Effects of β-Mannanase on Broiler Performance and Gut Morphology

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An experiment was designed to assess the effects of graded levels of β-mannanase on performance and gut morphology of broilers provided with diets based on corn and soybean meal. Four dietary treatments contained 0, 500, 700 and 900 g β-mannanase (Hemicell®) /ton. Each treatment contained 4 pen with 15 birds/pen. β-mannanase inclusion at 900 g/ton reduced (P<0.05) feed intake in finisher and total period, whereas inclusion at 500 or 700 g/ton resulted in no significant benefit. Enzyme supplementation had no effect on body weight and feed conversion ratio. β-mannanase inclusion at 900 g/ton improved gut morphology and increased (P<0.01) villus height and crypt depth and decreased (P<0.01) goblet cell number, epithelial thickness and ratio of crypt depth to villus height in different sections of small intestine.

Keywords: broiler, performance, gut morphology, β-mannanase

Introduction

Soybean meal contains a number of antinutritional factors. Among potential factors reducing nutrient bioavailability are the nonstarch polysaccharides (NSP). Among NSP, mannans occur in the forms of glucomannan, galactomannan, glucogalactomannan and glucurono- mannans in plant cell walls (Aman&Graham 1990). Hemicell® is a fermentation product of *Bacillus lentus*. It contains high amounts of β -mannanase that degrade β-mannan in feed. Mannan from guar gum, a galactomannan, has been shown to be a strong antinutritive factor for monogastric animals. It interferes with glucose metabolism and insulin secretion rates in swine (Leeds et al, 1980). The suppression of insulin secretion can impair the intestinal uptake and utilization of glucose and amino acids in peripheral tissues such as striated muscle by monogastric animals, resulting in reduced growth and feed efficiency (Jackson *et al*, 1999). NSP had the negative effects on the microscopic structure of gut, but published data on this aspect are limited and controversial. Wu et al, (2004) found that the addition of phytase and to wheat based diets reduced digesta viscosity, relative weight, length of small intestine, number of goblet cells in the jejunum and increased villus height in the duodenum. No published data are available on the effects of β-mannanase on gut morphology in broiler chickens. This experiment was designed to determine whether β-mannanase is capable of reducing the digesta viscosity in broiler intestine, and to evaluate the effects of β-mannanase on feed intake body weight, feed conversion rate and gut morphology.

Materials and methods

A total of 240 1-d-old Cobb broilers were used in this experiment. The chickens were allocated at random to the 4 experiment treatments. The experiment was carried out in 16 pens. The basal diet was based on corn and soybean meal *(Table 1)*. Treatments 1 to 4, respectively, contained 0, 500, 700 and 900 g Hemicell/ton. Body weights and feed intake were recorded on a pen basis. Mortality was recorded daily. The experimental design was completely randomized. The data were analyzed using the General Linear Model procedure of SAS to determine treatment effects. Significant mean differences were determined using a least significant difference test. Histological examinations were carried out according to the method of Iji et al. (2001). On d 42, two birds from each pen were selected, weighed, killed and the digestive tract was carefully excised. After removing the intestinal contents, approximately 5 cm lengths of duodenum, jejunum and ileum were removed for gut morphological measurements. The intestinal samples were flushed with ice-cold saline and immediately placed in Bouin's fluid. The samples were then transferred into 70% ethanol after 24 h. Each sample was then sectioned at a thickness of 6µm, stained with alcian blue/haematoxylin and eosin, and examined by light microscopy and analyzed for villus height, crypt depth, goblet cell number and epithelium thickness.

Fresh digesta were obtained from the chick's jejunum and centrifuged at 17,500×*g* for 3 min, and the viscosity of the supernatant (0.5 mL), expressed as centipoises (cP), was immediately measured with a Brookfield digital viscometer.

Table 1. Composition (%) and calculated analysis of the basal diet.

Ingredient	Starter (0-14 d)	Grower (15-28 d)	Finisher (29-42 d)
Corn	56.9	58.41	61.08
Soybean Meal (42% CP)	34	33	32
Soybean Oil	2.1	2.7	3.2
Fish Meal	3	2	0
Dicalcium Phosphate (18% P, 25% Ca)	1.60	1.66	1.72
Sodium Chloride	0.3	0.2	0.2
Vitamin Premix[1]	0.25	0.25	0.25
Mineral Premix[2]	0.25	0.25	0.25
DL- Methionine	0.32	0.29	0.16
L- Lysine	0.17	0.14	0.05
Oyster Shell	1.10	1.10	1.09
Calculated Analysis			
AME, (Kcal/kg)	2950	3000	3050
Crude Protein (%)	21.73	20.5	18
Lysine (%)	1.35	1.20	1.09
Methionine+Cysyeine (%)	0.98	0.53	0.49
Calcium (%)	0.99	0.95	0.90
Available Phosphorous (%)	0.50	0.45	0.46

[1,2] Vitamin and mineral premix was added at 0.025% to provide the following nutrients per kilogram of diet: 12000 IU of vitamin A, 5000 IU of vitamin D_3, 3 mg of vitamin K, 50 IU of vitamin E, 2 mg of B_1, 5 mg B_6, 7 mg B_2,15 mg of B_{12}, 200 mg of biotin,15 mg of B_3, 50 mg of B_5, 1 mg of folic acid, 10 mg Cu, 100 mg of Mn, 80 mg of Fe, 0.5 mg of Co, 1 mg of I, 0.2 mg of Se, 80 mg of Zn, and 0.5 mg of Mo.

Results and discussion

The effects of *β*-mannanase on the measured parameters of different sections of the small intestine of birds fed on a corn-soy diet are shown in *table 2*. *β*-mannanase inclusion at 700 g/ton significantly ($p<0.01$) increased the duodenal villus height, and crypt depth and decreased epithelial thickness, goblet cell numbers and ratio of crypt depth to villus height. Crypt depth and goblet cell numbers in the villi of the ileum were affected ($P<0.01$) by the *β*-mannanase supplementation at 700 g/ton. *β*-mannanase supplementation at 900 g/ton induced improvements ($P<0.01$) in villus height, goblet cell numbers, crypt

depth and epithelial thickness in different sections of small intestine, in except of, villus height and epithelial thickness in ileum. Silva and Smithard (2002) have demonstrated that supplementing diets based on rye with enzyme decrease crypt cell proliferation rate.

Table 2. Effects of different levels of β-mannanase on villus height, epithelial thickness, goblet cell number (per 100 villus height) and the ratio of crypt depth to villus height in different sections of the small intestine of birds fed corn-soybean diet[1]

	β-mannanase *content (g/ton)*				
	0	**500**	**700**	**900**	**SEM**
Duodenum					
Villus height	1756.20^{b}	1783.75^{b}	2018.30^{a}	2032.55^{a}	7.7887
Epithelial thickness	48.30^{a}	48.10^{a}	43.95^{b}	41.15^{b}	1.0129
Goblet cell number	9.45^{a}	8.50^{ab}	7.20^{b}	5.55^{c}	0.3480
Crypt depth	147.70^{b}	146.90^{b}	154.35^{a}	156.25^{a}	1.1128
Ratio[2]	0.084^{a}	0.082^{a}	0.076^{b}	0.077^{b}	0.0007
Jejunum					
Villus height	847.80^{b}	845.05^{b}	852.25^{b}	899.10^{a}	3.976
Epithelial thickness	38.70^{a}	37.70^{ab}	37.50^{ab}	35.55^{b}	0.67879
Goblet cell number	10.25^{a}	9.60^{a}	8.85^{ab}	7.75^{b}	0.37944
Crypt depth	119.75^{b}	121.85^{ab}	123.60^{a}	124.10^{a}	1.0916
Ratio	0.141^{ab}	0.144^{a}	0.145^{a}	0.138^{b}	0.0013
Ileum					
Villus height	797.15	809.15	811.65	820.20	8.0762
Epithelial thickness	34.60	33.95	33.40	32.05	1.1747
Goblet cell number	10.00^{a}	9.70^{a}	8.10^{b}	7.65^{b}	0.31886
Crypt depth	105.85^{b}	106.45^{b}	113.10^{a}	116.50^{a}	1.988
Ratio	0.133^{ab}	0.132^{b}	0.140^{ab}	0.142^{a}	0.0028
Viscosity (cP)	2.13^{a}	2.07^{ab}	1.96^{b}	1.90^{b}	0.0310

[a, b,] Means in row not sharing a common superscript differ (P<0.01).
[1,] Values are means of 20 observations. [2,] Ratio of crypt depth to villus height.

Increased numbers of goblet cells were observed in the villi of the different sections of small intestine of birds given the unsupplemented basal diet. The goblet cell number were, however, reduced (P<0.01) by *β*-mannanase addition at 700 or 900 g/ton in the different sections of small intestine. Reduced goblet cell numbers may be expected to lower mucin production and endogenous protein losses. Epithelium thickness in the duodenum and jejunum affected by enzyme supplementation. Johnson et al 1981, has been suggested that the absorption of nutrients may be impeded by an increase in the thickness of the unstirred layer in the small intestine (Silva & Smithard, 2002). It may be concluded that the anti nutritive effect of NSP is related to their ability to increase digesta viscosity which in turn causes changes in gut morphology and in the efficiency of nutrient utilization by the chicken.

The addition of *β*-mannanase had no effect on body weight, feed: gain ratio and feed intake (*table 3)*. *β*-mannanase inclusion at 900 g/ton significantly reduced (P<0.05) feed intake in finisher and overall periods. Several reasons have been proposed to explain the beneficial effects of *β*-mannanase in reducing feed intake: i) breakdown of *β*-mannan in the cell wall matrix and release of encapsulated nutrients. ii) increased villus height in the duodenum and jejunum and therefore in the surface area and increased in nutrient absorption. iii) decreased digesta viscosity. The viscous nature of *β*-mannan is a factor contributing to the reduction of absorption and utilization of nutrients. This occurs by slowing the gastric emptying, impairing the mixing of substrate with digestive enzymes and reducing the rate of contact of nutrients with the absorptive epithelium (read 1986).

The viscosity of digesta from the jejunum of birds was lowered (P<0.01) by the addition of *β*-mannanase at 700 or 900 g/ton to the control group *(table 2)*. Transport of nutrients, the interaction of enzymes with their substrates and diffusion of the digested products are all processes that might be impeded by that the high viscosity gels within the lumen of the gut, and there have been many studies that have shed light on the roles that these factors might play. Diffusion is a major component of the processes involved in the digestion and absorption of nutrient in the small intestine. The rate of diffusion decreases

as the viscosity of solution increases (Fengler & Marquard, 1988). NSP have an impact on gastro intestinal tract viscosity and digesta and absorption of nutrients (Annison *et al,* 1995).

Table 3. Effect of varying levels of *β*-mannanase inclusion on broiler performance[1]

	Enzyme, g/ton				
	0	**500**	**700**	**900**	**SEM[2]**
Starter					
Body Gain	404.77	409.35	422.67	411.10	6.14
Feed Intake	329.83	333.48	338.60	323.83	21.56
Feed: Gain Ratio[3]	0.814	0.814	0.799	0.788	0.045
Grower					
Body Gain	775.58	763.75	773.62	775.33	20.15
Feed Intake	1280.62	1266.67	1289.29	1266.67	12.37
Feed: Gain Ratio	1.65	1.66	1.67	1.64	0.037
Finisher					
Body Gain	1213.05	1164.05	1136.67	1140.21	24.96
Feed Intake	2513.10[a]	2432.60[ab]	2462.25[ab]	2373.80[b]	27.43
Feed: Gain Ratio	2.07	2.09	2.20	2.09	0.049
Overall					
Body Gain	2393.40	2337.18	2334.20	2326.65	20.69
Feed Intake	4123.55[a]	4032.75[ab]	4090.10[a]	3964.28[b]	35.69
Feed: Gain Ratio	1.72	1.72	1.76	1.70	0.019
Mortality	3.33	0	1.67	0	

[a-b] Rows without a common subscript differ significantly (P<0.05). [1] Values are the means of 4 replicates (15 birds each).
[2] Pooled standard error of means. [3] Feed: gain ratio values corrected for mortality.

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