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*Full Length Research Paper*

# Effects of β-Mannanase on broiler performance, gut morphology and immune system

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**An experiment was designed to assess the effects of graded levels of *β*-mannanase on performance, gut morphology and some blood proteins and leucocytes of broilers provided with diets based on corn and soybean meal. Broiler chickens are divided four group and supplied diet which contains 0, 500, 700, or 900 g/ton β-mannanase. Each treatment contained 4 pen with 15 birds/pen. 900 g/ton β-mannanase supplementation significantly reduced feed intake but did not influenced body weight gain and feed conversion ratio in both finisher and total period. 900 g/ton β-mannanase supplementation 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, suggesting that β-mannanase improves gut morphology in broiler chickens. The addition of *β*-mannanase at 700 and 900 g/ton to the diets significantly (P < 0.05) reduced jejunal viscosity compared with the control group. β-mannanase did not influence the blood serum proteins (albumin, alpha 1-, alpha 2-, beta and gamma-globulins) eosinophils and monocytes, but the addition β-mannanase increased lymphocyte, and decreased heterophil and heterophil: lymphocyte (H:L) ratio (p < 0.05). Thus it improved chickens immune system.**

**Key words:** Broiler, leucocytes, viscosity, serum proteins.

## INTRODUCTION

Soybean meal (SBM) is the conventional and relatively inexpensive protein source in broiler diets, but it contains a number of antinutritional factors. The identification and alleviation of factors inhibiting nutrient utilization are necessary for successful poultry production. Among potential factors reducing nutrient bioavailability are the nonstarch polysaccharides (NSP). NSPs are complex high molecular weight carbohydrates found in the structure of plant cell walls (Annison and Choct, 1991). SBM contains approximately 22.7% carbohydrates in the form of nonstarch polysaccharides (Chesson, 1987). Among NSP, mannans occur in the forms of glucomannan, galactomannan, glucogalactomannan and glucurono-mannans in plant cell walls (Aman and Graham, 1990). *β*-Mannans in SBM are linear polysaccharides composed of repeating *β*-1-4 mannose and α-1-6 galactose and glucose units attached to the *β*-mannans backbone. The *β*-mannans content of soybean meal is approximately 1.3 to 1.6% (Jackson et al., 1999).

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). The inclusion rate of 2 to 4% in feed severely retards growth and decreases feed efficiency in broilers (Couch et al., 1967; Ray et al., 1982; Verma and McNab, 1982).

NSP had the negative effects on the microscopic structure of gut, but published data on this aspect are

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limited and controversial (Wu et al., 2004). Viveros et al. (1994) reported that the jejunum of birds fed a diet containing 60% barley showed shortening, thickening and atrophy of the villi and an increased number of goblet cells compared with those on a corn-soybean diet.

Hemicell® is a fermentation product of *Bacillus lentus*. It contains high amounts of *β* -mannanase that degrade *β*-mannan in feed.

An important mode of action of *β*-mannanase is a reduction in innate immune stimulation associated with a reduction in the *β*-mannan content of substrate entering the intestinal tract (Jackson et al., 2004). *β*-Mannanase crossing the intestinal mucosa are potent stimulators of the innate immune system, resulting in increased proliferation of macrophages and monocytes and resultant cytokine production. These result in exacerbated disease symptoms and reduced nutrient use, which has been observed using galactomannans derived from fungi (Ross et al., 2002).

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. In a study with broiler chickens, Daskiran et al. (2004) reported that dietary *β*-mannanase inclusion significantly reduced water: feed ratio and total dry fecal output ($P < 0.01$) and improve the utilization of nutrients in diets containing *β*-mannan. 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, gut morphology and reduction in innate immune stimulation.

## MATERIALS AND METHODS

A total of 240 1-day-old Cobb broilers were used in this experiment. The chickens were allocated at random to the four groups. The experiment was carried out in 16 pens (2 × 1.5 m) with 15 chickens per pen. Starter, grower and finisher diets were offered to the birds from 0 to 14, 15 to 28 and 29 to 42 days of age, respectively. The basal diet was based on corn and soybean meal (Table 1). This diet was prepared according to the guideline of rearing Cobb broiler. Chickens fed diets contained 0 (as a control), 500, 700 or 900 g Hemicell/ton.

Room temperature was maintained at 32°C during the first week and gradually decreased to 24°C by the end of the third week. 24 h of lighting was provided throughout the trial. Body weights and feed intake were recorded on a pen basis at starter, grower and finisher periods. Feed and water were offered *ad libitum* at all times during the 42-day trial period. Mortality was recorded daily.

### Collection of intestinal tissue samples

On day 42, two birds from each pen (closest to the mean pen body weight) were selected and weighed. The birds were sacrificed by cervical dislocation and the digestive tract was carefully excised. After removing the intestinal contents, approximately 5 cm lengths of duodenum (mid point of the pancreatic loop), jejunum (mid point of jejunum) and ileum (5 cm after Meckel's diverticulum) were removed for gut morphological measurements. Intestinal samples from each section were immersed in formaldehyde, before fixation in Bouin's solution and paraffin embedding. The samples were then transferred into 70% ethanol after 24 h.

### Leukocyte differential count

Blood samples were collected from brachial vein of four broilers from each treatment at day 35. Blood smears were stained using May-Grunwald and Giemsa stain approximately 4 h after preparation with methyl alcohol fixation. Leucocyte cells (heterophils (H), lymphocytes (L), eosinophils and monocytes) were counted by the same person to 60 cells per individual smear, and the H: L ratios were calculated (Gross and Siegel, 1986).

### Cellulose acetate electrophoresis of plasma protein

The blood samples were taken by puncturing the wing vein at 35 days of age. The concentration of proteins was defined by spectrophotometry in the serum. The fractions of albumin, alpha 1-, alpha 2-, beta and gamma-globulins were defined by cellulose acetate electrophoresis, and their absolute concentrations were calculated using the total protein concentration.

### Intestinal viscosity

Fresh digesta were obtained from the chick's jejunum. Two Eppendorf tubes were filled with 1.5 g of sample 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.

### Histology

Histological examinations were carried out according to the method of Iji et al. (2001). Intestinal samples from each section were immersed in formaldehyde, before fixation in Bouin's solution and paraffin embedding. Paraffin sections at 6 µm thickness were made from each sample, stained with haematoxylin and eosin, and examined by light microscopy. Villus height, crypt depth, goblet cell number and epithelial thickness were analyzed from each preparation. The length of the intestinal villi and the depth of the intestinal crypt were measured with linear scaled graticule. The number of goblet cells/$\mu m^2$ area of the villus and crypts was measured by 25 squared graticule.

### Experimental design

The experimental design was completely randomized with four treatments. For gut morphology, individual birds were considered as the experimental unit. The data were analyzed using the General Linear Model procedure of SAS to determine treatment effects (SAS, 1991). Significant mean differences were determined using a least significant difference test.

## RESULTS

The results for body weight, feed intake, feed: gain ratio and mortality are presented in Table 2. Effects of *β*-

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

| Ingredient | Starter (0 - 14 days) | Grower (15 - 28 days) | Finisher (29 - 42 days) |
|---|---|---|---|
| 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 |
| DCP (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 vitamin $B_1$, 5 mg vitamin $B_6$, 7 mg vitamin $B_2$,15 mg of vitamin $B_{12}$, 200 mg of biotin,15 mg of pantothenic acid, 50 mg of nicotinic acid, 1 mg of folic acid, 10 mg copper,100 mg of manganese, 80 mg of iron, 0.5 mg of cobalt, 1 mg of iodine, 0.2 mg of selenium, 80 mg of zinc, and 0.5 mg of molybdenum.

**Table 2.** Effect of varying levels of $\beta$-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.

**Table 3.** 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 (μm) | 1756.20$^{b}$ | 1783.75$^{b}$ | 2018.30$^{a}$ | 2032.55$^{a}$ | 7.7887 |
| Epithelial thickness (μm) | 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 (μm) | 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 (μm) | 847.80$^{b}$ | 845.05$^{b}$ | 852.25$^{b}$ | 899.10$^{a}$ | 3.976 |
| Epithelial thickness (μm) | 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 (μm) | 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 (μm) | 797.15 | 809.15 | 811.65 | 820.20 | 8.0762 |
| Epithelial thickness (μm) | 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 (μm) | 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.

mannanase on body weight, feed: gain ratio and feed intake in starter and grower period was not significant. Addition of enzyme had no effect on body weight and feed: gain ratio in finisher period, whereas enzyme supplementation significantly reduced (P < 0.05) feed intake in compare with control group. In the overall period, body weight and feed: gain ratio were not affected by enzyme inclusion although feed intake significantly decreased (P < 0.05). *β*-Mannanase inclusion at 900 g/ton significantly reduced (P < 0.05) feed intake. The effect of enzyme on mortality was not significant. The effects of *β*-mannanase on the villus height, goblet cell numbers, epithelial thickness, crypt depth and the ratio of crypt depth to villus height of different sections of the small intestine in compare with control group shown in Table 3. Villi in the duodenum were lengthened and thinned in 700 g/ton β-Mannanase group (Figures 1 and 2). Villus height, epithelial thickness, goblet cell numbers, crypt depth and the ratio of crypt depth to villus height were no significant different between 0 or 500 g/ton treatments in the duodenum, jejunum and ileum. But *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. *β*-Mannanase inclusion at 500 or 700 g/ton had no effects on villus height, epithelial thickness, goblet cell numbers and the ratio of crypt depth to villus height in the jejunum, whereas, inclusion at 700 g/ton significantly increased (P < 0.01) crypt depth in compare with control group. There were no significant differences between 500 or 700 g/ton treatments. 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, whereas villus height and epithelial thickness were not affected. *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. The only exception was that 900 g/ton treatment had no effect on villus height and epithelial thickness in ileum. The ratio of crypt depth to villus height in duodenum of birds fed *β*-mannanase supplementation at 700 or 900 g/ton was lower (P < 0.01) than those in birds fed on diets with *β*-mannanase

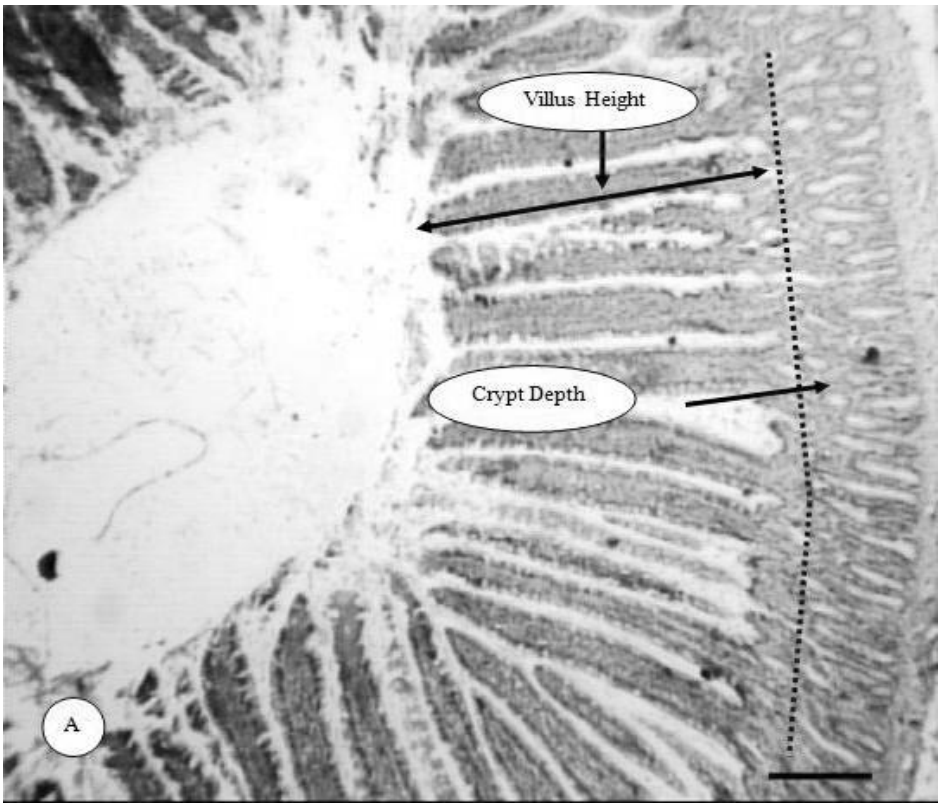


**Figure 1.** Section of duodenal tissue of broiler chicken. A: Control bird with basal diet. Hematoxilin and Eosin staining. Scale bar represent 500 µm.

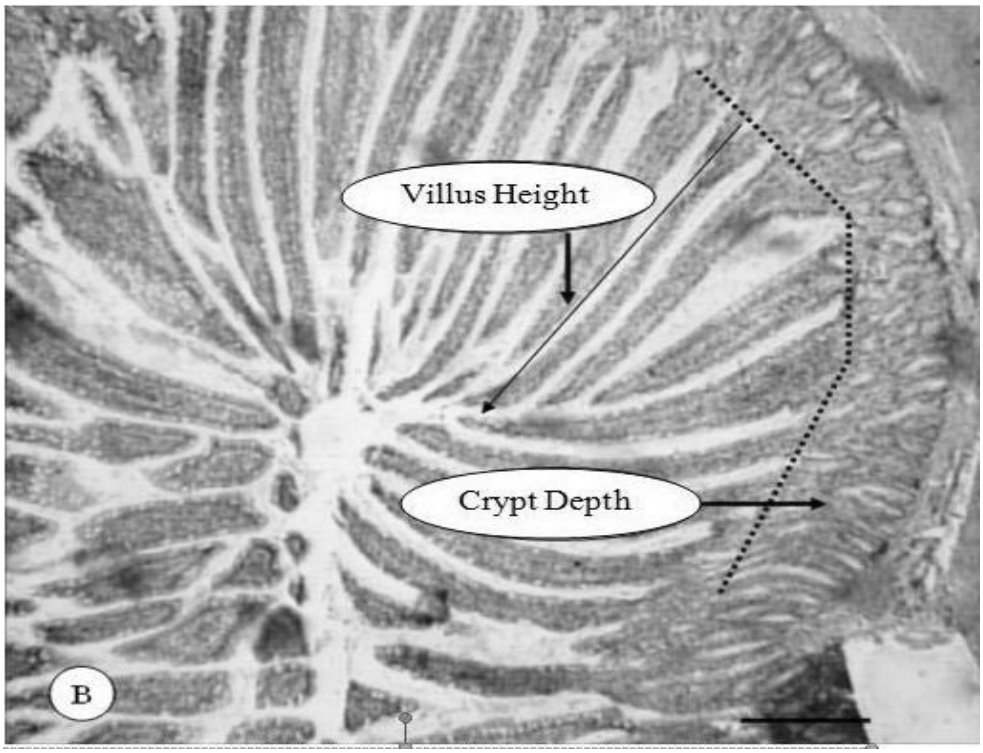


**Figure 2.** Bird from the treated group with β-mannanase (700 g/ton). Section of a duodenal tissue of broiler chicken. Scale bar represents 500 µm.

**Table 4.** Effect of varying levels of β-mannanase inclusion on blood serum proteins.

| | Enzyme, g/ton | | | | |
|---|---|---|---|---|---|
| | 0 | 500 | 700 | 900 | SEM[1] |
| Albumin | 44.42 | 46.30 | 43.30 | 45.52 | 2.16 |
| α 1-globulins | 3.65 | 3.52 | 2.93 | 3.15 | 0.85 |
| α 2-globulins | 4.5 | 4.27 | 4.55 | 5.05 | 0.42 |
| β-globulins | 14.85 | 15.25 | 14.65 | 15.05 | 1.63 |
| γ-globulins | 33.58 | 31.68 | 34.57 | 31.23 | 3.20 |

[1]Pooled standard error of means.

**Table 5.** Effect of varying levels of β-mannanase inclusion on leucocyte cells and heterophils: lymphocytes ratio.

| | Enzyme, g/ton | | | | |
|---|---|---|---|---|---|
| | 0 | 500 | 700 | 900 | SEM[1] |
| % heterophil | 41.250[a] | 31.286[b] | 34.375[ab] | 30.000[b] | 2.57 |
| % lymphocyte | 54.375[b] | 64.571[a] | 61.000[ab] | 65.375[a] | 2.68 |
| % monocyte | 2.500 | 2.286 | 2.625 | 2.375 | 0.182 |
| % eosinophil | 1.875 | 1.857 | 2.000 | 2.000 | 0.252 |
| H: L ratio[2] | 0.807[a] | 0.497[b] | 0.584[ab] | 0.472[b] | 0.078 |

[a - b] Rows without a common subscript differ significantly (P < 0.05).
[1] Pooled standard error of means.
[2] heterophils: lymphocytes ratio.

inclusion at 0 or 500 g/ton. There were no affects by β-mannanase supplementation in jejunum and ileum.

The effects of enzyme supplementation on the jejunal viscosity of digesta are shown in Table 3. 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.

As shown in Table 4, β-mannanase did not influence the blood serum proteins (albumin, alpha 1-, alpha 2-, beta and gamma-globulins). β-mannanase did not influence eosinophils and monocytes, increased lymphocyte, and decreased heterophil and H:L ratio (p < 0.05, Table 5).

## DISCUSSION

The objective of the present study was to examine the influence of β-mannanase on performance, digesta viscosity, and gut morphology in broilers fed corn-soy diet. Result of this study demonstrates an advantage with the use of β-mannanase at 900 g/ton on broiler performance with respect to decrease in feed intake in finisher period and total period (P < 0.05, Table 2). Several reasons have been proposed to explain the beneficial effects of β-mannanase in reducing feed intake: improvement of nutrients absorption by i) The release of encapsulated nutrients by breakdown in the cell wall matrix. ii) The decrease of digesta viscosity. iii) The increase of villus height in the duodenum and jejunum. The viscous nature of β-mannan is a factor contributing to the reduction of nutrients absorption. 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). Transport of nutrients, the interaction of enzymes with their substrates and diffusion of the digested products are all processes that might be impeded by 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. Since β-mannanase decreases the viscosity of digesta, probably it can increase the rate of diffusion. 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 and Marquard, 1988). NSP have an impact on gastro intestinal tract viscosity and digesta and absorption of nutrients (Ikegami et al., 1990; Salih et al., 1991; Annison et al., 1995).

Viscous polysaccharides also cause physiological and morphological changes to the digestive system in various species (Brown et al., 1979; Cassidy et al., 1981; Jacob, 1983). In the present study, β-mannanase inclusion at 700 g/ton to corn-soy diet significantly increased (P < 0.01) villus height and crypt depth in the duodenum, and significantly increased (P < 0.01) crypt depth in the jejunum. Enzyme supplement at 900 g/ton significantly increased (P < 0.01) villus height in the duodenum and

jejunum, and significantly increased ($P < 0.01$) crypt depth in the different sections of the small intestine. Across all groups, the villi heights in the duodenum were greater than those in the jejunum and ileum and this is consistent with the significant role that the duodenum plays in nutrient absorption. There is evidence that soluble fermentable polysaccharides with high viscosity stimulate the crypt cell proliferation rate in small intestine in rats (Johnson et al., 1988). Silva and Smithard (2002) have demonstrated that supplementing diets based on rye with enzyme decrease crypt cell proliferation rate. Yaghobfar et al. (2007) reported that the supplementing enzymes to broiler diets improved villus height, crypt depth and reduced goblet cell number of the duodenum and jejunum.

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 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. Silva and Smithard (2002) 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. 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 increase in lymphocyte percent in broilers received β-mannanase enzyme paralleled the decrease of heterophil percent and H: L ratio (Table 5) indicating a reduce of the stress effect by enzyme supplementation. Our findings show the first direct evidence suggesting that β-mannan increased H: L ratios are improved by β-mannanase supplementation.

Most important mode of action of β-mannanase is a reduction in innate immune stimulation associated with a reduction in the β-mannan content of substrate entering the intestinal tract in chickens (Jackson et al. 2004). In a study using rats, Johnson et al. (1988) reported that 10% inclusion guar gum to diets results in drastically increased production of mucosal cells in the large and small intestines as well as increased crypt length and basal width of the villi in rats. The response was not entirely dependent on physical properties of the intestinal chime. Acemannan, a major carbohydrate in the gel of the aloe vera leaf with similar chemical properties to β-mannanase in soybean meal, has large stimulatory effects on the innate immune system in rats. Zhang and Tizzard (1996) demonstrated that acemannan stimulates macrophage and cytokine production and nitric oxide release in murine macrophage cells. These result in exacerbated disease symptoms and reduced nutrient use, which has been observed using galactomannans derived from fungi. Ross et al. (2002), found that β-mannanase crossing the intestinal mucosa are potent stimulators of the innate immune system, resulting in increased proliferation of macrophages and monocytes and resultant cytokine production.

In summary, supplementation of β-mannanase change the quality of soybean meal, which in return improves morphological status in gut and immunological status in plasma in broiler chickens.

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