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Influence of Enzyme Supplementation on Intestinal Morphology in Broiler Chicken

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Abstract: *A study was carried out to evaluate the effect of enzyme supplementation (Multi-enzymes :Cellulose -400units/g; Xylanse-2000 units/g), Pectinase-600 units/g, Protease-2000units/g) in diets with 5% and 10% energy and protein deficient diets on the morphology of intestines in broiler chickens for 42 days. At 3rd and 6th week of study, ileal contents were assessed for microbial counts and small intestines for morphology. The microbial counts were comparable among all the treatment groups with non-significant reduction compared to control diet. The mean length of the small intestine of treatment groups was significantly reduced compared to control. At 3rd week, the intestinal weights were comparable in all groups but at 6th week, a significant decrease was observed in treatment groups fed reduced protein compared to other groups. Histomorphological features revealed significantly variable villi length, crypt length and width at 3rd and 6th weeks. Villi height, crypt length and width were increased in the all enzyme treated groups. It can be concluded from this study that incorporation of exogenous multi-enzymes during reduced energy or protein intake or both affects total microbial count and the intestinal gross and histomorphology in the broiler chicken*

Key words: *Enzyme supplementation, intestinal morphology, protein and energy deficiency, broiler chicken*

I. Introduction

Poultry, being mono-gastric lacks fiber degrading enzymes for complex molecules like cellulose, hemicellulose, pectin etc., Feed is the major component of expenditure in poultry industry and at the same time important for the growth of the animal. Several studies are addressing the issue of reducing cost of the feed by including various non-conventional ingredients [1]. Further, the effect of various contaminants like heavy metals [2], pesticides [3] and mycotoxins [4] are also addressed. Some studies incorporated phytochemicals and plant extracts in feed to improve productivity and ameliorate certain diseases [5-7]. In order to enhance the nutritive quality of various low-energy cereals and other corresponding dietary ingredients, specific enzyme products, which are capable of hydrolysing the relevant Non Starch Polysaccharides (NSP), have been used as a new class of feed additives in poultry. In recent years substantial progress has been achieved in understanding various metabolic and physiological effects of supplemental enzymes [8].

Supplementation of NSP – hydrolysing 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 [9]. Keeping these points in view a study on supplementation of the exogenous enzymes with different levels of energy and protein in broiler diet was undertaken to study the effect of enzyme inclusion on the morphological changes in the small intestine.

II. Materials And Methods

Broiler chicks, locally available feed ingredients and exogenous multienzymes (Neospark, Hyderabad) were utilized for this study.

The multienzymes had cellulase (400units/g), xylanase (2000units/g), pectinase (600units/g) and protease (2000units/g) activity and mixed at the rate of 500g/ton of feed. Eight experimental starter and finisher diets were formulated. The basal control diet was prepared as per NRC [10] and other diets were prepared by reducing either energy or protein or both as indicated below.

One hundred and forty four day old straight run Vencobb broiler chicks were wing banded, weighed individually and randomly distributed to the following groups with two replicates of nine chicks each.

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

The birds were maintained in cages with standard management practices for 42 days. At the 3rd and 6th week of the trial 6 birds from each group were slaughtered. Immediately after slaughter, one gram of ileal content of the small intestine was transferred to sterile tubes and diluted with 9mL of sterile distilled water. All the samples were subjected to six sequential dilutions and the microbial count was determined [11-12]. One mL of each dilution was poured separately into sterile plates. Agar was allowed to set and the plates were incubated in an inverted position at 37°C for 24 h. The colonies were counted with a colony counter.

Representative pieces of the small intestinal tissues were collected during slaughter and embedded in paraffin blocks. The paraffin embedded tissues were sectioned to 3 to 4 μm thickness and stained with hematoxylin and eosin for histomorphological studies. The mean length and width of the villi and crypt in jejunum of the broiler chickens at 3rd and 6th week of age were recorded using ocular micrometer calibrated with stage micrometer. All the parameters recorded were critically analyzed by completely randomized block design as per the method of Snedecor and Cochran [13] using one-way ANOVA followed by Tukey's *post-hoc* test SPSS software (19.0 V).

III. Results And Discussion

There was no significant variation in the ileal microbial count between enzymes supplemented groups and control. However, a slight reduction in the microbial count was observed in enzyme supplemented groups (4.67, 5.66, 4.70, 5.20, 6.67, 3.48 and 5.29 10^4/mL for the groups fed T2 to T8 respectively) when compared to control (7.66 10^4/mL T1). This may be due to better utilization of nutrients from the small intestine or limited availability of substrate for microbial growth [14].

The result of our study is in accordance with earlier observations of Cao *et al.* [15] and Silva *et al.* [16] who also reported to reduction in the number of starch and protein utilizing microflora in the small intestine of birds fed enzymes. Bedford [17] found that enzymes in the diet retarded microbial counts and increased absorption capacity of the intestine.

There was a significant reduction in the mean length of the small intestine of broilers at 3rd week ($P<0.05$) and 6th week ($P<0.01$) in the treatment groups compared to control (Table 1). However, the intestinal weight did not show any variation between the treatment groups and control at 3rd week of age, but there was a significant reduction ($P< 0.05$) in the weight at 6th week of age in the treatment groups fed reduced protein.

Table 1: Mean (± SE) small intestine length (cm/kg) and weight (g/kg) at 3rd and 6th week of age in broiler chickens fed different levels of energy and protein supplemented with exogenous enzymes

Treatment	*Intestine length (cm/kg)*		Intestine weight (g/kg)	
	3rd week	6th week	3rd week	6th week
T1	$118.15^{a} \pm 1.48$	$123.03^{A} \pm 1.90$	36.23 ± 1.75	$50.02^{a} \pm 2.62$
T2	$107.19^{b} \pm 2.75$	$110.30^{B} \pm 2.50$	29.96 ± 2.89	$45.28^{ab} \pm 2.77$
T3	$106.06^{b} \pm 1.97$	$106.68^{B} \pm 1.97$	28.67 ± 1.78	$46.12^{ab} \pm 2.92$
T4	$108.33^{b} \pm 3.57$	$106.49^{B} \pm 2.71$	32.53 ± 2.12	$46.85^{ab} \pm 3.52$
T5	$106.80^{b} \pm 1.73$	$102.37^{B} \pm 1.86$	33.70 ± 2.15	$39.68^{b} \pm 1.78$
T6	$106.59^{b} \pm 1.81$	$103.15^{B} \pm 2.97$	30.50 ± 3.51	$39.85^{b} \pm 2.00$
T7	$104.87^{b} \pm 1.10$	$109.28^{B} \pm 1.86$	30.72 ± 1.53	$41.66^{b} \pm 2.02$
T8	$105.38^{b} \pm 2.05$	$108.51^{B} \pm 2.32$	33.90 ± 2.55	$43.83^{ab} \pm 2.02$

Mean of Six observations; One way ANOVA followed by Tukey's *post-hoc* test using SPSS software (19.0 V)

Means with upper case ($P< 0.01$) and lower case ($P< 0.05$) letter with in a column differ significantly

Our findings agree with other reports [18-19] where the enzyme supplementation to broilers lowered the relative length and weight of the small intestine in the treatment groups fed reduced protein compared to control. On the contrary, Nageswara *et al.* [20] found that addition of enzyme had no effect on relative weight and length of the small intestine.

At 3rd week of age, villi length was significantly ($P<0.01$) lowest in T7 and T8 when compared to other enzyme treated groups (Fig 1) and control (Fig 2). At 6th week of age, there was significant ($P<0.01$) increase in villi length in the T2 to T4 than T1, T5, T6, T7 and T8 (Table 2), whereas, the villi width showed no significant variation among the treatments both at 3rd and 6th week of age.

Table 2: Mean (± SE) jejunal villi, crypt length and width (μm) at 3rd and 6th week of age in broiler chickens fed different level of energy and protein supplemented with exogenous enzymes

Treatment	*Villi length (μm)*		*Villi width (μm)*		*Crypt length (μm)*		*Crypt width (μm)*	
	3rd week	**6th week**	**3rd week**	**6th week**	**3rd week**	**6th week**	**3rd week**	**6th week**
T1	1163.53^{AB} ± 16.74	1415.03^{C} ± 27.00	92.48 ± 8.00	166.60 ± 8.39	112.79^{C} ± 4.42	125.87^{b} ± 3.04	57.82^{abc} ± 3.85	66.95^{bc} ± 1.92
T2	1222.83^{A} ± 26.67	1698.68^{A} ± 25.06	98.00 ± 7.16	169.73 ± 12.08	139.99^{A} ± 4.51	169.83^{a} ± 12.47	67.10^{a} ± 3.02	76.08^{a} ± 1.92
T3	1203.35^{A} ± 33.39	1692.13^{A} ± 37.07	94.73 ± 6.02	169.87 ± 6.53	135.51^{AB} ± 6.91	166.01^{a} ± 10.78	63.91^{ab} ± 4.71	76.08^{a} ± 3.85
T4	1260.15^{A} ± 26.85	1688.70^{A} ± 28.40	99.02 ± 6.65	173.00 ± 7.92	137.03^{A} ± 7.88	165.86^{a} ± 7.23	66.95^{a} ± 4.51	74.56^{ab} ± 4.36
T5	1091.13^{BC} ± 24.11	1571.83^{B} ± 23.95	94.87 ± 5.50	159.40 ± 9.55	118.21^{BC} ± 4.36	162.82^{a} ± 10.12	54.78^{bc} ± 5.27	66.95^{bc} ± 3.04
T6	1097.57^{BC} ± 11.32	1548.24^{B} ± 23.87	91.53 ± 4.14	150.27 ± 10.93	114.13^{C} ± 4.56	158.25^{a} ± 9.33	53.26^{bc} ± 4.36	65.43^{c} ± 3.66
T7	1045.33^{C} ± 15.73	1505.93^{BC} ± 30.96	93.68 ± 7.69	159.70 ± 8.08	112.79^{C} ± 4.42	149.12^{ab} ± 8.71	50.22^{c} ± 2.04	63.97^{c} ± 2.40
T8	1057.23^{C} ± 26.58	1519.00^{BC} ± 30.68	91.84 ± 7.91	163.00 ± 9.87	112.60^{C} ± 3.85	153.69^{a} ± 4.95	54.78^{bc} ± 2.36	63.91^{c} ± 2.36

Mean of Six observations; One way ANOVA followed by Tukey's *post-hoc* test using SPSS software (19.0 V)

Means with upper case ($P< 0.01$) and lower case ($P< 0.05$) letter with in a column differ significantly

Figure 1: The photomicrograph of the jejunum from the enzyme supplemented bird showing the greater surface area of the villi. H & E X 40

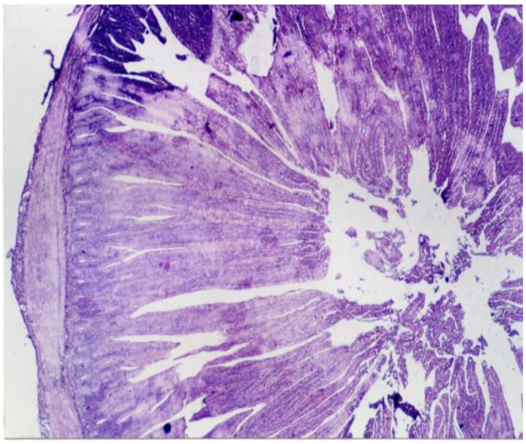

Figure 2: The photomicrograph of the jejunum from the control bird showing the lesser surface area of the villi. H & E X 40

The crypt length was significantly ($P<0.01$) higher in T2, T3 and T4 compared to other groups at 3rd week of age. At 6th week of age, significantly ($P<0.05$) higher crypt length was observed in all the enzyme treated groups except T7 when compared to control. A higher crypt width ($P<0.05$) was observed in T2 and T4 at 3rd week of age. The enzyme treated groups, T2 and T3 indicated significantly higher crypt width than other groups at 6th week of age.

This finding of our study agrees with Viveros *et al.* [21] who found that enzyme supplementation to broilers increased the villi and crypt length and width; increased the relative number of the goblet cells. Mathlouthi *et al.* [22] reported an increased bacterial activity in the gastrointestinal tract, which changed the morphology of gut wall in a rye based diet. The changes in the mucin content or composition might be the reason for increased nutrient absorption by the small intestine.

On the contrary, Iji *et al.* [23] and Wu *et al.* [19] noticed that addition of xylanase to wheat-based diets had no effect on villus height and crypt depth in the duodenum, jejunum and ileum of broilers.

It can be inferred from this study that incorporation of exogenous multienzymes at rate of 500g/ton in 5 to 10 per cent energy or protein or both reduced feed when fed to broilers influences the total microbial count, the intestinal morphology of the broiler chicken.

IV Conclusion

The influence of NSP degrading enzymes supplemented to broiler diets with reduced energy or protein or both content of broiler diets on total microbial count in the intestine showed non-significant difference among the treatment groups. Intestinal length was decreased in the treatment groups; whereas the intestinal weight was lower 6th week of age in the groups fed reduced protein. The intestinal morphology varied significantly in villi length, crypt length and width at 3rd week and 6th week. Villi height was increased in the entire enzyme treated groups and crypt length and width in the treatment groups were also higher than control.

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