who observed that the addition of Novozyme SP243 at 0, 15, 25 and 35 g/kg of layer diet increased ADF metabolizability significantly ( $P < 0.01$ ) in groups fed with 35g/kg of feed. Similarly Alloui *et al* (1994) who observed that a significant change in apparent digestibility of NDF, ADF when the enzyme was supplemented at 0.1% level to the diet containing 35% rapeseed meal compared with an unsupplemented enzyme group in broilers.

Ileal hemicellulose digestibility (%) was significantly ( $p < 0.01$ ) increased at 500 (45.65), 750 (47.35) and 1000g (45.42) of enzyme supplemented groups than control (39.82). The increase in hemicellulose digestibility was by 12.77 %, 15.9 %, and 12.33 % in 500, 750 and 1000 g/ ton of enzyme supplemented group respectively than control. Glamocic *et al.* (2011) also observed that the ileal hemicelluloses digestibility was significantly ( $p < 0.05$ ) improved while supplementation exogenous enzymes in broiler diet. A highly significant ( $P < 0.01$ ) increase in the ileal cellulose digestibility was observed in 250, 500, 750 and 1000g of enzyme supplemented groups than control. The increase of cellulose digestibility was by 22.64, 52.64, 56.23 and 55.95 % in 250, 500, 750 and 1000 g of enzyme supplemented groups respectively over that of control. Similarly Slominsky and Campbell (1990) observed an increase in cellulose digestibility from 0.1 to 13%

when the diet was supplemented with 1% enzyme in laying hens.

In commercial broiler production, the benefits of multi enzyme supplementation to NSPs rich diets are well documented (Bharathidhasan *et al.*, 2010; Shim *et al.*, 2017). In India, the maize and soybean meal are the major ingredients supplying the energy and protein in commercial broiler diets, however the other locally available feed ingredients like sunflower oil cake, rapeseed meal and de-oiled rice bran which contain higher level of NSPs and phytate phosphorus are also included in poultry ration to reduce the feed cost. Hence more poultry rations were formulated with locally available feed ingredients which contain higher level of NSPs and PP. The multi enzyme supplementation utilizes the unavailable portion of carbohydrate like NSPs and PP in poultry as documented early (Bharathidhasan *et al.*, 2010; Shim *et al.*, 2017) and also observed in the present study. The ileal digestibilities study of nutrients is an accurate measure to find out the efficacy of multi enzyme supplementation (Glamocic *et al.*, 2011). As the multi enzyme supplemented in the present study increased the ileal digestibilities of CP, PP and NSPs viz. NDF, ADF, Hemicellulose and cellulose. The earlier studies also reported that the exogenous enzyme supplementation of broiler diets based on corn and soybeans improved the ileal digestibility of the nutrients in energy reduced diets (Glamocic *et al.*, 2011) and total tract digestibility and performance (Bharathidhasan *et al.*, 2009, 2010) like present study.

It was concluded that the multi enzyme supplementation at minimum inclusion

level of 500 g per ton of feed increased the ileal digestibilities of CP, PP, NDF, ADF, hemicelluloses and cellulose and by 12.20 %, 18.17 %, 13.57 %, 55.63 % 12.77 % and 52.64 % respectively than control in broiler diet which could be due to the presence of feed enzymes like cellulase, xylanase, pectinase, protease, amylase and phytase in the diet releases the trapped nutrients in bound form.

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**Table-1: Ingredient and chemical composition of broiler starter and finisher diets**

Ingredients (%)	Broiler starter (Enzyme inclusion (g/ton))					Broiler Finisher (Enzyme inclusion (g/ton))				
	0	250	500	750	1000	0	250	500	750	1000
Maize	44	39.5	35	28	23	47.50	43.00	36.00	31.20	25.00
Broken rice	2.2	7.0	8.0	14.0	18.0	4.80	6.00	9.20	14.40	15.80
Cumbu	2.8	2.0	4.0	5.0	6.0	3.50	5.80	9.00	8.00	12.70
Deoiled rice bran	1.1	2.1	5.0	5.6	6.3	2.60	4.50	5.80	6.65	7.20
Sunflower meal	0.5	0.8	0.5	0.5	0.5	0.50	0.50	0.75	0.70	0.70
Deoiled groundnut cake	8.0	8.0	6.8	6.8	6.4	3.80	3.75	4.65	6.65	10.50
Soya bean oil cake	37.8	37.0	37.1	36.5	36.2	32.70	31.80	30.00	27.85	23.60
Calcite	1.45	1.52	1.59	1.62	1.66	1.35	1.47	1.47	1.48	1.51
Dicalcium phosphate	1.65	1.58	1.52	1.49	1.44	1.75	1.69	1.63	1.57	1.47
Oil	0.5	0.5	0.5	0.5	0.5	1.50	1.50	1.50	1.50	1.50
DLMethionine (g/100 kg)	267.8	268.5	272.6	274.4	275.0	140.00	135.00	140.60	140.30	137.00

Nutrients	Broiler starter (Enzyme inclusion (g/ton))					Broiler Finisher (Enzyme inclusion (g/ton))				
	0	250	500	750	1000	0	250	500	750	1000
Dry matter	91.69	91.72	91.62	91.72	91.69	90.44	90.70	90.77	90.63	90.49
Crude protein (% reduction)	22.99 (0)	22.80 (0.75)	22.56 (1.5)	22.46 (2.25)	22.30 (3)	20.07 (0)	19.83 (0.75)	19.70 (1.5)	19.56 (2.25)	19.39 (3)
Crude fibre	4.86	4.83	4.99	5.17	5.33	4.83	4.95	5.20	5.44	5.24
Ether extract	2.95	2.71	2.65	2.41	2.47	3.93	3.85	3.79	3.70	3.46
Total ash	9.13	8.99	9.02	9.06	8.87	9.31	9.47	9.20	9.89	9.93
NFE *	60.07	60.67	60.78	60.90	61.03	61.86	61.90	62.11	61.41	61.98
Acid insoluble ash	1.96	1.99	1.94	1.92	1.96	2.06	1.96	2.12	2.08	2.29
Calcium	1.14	1.14	1.04	1.15	1.19	1.05	1.09	1.05	1.05	1.09
Phosphorus	0.67	0.69	0.68	0.68	0.67	0.67	0.68	0.69	0.68	0.67
Available Phosphorus* (% reduction)	0.45 (0)	0.44 (2.2)	0.43 (4.4)	0.42 (6.6)	0.41 (8.8)	0.45 (0)	0.44 (2.2)	0.43 (4.4)	0.42 (6.6)	0.4 (8.8)
Cystine +methionine* (% reduction)	0.90 (0)	0.90 (0.5)	0.89 (1)	0.89 (1.5)	0.88 (2)	0.70 (0)	0.69 (0.5)	0.69 (1.0)	0.69 (1.5)	0.69 (2)
ME(kcal/kg)* (% reduction)	2799 (0)	2767 (1.25)	2732 (2.5)	2695 (3.75)	2669 (5)	2904 (0)	2869 (1.25)	2829 (2.5)	2794 (3.75)	2758 (5)

1. Mineral mixture 1g per kg feed added and supplied calcium-6.4 g, phosphorus-1.2 mg manganese-55 mg, iodine-2 mg, zinc-52 g, copper-2 mg and iron-20 mg

2. Vitamin A, B<sub>2</sub>, D<sub>3</sub>, K 0.2g per kg feed added and supplied vitamin A-8250 IU, B<sub>2</sub>-5 mg, D<sub>3</sub> 1200 IU and vitamin-K-1 mg.

3. Coccidiostat 0.5g per kg feed added and supplied 125 mg of Di-nitro-ortho Toluamide -

4. Antibiotic (TM 100) 0.5 g added per kg of feed.

5. Feed Enzyme at the level of cellulase 146 IU/g, xylanase 241 IU/g, Pectinase 98 IU/g, Protease 74 IU/g, Amylase 778

IU/g Phytase 33 IU/g

\* Calculated values