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Effects of multi-enzyme supplementation on gut morphology and histomorphology in broilers

R. Balamurugan, D. Chandrasekaran and A. Kirubakaran
Dept. of Animal Nutrition, Veterinary College and Research Institute,
Tamil Nadu Veterinary and Animal Sciences University, Namakkal, TN
nutritionbalu@gmail.com

Abstract

A biological trial in broilers was conducted with 7 treatments namely control (T1) as per nutrient requirements of poultry (NRC, 1994) specification, (T2) control plus 0.05 and (T3) 0.1% enzyme and control plus 0.05 and 0.1% enzyme, with (T4) 0.2%, (T5) 0.4% reduction in dicalcium phosphate with enzyme mixtures plus phytase with (T6) 2.5% and (T7) 5% reduction in metabolizable energy, crude protein, lysine and DL methionine with enzyme mixtures at 0.05 and 0.1% respectively. The villi height (μ) and villi width (μ) significantly ($P<0.01$) increased in enzyme treated groups (T2, T3, T4, T5 & T7 and T2, T3, T4, T5, T6 & T7 respectively) compared to that of control group. The number of crypts and goblet cells were decreased due to enzyme supplementation in T4, T5 and T7. The crypt height, crypt width and goblet cell height (μ) were significantly ($P<0.01$) reduced in all enzyme added groups than the control group. Similarly, the goblet cell width was reduced in T2, T4 and T5. No significant difference was noticed in intestinal weight (g/kg body wt.) and length (cm/kg body wt.) among the treatment groups.

Keywords: Enzyme, phytase, villi, crypt, goblet cell, intestine.

Introduction

Research findings have consistently reported the negative effects of non-starch polysaccharides on bird performance. The presence of soluble NSPs like β-glucans in the diet of chickens had been found to increase the viscosity of intestinal contents (Choct & Annison, 1990; Bedford *et al*., 1991). The NSPs have been found to alter the morphology of the gut like shortening and thickening, atrophy of the villi (Jaroni *et al.,* 1999), hyperplasia and hypertrophy of goblet cells (Viveros *et al.,* 1994). All these changes affect the absorption of nutrients. Chickens being monogastrics are unable to secrete the enzymes needed to breakdown some of the compounds such as non-starch polysaccharides and phytate present in the feed ingredients. These components are not only indigestible but also interfere with the utilization of other nutrients. Hence, supplementation of NSP hydrolyzing enzymes and phytase is expected to improve the nutritive value of feedstuffs and reduce the negative effects such as atrophy of the intestinal villi, enlarged digestive organs and increased size of gastro intestinal tract (Viveros *et al*., 1994). Supplementation of xylanase and β-glucanase in rye based diets has been reported to increase the villus size and the villus height-to-crypt depth ratio in the small intestine of broilers (Mathlouthi *et al*., 2002).

Materials and methods

Seven experimental starter and finisher diets were formulated (NRC, 1994). T1- Control (without enzyme); T2 - 500 g/ton NSP hydrolyzing enzyme; T3 -1000 g/ton NSP hydrolyzing enzyme; T4-500 g/ton NSP hydrolyzing enzyme + Phytase, 0.2% DCP reduction; T5-1000 g/ton NSP hydrolyzing enzyme + Phytase, 0.4% DCP reduction; T6-500 g/ton NSP hydrolyzing enzyme, 2.5% reduction of metabolizable energy, crude protein, lysine and methionine; T7 1000 g/ton NSP hydrolyzing enzyme, 5% reduction of metabolizable energy, crude protein, lysine and methionine. The total activity of cellulase, xylanase, pectinase and phytase, the ingredient and chemical composition of the diets used in the different experimental groups namely T1, T2, T3, T4, T5, T6 and T7 are furnished in Tables 1, 2, 3, 4 and 5 respectively.

1. Mineral mixture at the added level per kg feed supplied manganese-54 mg, zinc-52 mg, iron-20 mg, iodine-2 mg, copper-2 mg and cobalt-1mg.
2. Vitamin AB_2D_3K at added per kg feed supplied vitamin A-8250 IU, B_2-5 mg, D_3-1200 IU and vitamin K-1 mg.
3. Vitamin B complex at added level per kg feed supplied, thiamine 1 mg, pyridoxine 2 mg, cyanocobalamine 15 mcg, vitamin E 10 mg, Niacin 15 mg, calcium D pantothenate 10 mg and folic acid 1 mg.
4. Coccidiostat at the level added per kg feed supplied 125 mg of di-nitro-ortho-toluamide.

252 Vencobb broiler straight run chicks belonging to a single hatch were used for this experiment. The chicks were distributed randomly to 7 experimental diets with 3 replicates of 12 chicks each and reared for 42 d. 6 birds from each treatment were slaughtered and the intestinal length and weight were measured and recorded. The whole small intestine removed and milked out. The samples were taken from jejunum for histological studies. Jejunum was defined as the portion of the small intestine between the bile duct entrance to Meckel's diverticulum (Jaroni *et al*., 1999). Representative pieces of collected jejunum samples were processed by the routine method for histomorphological examination (Bancroft, 1996). The data collected on these parameters were statistically analyzed as per the method of Snedecor and Cochran (1989).

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Table 1. Total enzyme activity used in the broiler trial (IU/kg feed).

Activity	T1	T2 & T6	T3 & T7	T4	T5
Cellulase	-	199.6	402.82	199.6	402.82
Xylanse	-	1009.3	2020.02	1009.3	2020.02
Pectinase	-	299.62	600.37	299.62	600.17
Phytase	-	3.39	6.80	198.43	399.86

Table 2. Ingredient and chemical composition of broiler starter diet.

Ingredients (%)	Treatments						
	T1	T2	T3	T4	T5	T6	T7
Maize	42.5	42.5	42.5	42.8	43.2	46.9	50.8
Soya	46.7	46.7	46.7	46.8	46.7	44.4	42.5
Rice bran oil	7.8	7.8	7.8	7.6	7.5	5.7	3.68
Dicalcium phosphate	1.8	1.8	1.8	1.6	1.4	1.8	1.82
Calcite	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Salt (g/100 kg)	350	350	350	350	350	350	350
DL methionine (g/100 kg)	250	250	250	250	250	243.75	237.5
Mineral mixture (g/100 kg)	100	100	100	100	100	100	100
Vitamin A, B_2, D_3,K (g/100 kg)	10	10	10	10	10	10	10
B complex (g/100 kg)	12.5	12.5	12.5	12.5	12.5	12.5	12.5
Coccidiostat (g/100 kg)	50	50	50	50	50	50	50
Choline chloride (g/100 kg)	100	100	100	100	100	100	100
Anti-oxidant (g/100 kg)	12.5	12.5	12.5	12.5	12.5	12.5	12.5
Oxy tetracycline (g/100 kg)	50	50	50	50	50	50	50

Table 3. Nutrient composition of broiler starter diet.

Nutrients	Treatments						
	T1	T2	T3	T4	T5	T6	T7
Dry matter	90.29	90.11	90.56	90.27	90.22	90.14	90.15
Crude protein	23.10	22.99	23.09	23.02	23.00	22.41	21.92
NDF	9.08	9.08	9.08	9.11	9.14	9.25	9.39
Ether extract	8.98	8.85	9.02	9.07	9.09	8.21	5.58
Total ash	8.51	8.87	8.16	8.92	8.93	7.16	6.84
NFE*	55.63	55.52	55.94	55.24	55.21	58.3	61.70
Acid insoluble ash	1.85	1.83	1.84	1.85	1.84	1.86	1.87
Calcium	1.19	1.18	1.15	0.95	0.91	1.05	1.01
Avail-Phosphorus	0.45	0.45	0.45	0.42	0.38	0.45	0.45
Lysine*	1.49	1.48	1.49	1.50	1.49	1.44	1.39
Methionine	0.62	0.62	0.62	0.62	0.62	0.60	0.58
Cystine+ Methionine*	0.90	0.90	0.91	0.90	0.90	0.88	0.86
Metabolisable energy (kcal/kg)*	3201	3201	3201	3199	3202	3122	3040

**Calculated values.*

Table 4. Ingredient and chemical composition of broiler finisher diet.

Ingredients (%)	Treatments						
	T1	T2	T3	T4	T5	T6	T7
Maize	54.4	54.4	54.4	54.7	55.2	58.3	62.2
Soya	37.3	37.3	37.3	37.3	37.2	35.4	33.5
Rice bran oil	5.7	5.7	5.7	5.6	5.4	3.7	1.7
Dicalcium phosphate	1.3	1.3	1.3	1.1	0.9	1.3	1.3
Calcite	1.3	1.3	1.3	1.3	1.3	1.3	1.3
Salt (g/100 kg)	350	350	350	350	350	350	350
DL methionine (g/100kg)	150	150	150	150	150	146.2	142.5
Mineral mixture (g/100kg)	100	100	100	100	100	100	100
Vitamin A, B_2, D_3, K (g/100kg)	10	10	10	10	10	10	10
B complex (g/100 kg)	12.5	12.5	12.5	12.5	12.5	12.5	12.5
Coccidiostat (g/100 kg)	50	50	50	50	50	50	50
Choline chloride (g/100 kg)	100	100	100	100	100	100	100
Anti-oxidant (g/100 kg)	12.5	12.5	12.5	12.5	12.5	12.5	12.5
Virginamycin (g/100 kg)	50	50	50	50	50	50	50

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Table 5. Nutrient composition of broiler finisher diet.

Nutrients	Treatments						
	T1	T2	T3	T4	T5	T6	T7
Dry matter	90.44	90.42	90.47	90.55	90.62	90.77	90.64
Crude protein	20.02	20.05	20.10	20.05	20.11	19.52	19.04
NDF	9.13	9.13	9.13	9.16	9.20	9.28	9.43
Ether extract	8.62	8.45	8.52	8.59	8.61	7.45	4.64
Total ash	6.31	6.25	6.32	5.99	6.11	4.87	4.21
NFE *	61.71	61.87	61.78	61.9	61.75	64.67	68.58
Acid insoluble ash	1.90	1.91	1.90	1.94	1.93	1.92	1.89
Calcium	0.90	0.91	0.90	0.85	0.80	0.89	0.89
Avail-Phosphorus	0.35	0.36	0.34	0.32	0.29	0.35	0.35
Lysine *	1.25	1.23	1.25	1.25	1.24	1.20	1.15
Methionine	0.48	0.47	0.48	0.48	0.48	0.46	0.45
Cystine+ Methionine*	0.70	0.71	0.70	0.71	0.70	0.68	0.65
Metabolisable energy (kcal/kg)*	3200	3200	3200	3202	3200	3120	3041

**Calculated values.*

Table 6. Micrometric measurements of intestinal villi, crypts, goblet cells at jejunum of broilers fed multi enzyme supplemented diet.

Treatment groups	Villi height (μ) (80x)	Villi width (μ) (80x)	Number of crypts/field (40x)	Crypt height (μ) (80x)	Crypt width (μ) (80x)	Number of goblet cells/ field (40x)	Goblet cells Height (μ) (100x)	Goblet cells width (μ) (100x)
T1	141.84^{a} ± 6.68	7.32^{a} ± 0.28	18.50^{b} ± 1.90	47.55^{f} ± 1.24	7.71^{e} ± 0.08	118.90^{c} ±3.38	6.29^{b} ± 0.36	3.94^{b} ±0.17
T2	160.30^{bc} ± 4.44	16.59^{e} ± 0.36	15.10^{ab} ± 1.15	35.64^{d} ± 1.00	6.71^{d} ± 0.16	106.50^{abc} ±3.57	4.37^{a} ±0.22	3.16^{a} ±0.18
T3	180.36^{e} ± 1.57	17.69^{e} ± 0.30	15.20^{ab} ±1.20	18.49^{a} ± 1.30	6.63^{cd} ±0.23	105.50^{abc} ±4.32	4.43^{a} ±0.21	3.29^{ab} ±0.15
T4	172.22^{de} ± 1.65	13.64^{cd} ± 0.48	12.50^{a} ± 0.65	42.73^{e} ± 0.71	3.68^{a} ± 0.12	94.20^{a} ±3.58	4.27^{a} ±0.21	2.96^{a} ±0.16
T5	165.02^{bcd} ± 3.21	12.10^{b} ± 0.37	13.00^{a} ±0.74	22.17^{b} ± 0.56	6.05^{bc} ±0.13	101.10^{ab} ±4.16	4.17^{a} ±0.18	3.09^{a} ±0.12
T6	142.66^{a} ± 2.70	13.66^{cd} ± 0.34	14.80^{ab} ± 1.19	28.13^{c} ± 0.63	5.68^{b} ± 0.19	111.20^{bc} ±4.04	4.86^{a} ±0.34	3.58^{ab} ±0.27
T7	155.89^{b} ± 4.44	12.59^{bc} ± 0.43	11.80^{a} ± 0.55	38.34^{d} ± 0.90	3.80^{a} ± 0.17	96.30^{a} ±3.61	4.53^{a} ±0.28	3.45^{ab} ±0.21

Results and discussion

Gut morphology: The mean small intestinal weight, caecum weight and colo-rectum weight (g/kg body wt.) of different experimental group did not differ significantly. The weight of various intestinal segments in enzyme supplemented groups had numerically lower value when compared to that of control group. The mean length (cm/kg body wt.) of small intestine, caecum, colo-rectum did not differ significantly in between the treatment groups. The length of the various segments was numerically lower in the enzyme supplemented groups. Similarly Hanumantha Rao *et al.,* (2003) observed that supplementation of multi- enzyme to corn-soybean based diet did not significantly influence the intestinal length and weight compared to the control group. In this experiment it was observed that the intestinal length was nearly equal to that of the control group even in the groups fed with diluted diets indicating that the enzymes could have influenced in arresting the lengthening of the intestines.

Histomorphology: The micrometric measurements of jejunal sections of different treatments are presented in Table 6. Significantly (P<0.01) increased villi height was observed in the enzyme supplemented groups T2, T3, T4, T5 and T7 which was 160.3, 180.36, 172.22, 165.02 and 155.89 μ when compared to the control group 141.84μ. Among the enzyme supplemented groups significantly (P<0.01) lower in villi height (142.66μ) was observed in T6. The villi width of the different enzyme supplemented groups T2, T3, T4, T5, T6, T7 was 16.59, 17.69, 13.64, 12.10, 13.66, 12.59μ, which was significantly (P<0.01) more than the control group (7.32μ). The width in the T2 and T3 groups was significantly (P<0.01) higher than the other enzyme supplemented groups.

Similar trend was observed by Viveros *et al.* (1994). The jejunum of birds fed diets having 60% barley showed shortening, thickening and atrophy of the villi, addition of β-glucanase counteracted these effects. The number of intestinal crypts/field in experimental groups T1, T2, T3, T4, T5, T6 and T7 was 18.5, 15.1, 15.2, 12.5, 13, 14.8 and 11.8. Significantly (P<0.01) decreased number of intestinal crypt was observed in T4, T5 and T7 groups than the control. The intestinal crypt height of T2, T3, T4, T5, T6 and T7 groups was significantly (P<0.01) lower (35.64, 18.49, 42.73, 22.17, 28.13 and 38.34μ) than the T1 group (47.55μ). Among the enzyme supplemented groups significant difference was observed between all except between T2 and T7 groups. The crypt width of T1, T2, T3, T4, T5, T6 and T7 groups was 7.71, 6.71, 6.63, 3.68, 6.05, 5.68 and 3.8μ respectively. The crypt width was significantly (P<0.01) decreased in all enzyme supplemented groups compared to control group. Among the enzyme supplemented groups T4 and T7 recorded a significantly (P<0.01) lower crypt width. Wu *et al.* (2004) in his study found that addition of xylanase in wheat

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based broiler diet decreased crypt depth in the jejunum and combination of xylanase, phytase increased the crypt depth in the jejunum and ileum. But in this present study the crypt height is reduced with addition of NSP enzymes alone or in combination with phytase. The number of goblet cells/field in the T1, T2, T3, T4, T5, T6 and T7 groups was 118.9, 106.5, 105.5, 94.2, 101.1, 111.2 and 96.3 respectively. A significant ($P<0.01$) reduction in the number of goblet cells was observed in T4, T5 and T7 groups compared toT1 and in the other groups a numerical reduction was observed. The mean goblet cells height (μ) of different treatment groups T1, T2, T3, T4, T5, T6 and T7 was 6.29, 4.37, 4.43, 4.27, 4.17, 4.86 and 4.53 respectively. The goblet cells height was significantly ($P<0.01$) decreased in enzyme supplemented groups when compared to the control. No significant difference was observed between the enzyme supplemented groups. The goblet cells width (μ) was significantly reduced in the enzyme supplemented groups T2 (3.16), T4 (2.96), T5 (3.09) than the control (3.94). The T3, T6 and T7 groups showed a numerical decrease when compared to T1 group.

These results agree with the findings of the Viveros *et al.* (1994) who observed decreased number of goblet cells, goblet cell size in enzyme supplemented barley (60%) based broiler diet when compared to the unsupplemented group. Further, the reduction in the height and width of the crypt in the enzyme supplemented groups observed in this study could be due to the reduction in the numbers and size of the goblet cells.

Conclusion

Addition of non-starch degrading enzymes maintained the intestinal length of the birds fed diluted diets equal to that of the birds fed control diet. The enzymes were able to increase the villi height and width reduced the number, height and width of crypt and goblet cells compared to that of the control.

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