A dose–response study to evaluate the effects of pH-stable β-mannanase derived from *Trichoderma citrinoviride* on growth performance, nutrient retention, and intestine morphology in broiler chickens

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**ABSTRACT**

The objective of this trial was to determine the optimal supplementation level of β-mannanase preparation enzyme from *Trichoderma citrinoviride* on growth performance, apparent total tract retention of nutrients and gut health of broiler chickens. A total of 600 broiler chickens (Ross 308) were randomly allotted to four treatments on the basis of similar body weight. The dietary treatments included a corn soybean-based diet supplemented with 0 (control), 1200, 2400, or 4800 MNU/kg β-mannanase preparation. The experimental diets were fed in crumbles in phase 1 (1 to 14 days) and pellets in phase 2 (15 to 35 days). The linear and quadratic contrasts were used to compare effects of increasing dietary β-mannanase preparation levels. The overall result indicated a linear increase (*p* < .01) in overall body weight gain and feed conversion ratio. The retention of N, GE, soluble none starch polysaccharide, and insoluble none starch polysaccharide linearly improved in the phase 1 and phase 2 (*p* < .05). A linear response to increasing dietary β-mannanase was demonstrated for the digesta viscosity (*p* < .01). Increasing β-mannanase level in the diets increased the concentration of mannose, galactose, and glucose in ileum and caecum. An increase in villus height of the jejunum with increasing dietary levels of β-mannanase was observed (linear, *p* < .05). In addition, villus height of the duodenum and the ileum tended to increase (linear; *p* = .06) and with increase in dietary β-mannanase. In conclusion, this β-mannanase has potential to improve the growth performance, apparent total tract retention of nutrients and gut health of broiler chickens fed a corn SBM-based diet.

**HIGHLIGHTS**

- Dietary β-mannanase improved the growth performance of broiler chicks by increasing the retention of gross energy, soluble NSP and insoluble NSP.
- The viscosity of digesta in ileum was linearly increased as dietary β-mannanase level increased.
- An increase in villus height of the jejunum was observed with increasing dietary levels of β-mannanase.

**Introduction**

Among all the traditional feedstuffs, corn and soybean meal (SBM) are the most appropriate feed ingredients for broiler chickens. SBM is a popular protein source in the diets of poultry with low none starch polysaccharides (NSP) content, despite their semi-high β-mannan ratio. The β-mannan content of the SBM samples can be ranged from 1.02% to 2.14% based on the amount of hull in the meal (Hsiao et al. 2006). Generally, β-mannan accounts for 15% to 37% of the total NSP content of non-ruminant diets (CVB 1998). The complex molecular structure of β-mannan consist of β-1,4-mannopyranosyl residues or mannose residues (Buckeridge 2010). The low nutrient digestibility and growth performance in monogastrics is due to the lack of proper enzyme to cut the bonds in β-mannan (Kim et al. 2017). The greater growth performance and nutrient digestibility have observed by breaking the bonds in mannans.
by supplementing β-mannanase or enzyme complex to the diet of broiler chickens (Shim et al. 2017b), laying hens (Shim et al. 2017a; Ryu et al. 2017) or pigs (Kim et al. 2017, 2018). Many factors such as type of the substrate, the amount of dietary NSP content and physiological status of animals affect the response of exogenous enzymes on performance. The adverse effects of dietary β-mannan are not only referred to decreased nutrient digestibility, but also introduced potentially as the stimulator of innate immune system with the capability of wasting the energy with non-productive immune responses (Ross et al. 2002).

One of the approaches to improve the nutritional value of diets is dietary exogenous enzyme supplementation to degrade the structure of anti-nutrients (Kim et al. 2018). The supplementation enzymes in to broiler chickens diet has been extensively reviewed and well documented to enhance the growth performance and feed efficiency (Shim et al. 2017b; Kim et al. 2017, 2018). However, the inability of enzymes to tolerate the high temperature of pelleting during feed processing and low pH of stomach may be contributed to the inconsistent reported results in the literatures. Therefore, this study has been developed based on a novel pH-stable and thermostable enzyme. The objectives of this experiment were to evaluate the influence of β-mannanase supplementation in corn-SBM-based diet on growth performance, apparent total tract retention (ATTR) of energy and nutrients, and intestine morphology in broiler chickens.

**Materials and methods**

The protocol for the present experiment was approved by the Institutional Animal Care and Use Committee of Kangwon National University, Republic of Korea.

**Enzyme preparation**

The mannanase (DigeGrain M 500) used in the present study was provided by Advanced Enzyme Technologies Ltd. (Thane, India). The enzyme β-mannanase was produced by the controlled fermentation of *Trichoderma citrinoviride* and contained 9,600,000 U of endo-1,4-β-mannanase/kg. One unit of β-mannanase is defined as the amount of enzyme which liberate 1 μmol of reducing sugar (β-mannanase equivalent) from β-mannan substrate (Locust bean gum) per minute at 5.3 pH and 50 °C.

**Animals and experimental design**

A total of 600 as-hatched broiler chickens (Ross 308, initial body weight: 45.1 ± 0.8 g) were randomly allotted to four treatments on the basis of body weight (10 pens per treatment with 15 broiler chickens in each pen). The dietary treatments included a corn-SBM-based basal diet (control) and control diet supplemented with 1200, 2400, or 4800 MNUU/kg β-mannanase preparation. The experimental diets were offered in two feeding phases, starter and grower, in crumbles from 1 to 14 days and pellets from 15 to 35 days of age. Diets for phase 1 were formulated to contain 3100 kcal/kg ME and 1.15% SID lysine. Diets for phase 2 were formulated to contain 3150 kcal/kg ME and 1.03% SID lysine. All diets met or exceeded the nutrient requirements recommended by Aviagen (2014) (Table 1). The birds were housed in rice hull-covered floor pens. Each pen was provided with a self-feeder and a hanging bell drinker to allow free access to feed and water. The house temperature was maintained at 34 °C for the first 5 days and was then gradually reduced according to normal management practices, until a temperature of 23 °C was achieved. Lighting was provided for 23 hr/d.

**Experimental procedure and sampling**

The birds were individually weighed at the start of the trial, day 14 and on day 35. Feed that was not consumed was weighed at end of experiment and feed intake was calculated. Body weight gain (BWG), average

| Table 1. Ingredient and composition of basal diet, as-fed basis. |
|---------------------------------------------------------------|
| Ingredients | Phase 1 | Phase 2 |
| Corn          | 52.74   | 58.41   |
| Soybean meal  | 37.80   | 31.28   |
| Animal fat    | 4.77    | 4.85    |
| Tricalcium phosphate | 1.84 | 1.66    |
| Rapeseed meal | 1.00    | 2.00    |
| Limestone     | 0.62    | 0.56    |
| ɑ-methionine (84%) | 0.28 | 0.28    |
| L-lysine (55%) | 0.26    | 0.28    |
| Salt          | 0.20    | 0.20    |
| Vitamins*     | 0.15    | 0.15    |
| Mineralsb     | 0.15    | 0.15    |
| l-threonine (78%) | 0.14 | 0.13    |
| Choline chloride (50%) | 0.05 | 0.05    |
| Total         | 100.00  | 100.00  |
| Calculated composition |       |         |
| ME (MJ/kg)    | 12.97   | 13.18   |
| CP (%)        | 21.50   | 19.50   |
| Ca (%)        | 0.87    | 0.79    |
| Av. P (%)     | 0.44    | 0.40    |
| Lys (%)       | 1.15    | 1.03    |
| Met + Cys (%) | 0.87    | 0.80    |
| The (%)       | 0.77    | 0.69    |
| Tryp (%)      | 0.18    | 0.16    |

*aSupplied per kg diet: 10,000 U vitamin A, 4500 U vitamin D₃, 65 mg vitamin E, 1.5 mg vitamin B₁, 12 mg vitamin B₂, 3.2 mg vitamin B₆, 0.011 mg vitamin B₁₂, 3.0 mg vitamin K₃, 60 mg niacin, 0.18 mg biotin, 1.9 mg folic acid, 18 mg ethoxyquin.
bSupplied per kg diet: 20 mg Fe, 16 mg Cu, 110 mg Zn, 120 mg Mn, 1.25 mg I, 0.9 mg Co, 0.3 mg Se.
daily feed intake (ADFI), and feed conversion ratio (FCR) were corrected for the weight of dead birds. Nutrient balance trials were conducted during the end of phase II of the feeding trial to determine the apparent ileal and total retention of dry matter (DM), nitrogen (N), and gross energy (GE). From day 33 onwards, the diets containing 2.5 g/kg chromium as an indigestible marker was given and two birds from each replicate 80 birds (20 birds/treatment and 2 birds/cage) were allocated in individual cages (one bird/cage) to facilitate the collection of excreta samples. At the end of the experiment, 160 birds were slaughtered and ileal and caecal digesta were collected from the distal two-thirds of ileum. Ileal digesta of the selected birds in a replicate were pooled to evaluate the apparent ileal digestibility of nutrients, concentration of monosaccharides, and digesta viscosity. Cecal samples for each cage were pooled to evaluate the concentration of monosaccharides. The digestibility samples were dried in a forced air drying oven at 60°C for 72 h and ground in a Wiley laboratory mill (Thomas Model 4 Wiley® Mill, Thomas scientific, Swedesboro, NJ) using a 1 mm screen. The total nutrient utilisation was calculated as:

Total nutrient utilisation (% of dry matter) = 100 – [100 × (Cr in feed/ Cr in excreta)] × (% nutrient in excreta/ % nutrient in feed)]. The apparent ileal digestibility (AID) was calculated as: nutrient AID (%) = 100 – [100 × (Cr in feed/ Cr in ileum)] × (% nutrient in ileum/ % nutrient in feed).

Indigestibility factor (IF) = markerfeed / markerexcreta

AMEn (MJ/kg) = GEdiet − (GEexcreta × IF) + 8.22 × (Ndiet − (Nexcreta × IF))

Samples of digesta from the ileum and caeca of each of the four birds were collected and stored at -20°C for the analyses of monosaccharides. Two Eppendorf tubes (per replicate) were filled (1.5 g) with ileal digesta and centrifuged (4°C, 3500 g, 10 min) and the viscosity (in centipoises, cP) of the supernatant (1 ml) was measured at 25°C using a digital viscosimeter (Brookfield Digital DV-III Ultra Programmable Rheometer). A subsample of the ileal digesta was dried at 60°C and ground for chemical analyses.

To study the effects of dietary treatments on small intestinal morphology, two samples per replicate were collected from the region of duodenum (2 cm after the gizzard), jejunum (just before Meckel’s diverticulum), and ileum (2 cm before the ileo-caecal transition) and after removal of its contents flushed with physiological saline and submersed in a fixative solution (0.1 M collidine buffer, pH 7.3) containing 3% glutaraldehyde, 2% paraformaldehyde, and 1.5% acrolein and then brought to laboratory to study morphological changes.

**Chemical analyses**

Experimental diets, ileal and excreta samples were analysed in triplicate for dry matter (method 930.15; AOAC 2007), crude protein (method 990.03; AOAC, 2007), ash (method 942.05; AOAC 2007), and calcium and phosphorus (method 985.01; AOAC 2007). The gross energy (GE) of diets and faeces were measured by a bomb calorimeter (Model 1261, Parr Instrument Co., Moline, IL), and chromium concentration was determined with an automated spectrophotometer (Jasco V-650, Jasco Corp., Tokyo, Japan) according to the procedure of Fenton & Fenton (1979). Non-starch polysaccharides of diets were estimated according to the method of Englyst and Cummings (1984).

Monosaccharide content (arabinose, xylose, mannose, galactose, and glucose) of the samples were determined according to Kim et al. (2018). In brief, 10 mg of sample was pre-treated with H2SO4 (72:100 w/w; 125 µL) at room temperature for 45 min. Hydrolysis was performed with 1.35 mL distilled water for 3 h at 100°C. The mixtures were saponified using hydroxylamine (15 M; 320µL), then reduced using 1 mL 2% NaBH4-DMSO solution at 40°C for 90 min. Acetic acid (100 µL, 18 M) was added, and then the mixtures were acetylated using 200 µL 1-methylimidazole followed by 2 mL acetic anhydride and incubated at room temperature for 10 min. The mixtures were quenched with 5 mL distilled water and acetylated sugars were extracted in 1.0 mL dichloromethane. The alditol acetates were extracted with dichloromethane, and the organic phase was analysed by gas liquid chromatography (GLC, Agilent 6890 N, Palo Alto, CA).

**Small intestinal morphology**

Three cross-sections for each intestinal sample (duodenum, jejunum, and ileum) were prepared after staining with azure A and eosin using standard paraffin embedding procedures (Hosseindoust et al. 2017). A total of 10 intact, well-oriented crypt-villus units were selected in triplicate for each intestinal cross-section. Villus height (VH) was measured from the tip of the villi to the villus crypt junction, crypt depth (CD) was defined as the depth of the invagination between adjacent villi and villus width was measured at the
mid of the villus. All morphological measurements (villus height or crypt depth) were made in 10 μm increments by using an image processing and analysis system (Optimus software version 6.5, Media Cybergenetics, North Reading, MA).

Statistical analyses

Data generated in the present study were subjected to statistical analysis using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC) in a randomised complete block design. The linear and quadratic contrasts were used to compare effects of increasing dietary β-mannanase preparation levels. The pen was used as the experimental unit for the analysis of growth performance and nutrient retention. Individual broiler chickens used as experimental unit for analysis of intestinal morphology. Probability values of ≤0.05 were considered significant.

Results

Growth performance

As shown in Table 2, there was a linear increase (p < .01) in BWG and ADFI during the first phase. During the phase 2, there were no significant effects of dietary β-mannanase on BWG, ADFI, and FCR. A linear response to increasing dietary β-mannanase was demonstrated for the overall BWG (p < .01) and FCR (p < .05). However, β-mannanase supplementation did not affect the overall FCR. There was a tendency (linear; p = .075) for a lower feed cost per kg weight gain with increasing dietary levels of β-mannanase.

Nutrient retention and digesta viscosity

Polynomial contrasts analysis showed no significant difference in the ATTR of DM in the first phase, but the retention of N, GE, soluble NSP and insoluble NSP linearly improved in the first phase (p < .05; Table 3). Increasing dietary supplementation of β-mannanase broiler chickens linearly increased (p < .05) the ATTR of DM, GE, soluble NSP and insoluble NSP during phase 2. A linear increase was observed in the value of AMEn with increasing dietary levels of β-mannanase. A linear response to increasing dietary β-mannanase was demonstrated for the digesta viscosity (p < .01).

Monosaccharide concentrations in digesta

Increasing β-mannanase level in the diets had no significant effect on concentration of arabinose and xylose in ileum and caecum (Table 4). A linear increase was observed in concentration of mannose, galactose and glucose in ileum and caecum with increasing dietary levels of β-mannanase.

Morphology

An increase in VH of the jejunum with increasing dietary levels of β-mannanase was observed (linear, p < .05; Table 5). In addition, VH of the duodenum and the ileum tended to increase (linear; p = .06) and with increase in dietary β-mannanase, however, VH was not affected in ileum. Crypt depth and villus height to crypt depth ratio were unaffected by supplementation of β-mannanase in diets.

Discussion

The findings of current study showed that the supplementation of β-mannanase levels to the diet improved the growth performance and FCR in broiler chickens fed a corn SBM-based diet. This result is in agreement with the results of Latham et al. (2018) who indicated

| Item               | β-mannanase (MNUU/kg) | SEM | p  |
|--------------------|-----------------------|-----|----|
|                    | 0  | 1200 | 2400 | 4800 |     |
| 1–21 d             |    |      |      |      |     |
| BWG (g)            | 779 | 792  | 872  | 863  | 13.11| .001 | .425 |
| FI (g)             | 1,087 | 1,064 | 1,173 | 1,150 | 19.06 | .001 | .993 |
| FCR                | 1.40 | 1.36  | 1.35  | 1.33  | 0.04 | .215 | .707 |
| 22–35 d            |    |      |      |      |     |
| BWG (g)            | 1,339 | 1,360 | 1,387 | 1,376 | 28.95 | .293 | .593 |
| FI (g)             | 2,537 | 2,504 | 2,547 | 2,518 | 65.30 | .965 | .970 |
| FCR                | 1.89 | 1.84  | 1.84  | 1.83  | 0.03 | .163 | .501 |
| Overall            |    |      |      |      |     |
| BWG (g)            | 2,119 | 2,152 | 2,258 | 2,240 | 31.35 | .002 | .409 |
| FI (g)             | 3,624 | 3,568 | 3,720 | 3,669 | 65.27 | .335 | .968 |
| FCR                | 1.71 | 1.66  | 1.65  | 1.64  | 0.02 | .033 | .377 |
| Feed cost/kg weight gain | 100.0 | 97.25 | 96.56 | 96.46 | 1.38 | .075 | .343 |

Each mean represents values from 10 replicates (15 birds/replicate).
SEM: Standard error mean; BWG: body weight gain; FI: feed intake; FCR: feed to gain conversion ratio.

Table 2. Effects of broilers fed diets containing graded levels of β-mannanase on growth performance and feed costa.
Table 3. Effects of broilers fed diets containing graded levels of β-mannanase on ileal and total tract apparent digestibility of nutrients and digesta viscosity*.

| Item                        | β-mannanase (MNUU/kg) | 0   | 1200 | 2400 | 4800  | SEM | Linear | Quadratic |
|-----------------------------|-----------------------|-----|------|------|-------|-----|--------|-----------|
| Ileum (g/kg dry matter)     |                       |     |      |      |       |     |        |           |
| Arabinose                   | 3.55                  | 3.68| 3.75 | 3.87 | 0.18  | .198| .978   |           |
| Xylose                      | 4.02                  | 4.01| 4.13 | 4.21 | 0.11  | .190| .691   |           |
| Mannose                     | 0.35                  | 0.36| 0.40 | 0.39 | 0.02  | .016| .643   |           |
| Galactose                   | 6.03                  | 6.49| 6.49 | 6.51 | 0.12  | .014| .079   |           |
| Glucose                     | 3.60                  | 3.96| 3.78 | 4.59 | 0.22  | .003| .744   |           |
| Jejunum (g/kg dry matter)   |                       |     |      |      |       |     |        |           |
| Arabinose                   | 2.40                  | 2.44| 2.63 | 2.59 | 0.15  | .260| .814   |           |
| Xylose                      | 3.90                  | 3.79| 3.97 | 3.92 | 0.08  | .527| .776   |           |
| Mannose                     | 0.30                  | 0.31| 0.37 | 0.36 | 0.02  | .007| .735   |           |
| Galactose                   | 5.74                  | 6.26| 6.04 | 6.16 | 0.11  | .037| .075   |           |
| Glucose                     | 2.50                  | 2.55| 2.62 | 2.68 | 0.06  | .017| .982   |           |

*Each mean represents values from 10 replicates (four birds/replicate). SEM: Standard error mean.

Table 4. Effects of broilers fed diets containing graded levels of β-mannanase on concentrations of monosaccharide in ileum and caecum of broilers*.

| Item                        | β-mannanase (MNUU/kg) | 0   | 1200 | 2400 | 4800  | SEM | Linear | Quadratic |
|-----------------------------|-----------------------|-----|------|------|-------|-----|--------|-----------|
| Ileum (g/kg dry matter)     |                       |     |      |      |       |     |        |           |
| Arabinose                   | 2.40                  | 2.44| 2.63 | 2.59 | 0.15  | .260| .814   |           |
| Xylose                      | 3.90                  | 3.79| 3.97 | 3.92 | 0.08  | .527| .776   |           |
| Mannose                     | 0.30                  | 0.31| 0.37 | 0.36 | 0.02  | .007| .735   |           |
| Galactose                   | 5.74                  | 6.26| 6.04 | 6.16 | 0.11  | .037| .075   |           |
| Glucose                     | 2.50                  | 2.55| 2.62 | 2.68 | 0.06  | .017| .982   |           |

*Each mean represents values from 10 replicates (four birds/replicate). SEM: Standard error mean.

Table 5. Effects of broilers fed diets containing graded levels of β-mannanase on intestinal morphology*.

| Item                        | β-mannanase (MNUU/kg) | 0   | 1200 | 2400 | 4800  | SEM | Linear | Quadratic |
|-----------------------------|-----------------------|-----|------|------|-------|-----|--------|-----------|
| Villus height (μm)          |                       |     |      |      |       |     |        |           |
| Duodenum                    | 638                   | 640 | 673  | 687  | 20.94 | .062| .787   |           |
| Jejunum                     | 774                   | 801 | 831  | 835  | 17.47 | .010| .513   |           |
| Ileum                       | 525                   | 527 | 551  | 562  | 18.96 | .120| .819   |           |
| Crypt depth (μm)            |                       |     |      |      |       |     |        |           |
| Duodenum                    | 423                   | 439 | 427  | 450  | 14.47 | .279| .826   |           |
| Jejunum                     | 468                   | 473 | 503  | 496  | 16.83 | .140| .744   |           |
| Ileum                       | 343                   | 356 | 362  | 355  | 7.49  | .213| .174   |           |
| Villus height: crypt depth  |                       |     |      |      |       |     |        |           |
| Duodenum                    | 1.51                  | 1.46| 1.60 | 1.54 | 0.07  | .456| .956   |           |
| Jejunum                     | 1.66                  | 1.70| 1.68 | 1.71 | 0.06  | .606| .933   |           |
| Ileum                       | 1.54                  | 1.48| 1.53 | 1.59 | 0.07  | .501| .399   |           |

*Each mean represents values from 10 replicates (two birds/replicate). SEM: Standard error mean.

Improvement in body weight gain of broiler chickens fed diets supplemented with increasing levels of β-mannanase. Similarly, it was reported that broiler chickens fed corn SBM-based diets supplemented with multienzymes including β-mannanase had greater FCR and ADG (Shim et al. 2017b; Kim, et al. 2017). Found that the addition of β-mannanase to a corn-SBM-based low energy diet improved ADG of broiler chickens. Contrary to the present results, Klein et al. (2015) reported no improvement in overall ADG and FCR of broiler chickens fed a corn–SBM-based diet containing exogenous enzyme. It is important to note that these contradictions in the reported results might be due to variation in physiological status of the animal, β-mannan contents in the diet, dose of supplemented β-mannanase, and more importantly the stability of exogenous enzymes in high temperature feed processing or low pH of stomach. The β-mannanase in the present study was approved to be highly stable in low pH and high temperature. The major role of dietary β-mannanase is attributed to their contribution in β-mannan degradation. The improved nutrients retention and NSP digestibility in the present study with dietary supplementation of a β-mannanase is suggested to be the reason contributing to the increase of ADG. Moreover, the increased mannan and galactose hydrolysis may increase the accessibility of endogenous enzyme to the nutrients. The enzymatic reaction of β-mannanase may influence the long-chain polysaccharides in the cell walls. It has been well documented that β-mannan is one of the NSPs that is able to increase the viscosity of digesta (Lee et al. 2003). The digesta viscosity is decreased in the current study by supplementation of β-mannanase enzyme. The reduced digesta viscosity is one of the main modes of action of β-mannanase enzyme by which they affect the digesta profile and consequently they increase nutrient utilisation and growth performance (Lee et al. 2003). NSPs are poorly digested by broiler
chickens due to the lack of necessary enzymes to degrade the non-starch fibre content in the diet (Gheisar et al. 2016; Ghayour-Najafabadi et al. 2018). Dietary supplementation of β-mannanase was beneficial for broiler chickens growth performance.

There are two hypotheses for the enzymatic influences of β-mannanase on the retention of nutrients. First, the role of enzyme on the breaking the bonds between the sub-units in NSP to decrease the viscosity of digesta and release the encompassed nutrients in their complex structure (Lee et al. 2003; Kim et al. 2017; Senkoylu et al. 2009). Another reason may be associated to the usage of the released NSP monomers such as arabinose, mannose, galactose, and glucose as nutrients. The present study indicated that broiler chickens fed increasing dietary levels of β-mannanase improved the N and GE retention. This greater nutrient retention may be due to the increased digestibility of both soluble and insoluble NSP. Furthermore, the rate of released ileal and caecal mannose, galactose, and glucose were linearly improved as β-mannanase dose increased in the diet. β-Mannans in composed of repeating mannose (β-1-4) and galactose (α-1-6) and glucose units attached to the β-mannan backbone (Jackson et al. 2004). Therefore, the increased in cleaving rate of β-mannan might have increased the release of galactose and glucose. The observation by Ryu et al. (2017) in laying hens indicated that β-mannanase increased mannose digestibility either in high mannan or in low mannan diets. Similarly, Latham et al. (2018) reported that the supplementation of β-mannanase to the male broiler chickens ration led to an increase in ileal digestible energy regardless of the mannan levels in the diet. Our results concur with data reported by Cowieson and Ravindran (2008), who observed an increase in energy utilisation and permeability of cell wall in a corn-based diet when targeting NSP bonds by the addition of exogenous enzymes in to the broiler chickens diet. They also noted an increased retention of DM, nitrogen and energy. Corn and SBM generally contain relatively lower NSP than most of common feed materials and are highly digestible for broiler chickens. Accordingly, the supplemented enzymes need to be selected on the basis of the type of NSP in the diets. Comparing the result of the present study with the other studies, it may be hypothesised that the supplementation of only β-mannanase may be sufficiently effective to improve the utilisation of nutrients in a corn SBM-based diet other than other dietary exogenous enzymes. This is presumably due to the fact that β-mannan is the most important NSP content of SBM that can include around 2% of the SBM content (Hsiao et al. 2006). Moreover, it is likely that the degradation of β-mannan in the digesta decreased the viscosity, consequently increasing nutrient retention. High NSP content in broiler chickens diets decrease the accessibility by impairing the mixing of digestive enzymes with substrates by increasing viscosity of digesta (Lee et al. 2003; Montagne et al. 2003). Exogenous NSP enzymes in poultry diets has been widely reviewed and well documented to decrease the digesta viscosity and increase nutrient digestibility (Lee et al. 2003; Ryu et al. 2017; Kim et al. 2017). The greater nutrients retention in the present study may be related to improvements of digesta viscosity, effective degradation of nondigestible NSP and greater intestinal villus height.

The high viscose digesta is a factor contributing to the adverse effects on diffusion rate that dramatically decreases the diffusion of digestive enzymes and the flow of solid nutrient through the intestine. The high viscosity directly decreases the digestibility of nutrient due to the restriction on the reaction of digestive enzymes on their substrates. The viscous nature of mannans is associated to the reduction of nutrient utilisation (Lee et al. 2003; Shim et al. 2017b; Kim et al. 2017) by trapping the dietary nutrients in their complex structure. Other studies using broiler chickens (Sharifi et al. 2013) also show that enzyme supplementation has the potential to decrease the viscosity of digesta. Results from this experiment indicated that the β-mannanase supplemented diet decrease digesta viscosity.

In the present study, a linear increased villus height was observed in jejunum with supplementation of β-mannanase. Moreover, villus height in duodenum tended to increase with the addition of dietary β-mannanase. However, no variation was observed in crypt depth. These results are contrary to data reported by Karimi and Zhandi (2015), who observed a greater crypt depth in duodenum, jejumun, and ileum of broiler chickens fed a barley-based diet supplemented with β-mannanase. However, they also reported that the β-mannanase improved villus height in duodenum and ileum, which is in agreement with the result of the present study. A significant improvement in Villus height, crypt depth and VH:CD with dietary β-mannanase supplementation is an indicator of better absorption of nutrients as previously noted in other studies (Choi et al. 2016; Lee et al. 2016). The increase in villus height enhances surface area of epithelium and may indirectly contribute to the rate of nutrients absorption. Current findings indicate that dietary enzymes
decrease crypt depth in duodenum and increase villus height and VH:CD in jejunum and ileum in typical corn-SBM-based diets (Sharifi et al. 2013). There is a correlation between dietary NSP and poor gut health through adverse influences on cell turnover of gut mucosa and epithelial morphology (Montagne et al. 2003). The mannan hydrolyzation and decreased digesta viscosity in the current study may reverse the adverse effects on intestine morphology. In addition, the increased villus height may be an indicator of improved absorption capacity that may have associated to the increased nutrients utilisation.

Conclusions

Overall, data from this experiment show that increasing β-mannanase inclusion effectively yield greater performance by 6.5%, as well as higher ileal retention of N, GE, soluble and insoluble NSP. Dietary supplementation of β-mannanase has potential to improve the villus height in jejunum.

Disclosure statement

No potential conflict of interest was reported by the authors.

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