

β -Mannanase Derived from *Bacillus Subtilis* WL-7 Improves the Performance of Commercial Laying Hens Fed Low or High Mannan-Based Diets

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A trial was conducted to investigate the effects of dietary mannan level and β -mannanase supplementation on egg production performance, nutrient retention and blood metabolites of laying hens. Two hundred and forty Hy-Line Brown layers (52 wk-old) were randomly allotted to 6 treatments on the basis of laying performance. Each treatment had 8 replicates with 5 birds (40 birds per treatment). Laying hens were fed low or high mannan diets containing 0, 0.4 or 0.8 g β -mannanase/kg diet in a 2×3 factorial arrangement during 56 d feeding period. Laying hens fed diets supplemented with high β -mannanase level had greater ($P<0.05$) overall egg production, egg weight, egg mass, retention of gross energy, crude protein and mannan than hens fed the diets without β -mannanase. Laying hens fed diets without β -mannanase or supplemented with high β -mannanase level had greater ($P<0.05$) retention of dry matter than hens fed diets with low β -mannanase level. Moreover, laying hens fed high mannan diets had higher ($P<0.05$) feed intake and feed conversion ratio than that of hens fed low mannan diets. Furthermore, laying hens fed diets supplemented with a high level of β -mannanase had increased serum glucose ($P<0.05$) concentrations but these diets had no effect on total cholesterol, total protein or blood urea nitrogen. The results obtained in the present study indicate that a high mannan content in diets had adverse effect on the performance of laying hens and that dietary supplementation with β -mannanase has the potential to improve laying hen performance and nutrient retention.

Key words: β -mannanase, blood metabolite, egg production, laying hen, mannan, nutrient retention

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Introduction

The poultry feed industry as a whole has been working toward solutions to lower costs up front, and as a result has lowered costs of the end product for consumers. Considering the high fluctuations in the price of main feed ingredients, agro-industrial byproducts like palm kernel meal, rapeseed meal, and distiller dried grains have recently received considerable attention because of their relatively similar nutritional value and lower price compared with soybean-meal (SBM) and maize (O'Doherty and Mckeon, 2000; Fang *et al.*, 2007). However, these low cost byproducts contain non-starch polysaccharides (NSPs), which are considered major anti-nutritional factors for poultry and monogastric animals (Campbell and Bedford, 1992). The common NSPs

include β -mannan, β -glucans, arabinoxylans and fructans (Aman and Graham, 1990; Classen and Bedford, 1991), which increase intestinal viscosity, impair endogenous enzyme action and absorption which leads to poor utilization of nutrients and performance. In order to reduce the levels of NSPs, various exogenous enzymes are added to poultry diets. However, although NSP hydrolyzing enzymes show promise, they tend to be inconsistent in their effects on growth and nutrient utilization, and therefore it is imperative to closely match both the types and amounts of NSPs with appropriate enzymes for beneficial effects (Adeola and Cowieson, 2011). Accordingly, the selection of exogenous enzymes depends upon the type of diet and the NSP levels within diets.

β -mannan accounts for 15%-37% of the total NSP content in non-ruminant diets (CVB, 1998). The backbone of β -mannan comprises β -1, 4-mannopyranosyl residues or mannanose residues substituted by single units of α -1, 6-linked galactoses (Buckeridge *et al.*, 2000). The poultry digestive tract lacks enzymes that target the β -1, 4-mannosyl and α -1, 6-galactosyl bonds, which limits nutrient utilization and

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resulting performance. Ray *et al.* (1982) and Daskiran *et al.* (2004) reported that mannan significantly reduced growth and increased feed conversion ratio in broiler chickens. Previous studies have reported that supplementation of laying hen diets with β -mannanase could improve the egg production performance and nutrient utilization of laying hens (Wu *et al.*, 2005; Zangiabadi and Torki, 2010; Lee *et al.*, 2013). Among the different types of β -mannanase available, bacterial β -mannanase appears to be the best option due to its high production rate, ease of control, and overall lower cost. To the best of our knowledge, however, there have been no reports on the effects of β -mannanase supplementation of high or low mannan diets on the performance of laying hens. Therefore, the objective of the present study was to investigate the effects of dietary mannan level and β -mannanase supplementation on the egg production performance, nutrient retention and blood metabolites of laying hens.

Materials and Methods

The protocol for the experiment was approved and laying hens were cared according to the guidelines of the Institutional Animal Care and Use Committee of Kangwon National University, Chuncheon, Republic of Korea. The exogenous enzyme, β -mannanase (patent 100477456-0000, CTCBIO Inc.) was produced by using *Bacillus subtilis* (WL-7) grown on Luria broth and contained 800,000 U of β -mannanase/kg. One unit of β -mannanase is the amount of enzyme, which liberates 1 μ mol of total reducing sugar (glucose equivalence) per minute at pH 4.0 and 30°C.

Birds, Diets and Management

In total, 240 Hy-Line Brown layers (52 wk-old) were randomly allotted to 6 treatments on the basis of laying performance. Each treatment had 8 replicates with 5 birds each (40 birds per treatment). Laying hens were fed low or high mannan diets with 0, 0.4 and 0.8 g β -mannanase/kg diet in a 2 \times 3 factorial arrangement respectively. All birds were fed isocaloric and isoproteineous diet in mash form for 56 d.

In this experiment, diets were formulated to meet or exceeded the nutrient requirements recommended by NRC (1994). Ingredients and chemical composition of experimental diets are shown in Table 1. Two hens were confined with a cage size 35 \times 35 \times 40 cm and each 10 birds (5 cages) shared a common feed trough between them forming one experimental unit. The birds were provided daily ad libitum feed and clean drinking water during 56 d feeding period. Laying hens were exposed to a 16-h incandescent light period.

Experimental Procedure

The laying hens were weighed at the beginning and on d 28 and 56 of experimental feeding. Feed consumption was recorded weekly and feed conversion ratio (feed intake/egg mass) was calculated during 56 d experimental period. Daily egg production and egg weight per treatment group were recorded to determine hen day egg production and the egg mass production (g/d/hen). To analyze nutrient retention, 2 hens (d 48 onward during the experiment) from each re-

plicate were allocated in individual cage (one bird/ cage), to facilitate collection of excreta. Chromium oxide (Cr₂O₃) was added to the diet at 0.20% as an indigestible marker at the beginning of the 48th day to calculate the digestibility coefficient. The samples for measuring the digestibility were collected 7 days after adding Cr₂O₃ to the diets. Total amount of excreta voided at the end of the collection was weighed and homogenized, followed by representative sampling of excreta (250-g) for analysis. The excreta were then dried using a forced-air drying oven at 60°C and ground in Wiley mill (Thomas Model 4 Wiley Mill, Thomas Scientific, Swedesboro, NJ, USA) using a 1-mm screen and used for chemical analysis.

On the last day of each experiment, a 5 mL blood sample was collected by bronchial wing vein puncture from 2 randomly selected hens in each replicate using sterilized syringes and needles. After 45 min standing at room temperature, serum was isolated by centrifugation (3,000 \times g for 15 min at 4°C), stored at -20°C and later analyzed for concentrations of total cholesterol, triacylglycerides, glucose, total protein, and blood urea nitrogen. To measure the concentration of VFA and ammonia N, fecal samples were collected directly from rectum massage of five randomly selected laying hens in each treatment to minimize the air contact. These samples were immediately sealed in vinyl bags and placed on ice.

Chemical Analysis

Experimental diets and excreta samples were analysed in triplicate for dry matter (DM, method 930.15), crude protein (CP, Method 990.03) and calcium, and phosphorus (method 985.01) according to AOAC (2007). Gross energy (GE) of diets and excreta were measured by a bomb calorimeter (Model 1261, Parr Instrument Co., Molin, IL), and chromium concentrations was determined with an automated spectrophotometer (Jasco V-650, Jasco Corp., Tokyo, Japan) according to the procedure of Fenton and Fenton (1979). The content of β -mannan in feed ingredients, diets and excreta samples was estimated from the total mannose contents according to the method of Englyst and Cummings (1984). In brief, after removing fat, a stock buffer solution was made by adjusting the pH to 5.2 by acetate buffer solution using of α -amylase at 45°C for 16–18 h to hydrolysis of starch. The mannose separated and identified on Rtx-2330 using gas liquid chromatography (HP 6890 Plus; Hewlett Packard, Houston, TX, USA). Commercial kits (Fujifilm Corp., Saitama, Japan) were used for analysis of serum metabolites (total cholesterol, triacylglycerides, glucose, total protein, and blood urea nitrogen) using an automated chemistry analyzer (Fuji Dri-chem 3500i, Fujifilm Corp.).

Statistical Analyses

Data were analyzed as a 2 \times 3 factorial arrangement of treatments in randomized blocks. The main effects of dietary mannan level, β -mannanase supplementation and their interactions were determined by the MIXED procedure of SAS. Probability values of ≤ 0.05 were considered significant.

Table 1. **Ingredients and chemical composition of experimental diets (as-fed basis)**

Item	Low mannan diet	High mannan diet
Ingredients, g/kg		
Corn	510.6	384.3
Wheat	50.0	50.0
Soybean meal (450 g/kg)	202.0	215.0
Rapeseed meal	20.0	8.0
Corn gluten meal	44.0	30.0
Palm kernel meal	0	87.0
Distiller dried grains with solubles	30.0	30.0
Rice bran	30.0	50.0
Animal fat	11.3	45.0
Choline chloride (500 g/kg)	0.5	0.5
L-Lysine (780 g/kg)	1.5	1.2
DL-Methionine (980 g/kg)	1.1	1.3
Limestone	84.0	83.4
Dicalcium phosphate	10	9.3
Salt	2.0	2.0
NaHCO ₃	1.0	1.0
Vitamin premix ^a	1.0	1.0
Mineral premix ^b	1.0	1.0
Analyzed chemical composition		
Gross energy, MJ/kg	17.39	17.63
Crude protein, g/kg	183.4	181.9
Calcium, g/kg	38.9	39.3
Phosphorus, g/kg	5.6	5.4
Mannose, g/kg	10.5	23.3
Calculated chemical composition		
Metabolizable energy, MJ/kg ^c	11.51	11.51
Crude protein, g/kg	180.0	180.0
Calcium g/kg	38	38
Available phosphorus, g/kg	3.6	3.6
Lysine, g/kg ^c	9.3	9.3
Methionine, g/kg ^c	4.3	4.3

^a Supplied per kilogram of diet: 16,000 IU vitamin A, 3,000 IU vitamin D3, 40 IU vitamin E, 5.0 mg vitamin K3, 5.0 mg vitamin B1, 20 mg vitamin B2, 4 mg vitamin B6, 0.08 mg vitamin B12, 40 mg pantothenic acid, 75 mg niacin, 0.15 mg biotin, 0.65 mg folic acid.

^b Supplied per kilogram of diet: 45 mg Fe, 0.25 mg Co, 50 mg Cu, 15 mg Mn, 25 mg Zn, 0.35 mg I, 0.13 mg Se.

^c Values were calculated according to NRC (1994).

Results

Laying Performance

The egg weight of hens fed diets containing a high level of β -mannanase (0.8 g/kg diet) was greater than that of hens fed diets lacking β -mannanase (Table 2). Laying hens fed diets supplemented with β -mannanase had greater overall egg mass and feed conversion ratio than hens fed the diets without β -mannanase supplementation. Egg production also tended to be higher in hens fed β -mannanase-supplemented diets. Moreover, laying hens fed either low mannan diets or β -mannanase-supplemented diets had a greater feed conversion ratio, although a higher feed intake was observed in birds fed high mannan diets. No interactions among the mannan level of diets and β -mannanase supplementation

were observed for any of the measurements.

Nutrient Retention

Laying hens fed diets without β -mannanase or diets supplemented with a high β -mannanase level had greater ($P < 0.05$; Table 3) retention of DM than hens fed diets with a low β -mannanase level. The digestibility of CP in hens fed diets with a high level of β -mannanase was greater ($P < 0.05$) than in hens fed diets without β -mannanase. Moreover, retention of mannose was greater ($P < 0.05$) in laying hens fed diets supplemented with β -mannanase. No interactions among the mannan level of diets and β -mannanase supplementation were observed.

Blood Metabolites

Laying hens fed diets supplemented with high β -mannanase had increased serum glucose concentrations ($P <$

Table 2. Effects of mannan level of diet and β -mannanase supplementation on egg performance in laying hens¹

Items	Mannan levels		β -mannanase			SEM ³	P-value ²		
	Low	High	0	0.4	0.8		M	E	M×E
Overall (0–8 weeks)									
Egg production (%)	72.0	71.6	70.9	72.3	72.3	0.63	0.42	0.06	0.85
Egg weight (g)	65.1	65.0	64.4 ^b	65.3 ^{ab}	65.6 ^a	0.47	0.75	0.03	0.35
Egg mass (g/d)	46.9	46.6	45.6 ^b	47.3 ^a	47.4 ^a	0.46	0.35	<0.01	0.4
Feed intake (g/d)	110.3 ^b	112.8 ^a	112.9	110.8	110.9	1.19	0.02	0.18	0.46
Feed conversion (g feed/g egg)	2.35 ^b	2.43 ^a	2.48 ^a	2.35 ^b	2.34 ^b	0.03	<0.01	<0.01	0.53

¹ Values are mean of 8 replicates of 5 hens each.

² P-values: M: effect of mannan level of diets; E: effect of enzyme (β -mannanase) and M×E: interaction between mannan level of diet and β -mannanase.

³ Standard error of the mean.

Table 3. Effects of mannan level of diet and β -mannanase supplementation on the apparent digestibility (%) of laying hens¹

Items	Mannan levels		β -mannanase			SEM ³	P-value ²		
	Low	High	0	0.4	0.8		M	E	M×E
DM	87.0	84.0	86.2	83.1	87.3	0.46	0.46	0.05	0.74
GE	83.9	83.4	83.0	83.9	84.1	0.56	0.38	0.12	0.88
CP	72.6	72.1	71.2 ^b	72.6 ^{ab}	73.4 ^a	0.54	0.26	<0.01	0.87
Mannose	36.7	36.1	32.4 ^c	35.2 ^b	41.6 ^a	1.17	0.58	<0.01	0.06

¹ Values are mean of 8 replicates of 5 hens each.

² P-values: M: effect of mannan level of diets; E: effect of enzyme (β -mannanase) and M×E: interaction between mannan level of diet and β -mannanase.

³ Standard error of the mean.

Table 4. Effects of mannan level of diet and β -mannanase supplementation on blood metabolites (d 56) of laying hens¹

Items	Mannan levels		β -mannanase			SEM ³	P-value ²		
	Low	High	0	0.4	0.8		M	E	M×E
Total cholesterol (mg/dl)	201	199	201	201	198	10.12	0.79	0.96	0.96
Triacylglycerides (mg/dl)	1204	1203	1,203	1,207	1,201	21.58	0.94	0.95	0.9
Glucose (mg/dl)	251	258	245 ^b	253 ^{ab}	265 ^a	7.28	0.2	0.04	0.98
Blood urea nitrogen (mg/dl)	2.56	2.83	2.69	2.81	2.58	0.16	0.06	0.39	0.49
Total protein (g/dl)	5.95	6.11	6.05	6.05	6.00	0.13	0.13	0.92	0.51

¹ Values are mean of 8 replicates of 5 hens each.

² P-values: M: effect of mannan level of diets; E: effect of enzyme (β -mannanase) and M×E: interaction between mannan level of diet and β -mannanase.

³ Standard error of the mean.

0.05; Table 4) compared to laying hens fed diets without β -mannanase. The mannan level of diets and the β -mannanase supplementation had no effect ($P > 0.05$) on the concentrations of other serum metabolites and no interactions between mannan level of diets and β -mannanase were observed.

Fecal Volatile Fatty Acids and Ammonium

There were no significant differences between β -mannanase and control with regard to acetic acid, propionic acid, butyric acid and $\text{NH}_3\text{-N}$ in feces, although in the β -mannanase group, the mean values were numerically higher ($P =$

0.09) for propionic acid (Table 5).

Discussion

The exogenous enzymes are less consistent in their effects on performance and nutrient utilization, as their beneficial effects depend upon the type of substrate and amount of NSP contained in diets (Adeola and Cowieson, 2011). Accordingly, exogenous enzymes should be selected on the basis of the type and quantity of NSP in poultry diets. In the present study, we initially analyzed the mannan contents of all feed

Table 5. Effects of mannan level of diet and β -mannanase supplementation on fecal volatile fatty acids and ammonium concentrations¹

Items	Mannan levels		β -mannanase			SEM ³	P-value ²		
	Low	High	0	0.4	0.8		M	E	M×E
Acetic acid (μ mol/N)	83.6	83.3	82.7	84.2	83.5	2.59	0.88	0.84	0.9
Propionic acid (μ mol/N)	19.1	19.4	19.2	17.8	20.8	1.17	0.77	0.09	0.13
Butyric acid (μ mol/N)	7.11	7.24	7.27	7.04	7.23	0.23	0.37	0.72	0.87
NH ₃ -N (ppm)	319	320	318	320	321	2.5	0.84	0.62	0.78

¹ Values are mean of 8 replicates of 5 hens each.

² P-values: M: effect of mannan level of diets; E: effect of enzyme (β -mannanase) and M×E: interaction between mannan level of diet and β -mannanase.

³ Standard error of the mean.

ingredients and diets with low or high dietary mannan level were formulated to investigate the effects of the mannan level of diets and β -mannanase supplementation on egg production performance, nutrient retention, and blood metabolites in laying hens. The improved egg production performance of laying hens fed diets supplemented with β -mannanase was observed in the present experiment, which is in agreement with the data reported by Lee *et al.* (2013), who observed the improved egg production performance of laying hens fed maize-soybean or palm kernel meal-based diets that were supplemented with β -mannanase. Similarly, it was reported that dietary supplementation with β -mannanase improved egg production and growth performance in layers and broilers (Wu *et al.*, 2005; Zangiabadi and Torki, 2010). Laying hens fed diets containing a higher level of mannan had a higher feed intake and feed conversion ratio than laying hens fed low mannan diets. However, the inclusion of β -mannanase in laying hen diets, regardless of dietary mannan level, decreased the feed conversion ratio. These observations are consistent with those of Wu *et al.* (2004) who observed that dietary supplementation with β -mannanase improved the feed conversion of laying hens. Similarly, Cheraghi *et al.* (2014) and Daskiran *et al.* (2004) reported that dietary inclusion of β -mannanase improved the feed/gain ratio in broilers. Mannanases improve the nutritive value of maize and soybean meal based diets for broiler chicks as noted by Centeno *et al.* (2006). The improved performance of laying hens fed diets supplemented with β -mannanase observed in these trials might be associated with the greater retention of DM, GE, CP and mannan. Higher levels of NSP in poultry and monogastrics diets can cause an increase in digesta viscosity, which prevents interaction between substrates and digestive enzymes and thus reduces nutrient digestibility (Campbell and Bedford, 1992). Previously, it has been reported that supplementation of high NSP diets with exogenous enzymes could reduce the viscosity of diets and enhanced nutrient utilization in broilers (Lee *et al.*, 2003). In the present study, laying hens fed diets supplemented with β -mannanase had greater retention of mannanose, which might have reduced digesta viscosity and enhanced the retention of DM, GE and CP. Our findings are consistent with those of Kong *et al.* (2011), who observed reduced

intestinal viscosity and greater ileal and total tract digestibility of DM and energy in boiler chickens that were fed diets supplemented with β -mannanase. A recent study conducted in the author's laboratory also reported an improvement in total tract digestibility of DM, GE and mannanose in pigs fed diets supplemented with β -mannanase (Kim *et al.*, 2013). Viscosity reduction has been suggested to be the primary reason for improved performance following dietary supplementation with exogenous enzymes in chickens (Lee *et al.*, 2003). Increased mannanose digestion in the present study might be associated with improvement in energy metabolism. Wu *et al.* (2005) reported improved energy utilization in commercial leghorns fed diets supplemented with β -mannanase. NSPs are able to encompass nutrients within their complex structure, and thereby suppress their absorption in the gastrointestinal tract because of their viscous nature. Additionally, β -mannan also stimulates innate immunity, which could potentially lead to unnecessary energy expenditure and result in less efficient nutrient utilization (Jackson *et al.*, 2003). Another recent study in the author's laboratory also reported that dietary supplementation with β -mannanase provided the equivalent of 0.36 MJ/kg of metabolism energy to the diets of growing pigs. The reduction in digesta viscosity and improved energy utilization may thus be contributory factors in the improved performance and nutrient retention of laying hens fed a diet supplemented with β -mannanase.

In the present study, laying hens fed diets supplemented with β -mannanase showed improvement in their blood glucose concentrations, but β -mannanase supplementation had no effects on total cholesterol, total protein, or blood urea nitrogen. These findings are consistent with those of our previous study (Kim *et al.*, 2013), in which pigs fed diets supplemented with β -mannanase had increased blood glucose concentrations but unaltered blood total cholesterol, total protein, and blood urea nitrogen. The ability of glucomannan to lower blood glucose is related to its viscous and soluble nature (Vuksan *et al.*, 1999). Unhydrolyzed β -mannan in the diet has been shown to interfere with glucose metabolism and insulin secretion rates in swine (Torki and Chegeni, 2007). In the current experiment, dietary supplementation with β -mannanase might have ameliorated the

glucose absorption and energy metabolism of laying hens, which could explain the observed laying performance response to dietary supplementation with β -mannanase.

In conclusion, the results obtained in the present study indicate that β -mannanase has a positive effect on performance and digestibility of nutrients in laying hens, regardless of the amount of mannan in the diet.

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