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Influence of enzyme supplementation on intestinal physio chemical characteristics and nutrient digestablity in broilers

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Abstract
Non-Starch Polysaccharide (NSP) in animal feeds is considered as anti nutritive factors in the cell wall of endosperm. Supplementation of NSP hydrolysing enzymes improved metabolizable energy, increased utilization of fat and protein, decreased viscosity of intestinal digesta. The present study was undertaken to find out the effect of supplementation of the NSP hydrolyzing exogenous enzymes to lower the recommended levels of energy and protein in the broiler diet. One hundred and forty four day old straight run Vencobb broiler chicks were randomly distributed to eight treatment groups with two replicates of nine chicks each. The treatment included reduction of 5 or 10% energy or protein or both with and without exogenous multienzyme mix containing cellulose, xylanase, pectinase and protease. The pH of the intestinal content, the relative viscosity of the digesta, amylase activity, tryptic activity, disaccharidase activity, lipase activity and ileal digestibility were determined in the intestinal digesta. The pH was significantly ($P<0.05$) low in 10% less energy and protein with enzyme supplementation group (5.84) followed by standard diet with enzyme (5.88) at 3rd week of age and at the 6th week of age 5% less energy and protein diet with enzyme recorded the lowest pH (5.98). The digesta viscosity was significantly ($p<0.05$) lowered in all the enzyme supplemented groups than the control. There was a significant ($P<0.05$) increase in amylase, maltase and lipase activities but no significant variation in the mean tryptic activity and the nutrient (dry matter, crude protein, ether extract and crude fibre) digestibilities between enzymes supplemented groups and control.

Keywords: NSP, pH, digesta viscosity and digestablity

1. Introduction
One of the impediments in growth of poultry industry is the availability of feed that accounts for 70% of the cost of production. Enzymes are naturally occurring proteins of all living materials and act as catalysts controlling the biological processes essential for life. The major feed ingredients used in the poultry ration are of plant origin, which contain considerable amount of non-starch polysaccharides (NSP). Non-Starch Polysaccharide (NSP) in animal feeds based on cereals is cellulose, 1-3, 1-4-ß-glucans and pentosans of the arabinoxylan type. ß-glucans and pentosans, are considered as anti nutritive factors because of their localisation in the cell wall of endosperm. [1, 2] Antoniou and Marquardt (1981) and White *et al*. (1983) reported that NSP components in feed have reduced the energy utilization, protein digestion and also decreased the absorption of other nutrients. Solubilised NSP is known to produce high viscosity of digesta and it is assumed that elevated digesta viscosities in the upper digestive tract cause impaired nutrient availability. Supplementation of NSP hydrolysis enzymes usually results in various benefits such as improved metabolizable energy, increased utilization of fat and protein, improved feed/gain ratio, increased growth rate, decreased viscosity of intestinal digesta and modification of intestinal microflora [3]. Keeping this in view, the present study was undertaken to find out the effect of supplementation of the NSP hydrolyzing exogenous enzymes to lower the recommended levels of energy and protein in the broiler diet.

2. Materials and Methods
The activities of cellulase, xylanase, pectinase and protease of the multienzyme preparation (Neospark, Hyderabad) were analysed by *in vitro* techniques. The cellulase, xylanase and pectinase activities were estimated as per the method of [4] and protease activity was estimated according to the method [5]. Two control starter and finisher diets were formulated [6]

and experimental diet with 5 to 10 percent reduction in metabolisable energy or protein or reduction of both metabolisable energy and crude protein. The multienzyme containing cellulase (400units/g), xylanase (2000units/g), pectinase (600units/g) and protease (2000units/g) were mixed at the rate of 500g/ton of feed for all the diets except the control feed (Treatment 1 - T1). One hundred and forty four day old straight run Vencobb broiler chicks randomly distributed to eight treatment groups (T1 to T8) with two replicates of nine chicks each. The treatment groups were T1-control diet (without enzyme), T2-control diet + multienzymes, T3- 5% less energy diet + multienzymes, T4-10% less energy diet + multienzymes, T5 -5% less protein diet + multienzymes, T6-10% less protein diet + multienzymes, T7 -5% less energy and protein diet + multienzymes, T8 -10% less energy and protein diet + multienzymes. At the end of third week and sixth week of age, six birds from each experimental group were randomly selected and slaughtered. The intestinal contents were collected for the estimation of intestinal pH, viscosity and enzyme activities.

The pH of the intestinal content was recorded by digital pH meter immediately after slaughter. The relative viscosity of the digesta was calculated by the method [7] using Ostwald U-tube viscometer. Amylase activity [8], tryptic activity [9], disaccharidase activity [10], and lipase activity [11] were determined in the intestinal digesta. All the data were analyzed by completely randomized block design [12].

To determine ileal digestibility ten birds from each group were fed test diets containing titanium dioxide (TiO_2) as marker @ 5g/kg feed for six days and on day six, six birds from each group were slaughtered and the ileal contents were squeezed out immediately into a container. The samples were pooled across group and analyzed for dry matter, crude protein, ether extract, and crude fibre contents. The level of titanium dioxide was estimated according to the method [13].

3. Result and Discussion

The pH was significantly (P<0.05) low in T8 (5.84) followed by T2 (5.88) at 3rd week of age (Table 1) and at the 6th week of age T7 recorded the lowest pH (5.98). This finding agrees with [14] who found reduction in the duodenal pH in chickens, fed soybean meal. [15] suggested that decreased digesta pH might be due to the H^+ release from the carboxyl groups in the hydrolysed peptide when an exogenous enzyme was included in the diet.

The digesta viscosity was singnificantly (p<0.05) lowered in the enzyme supplemented groups (T2 to T8) than the control (Table 1). The reduction in the viscosity of the treatment groups might be due to breakdown of NSP by multienzymes. The viscosity of intestinal digesta directly reflects partial or complete hydrolysis of NSP [1]. This finding of our study concurs with that of [16]. They stated that NSP could be broken down to release starch and smaller polymers to prevent the viscous net work in the intestine and this reduced the water holding capacity[17]. The breakdown of NSP to small molecules could be the reason for reduced viscosity of the present study.

All the enzyme treated groups showed a significant (P<0.05) increase in amylase activity compared to control (Table 2). The result of our study is in accordance with that of [18]. Increased amylase activity might be due to reduction in digesta viscosity brought about by the supplemented enzymes on NSP which might have released bound sugars from NSP into intestine. These sugars could be the stimulant for the enhanced amylase activity observed in our study.

There was no significant variation in the mean tryptic activity between enzymes supplemented groups and control. Our observations indicate that the trypsin activity was not influenced by the multienzyme supplementation on the feed ingredients used in our study. However, [18] observed highest tryptic activity in broiler chicks, fed high viscosity barley diet.

There was a significant (P<0.05) increase in maltase activity in the enzyme supplemented groups compared to control. This is in accordance with the report of [19] who found that rapid increase of disaccharidase activities in the small intestine might be due to diet composition. [20] Reported that specific activity of maltase and sucrase were increased through 43 days of age of chicks fed carbohydrate diet than non-carbohydrate diets. The result of the present study revealed that more availability of digestible sugars due to NSP break down by exogenous enzymes might have stimulated the production of maltase in the small intestine.

There was a significant (P<0.05) increase in lipase activity in all the enzyme treated groups compared to control. This result agrees with that of [18]. They found highly specific lipase activity in the small intestine which could be due to decreased dietary fibre in the small intestine of broiler chickens [21] and lower intestinal pH [22].

There was no significant increase in the dry matter digestibility in the enzyme treated groups compared to control group. The finding of our study concurs with [23], who reported no change in ileal dry matter digestibility in enzyme supplemented birds.

The ileal protein, ether extract and crude fibre digestibilities of the treatment groups did not differ significantly compared with the control. However, the protein digestibility showed apparent increase of 9.96, 8.49, 11.42, 6.32, 5.74, 7.67 and 6.27 per cent in the enzyme supplemented groups (T2 to T8) compared to the control. This observation of the study is in accordance with [24] who reported increased apparent protein digestibility in the groups supplemented with exogenous enzymes than in the control. This might be due to reduced ileal digesta viscosity and enhanced nutrient utilization in broiler chickens. The ileal ether extract digestibility showed an apparent increase of 3.9 and 4.6% in T2 and T4 groups respectively. This finding agrees with the report of [25]. The enhanced ileal ether extract digestibility in our study might be due to increased lipase activity.

T2 and T4 groups recorded an increased crude fibre digestibility of 6.37 and 6.60% respectively when compared to control. These small increases in digestibility of the nutrients might be due to increased contact between the feed and digestive enzymes caused by reduced viscosity of the digesta.

4. Conclusion

An investigation was undertaken to study the effect of enzyme supplementation with different levels of energy and protein on nutrient digestibility, intestinal digesta pH, viscosity and intestinal enzyme activities in broiler chickens. Supplementation of protein and energy at different levels significantly reduced the intestinal pH at 3rd and 6th week of age. The viscosity of the small intestinal digesta decreased significantly in all the treatment groups compared to control. Among the treatment groups, the lowest viscosity was recorded in group fed 5% less energy and protein with enzyme compared to control. Significantly increased amylase, disaccharidase and lipase activities were recorded in all the treatment groups compared to control, whereas tryptic activity was not influenced by enzyme addition. The percentage of dry matter, protein, ether extract, and crude fibre digestibility in the ileum of treatment groups were slightly higher than the control.

Table 1: Mean (± SE) digesta pH,Viscosity (cP) at 3rd and 6th and small intestinal amylase (U/ml), tryptic (U/ml), disaccharidase (U/ml) and lipase (U/ml) activities at 6th week of age week of age in broiler chickens fed different levels of energy and protein supplemented with exogenous enzymes

Treatment	pH		Viscosity (6th week)	Amylase	Tryptic activity	Disaccharidase	Lipase
	3rd week	6th week					
T1	$6.53^{a} \pm 0.20$	$6.51^{a} \pm 0.07$	$2.02^{a} \pm 0.06$	$358.70^{b} \pm 37.65$	56.00 ± 16.40	$3.53^{b} \pm 0.23$	$5.75^{b} \pm 0.29$
T2	$5.88^{b} \pm 0.09$	$6.40^{a} \pm 0.10$	$1.59^{a} \pm 0.08$	$531.77^{a} \pm 42.39$	128.00 ± 28.62	$5.56^{a} \pm 0.37$	$7.73^{a} \pm 0.78$
T3	$6.20^{ab} \pm 0.08$	$6.46^{a} \pm 0.11$	$1.59^{b} \pm 0.02$	$535.08^{a} \pm 44.49$	112.00 ± 32.79	$5.44^{a} \pm 0.21$	$7.03^{a} \pm 0.63$
T4	$6.26^{ab} \pm 0.11$	$6.33^{a} \pm 0.08$	$1.55^{b} \pm 0.03$	$539.18^{a} \pm 31.42$	112.00 ± 32.79	$5.61^{a} \pm 0.48$	$8.87^{a} \pm 0.61$
T5	$6.30^{ab} \pm 0.16$	$6.23^{ab} \pm 0.06$	$1.54^{b} \pm 0.06$	$446.38^{a} \pm 41.58$	138.67 ± 25.69	$5.23^{a} \pm 0.29$	$7.66^{a} \pm 0.55$
T6	$6.10^{ab} \pm 0.12$	$6.31^{a} \pm 0.14$	$1.51^{b} \pm 0.04$	$459.57^{a} \pm 42.49$	128.00 ± 28.62	$5.15^{a} \pm 0.29$	$6.93^{a} \pm 0.33$
T7	$6.40^{ab} \pm 0.16$	$5.98^{b} \pm 0.08$	$1.50^{b} \pm 0.04$	$447.86^{a} \pm 38.12$	61.33 ± 15.69	$5.24^{a} \pm 0.20$	$9.16^{a} \pm 0.70$
T8	$5.84^{b} \pm 0.21$	$6.35^{a} \pm 0.16$	$1.69^{b} \pm 0.06$	$467.31^{a} \pm 39.58$	61.33 ± 15.69	$5.27^{a} \pm 0.28$	$8.31^{A} \pm 0.63$

Mean of 6 observations
Means bearing different alphabets in a column differ significantly ($p<0.05$)

Table 2: Effect of enzymes supplementation on ileal digestibility of dry matter, crude protein, ether extract and crude fibre at 6th week of age in broiler chickens fed different levels of energy and protein

Treatment	Dry matter (%)	Protein (%)	Ether extract (%)	Crude fibre (%)
T1	62.85	72.41	70.33	37.75
T2	65.63	80.42	73.19	40.32
T3	64.24	79.13	71.16	39.87
T4	66.33	81.75	73.62	40.42
T5	64.01	77.30	71.04	38.99
T6	64.83	76.82	71.68	39.12
T7	64.02	78.43	71.18	39.98
T8	64.79	77.26	71.98	38.01

Pooled sample values of 6 birds from each group

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