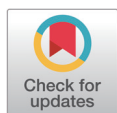


# Effects of varying levels of $\beta$ -mannanase on growth performance, nutrient digestibility, blood metabolites and diarrhea incidence in weaning pigs

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## Abstract

This study was carried out to examine the effects of varying levels of  $\beta$ -mannanase supplementation in corn-soybean meal (SBM)-based diet on growth performance, nutrient digestibility, blood metabolites, and diarrhea incidence of weaning pigs. A total of 160 pigs with an initial body weight (BW) of  $8.66 \pm 0.060$  kg were used in the experiment. Using a randomized complete block (RCB) design, they were assigned to 4 treatments with 5 replicates and 8 pigs per pen, considering sex and initial BW. Treatments for early (0–2 weeks) and late weaning phases (2–5 weeks) were as follows:  $\beta$ -Man0: corn-SBM-based basal diet +  $\beta$ -mannanase 0%;  $\beta$ -Man0.05: basal diet +  $\beta$ -mannanase 0.05%;  $\beta$ -Man0.1: basal diet +  $\beta$ -mannanase 0.1%; and  $\beta$ -Man0.15: basal diet +  $\beta$ -mannanase 0.15%. During the early weaning phase, average daily gain tended to increase when  $\beta$ -mannanase level increased (linear,  $p = 0.07$ ). When  $\beta$ -mannanase level increased in the late weaning phase, the average daily feed intake tended to decrease (linear,  $p = 0.08$ ), and gain to feed ratio (G:F ratio) increased (linear,  $p = 0.02$ ). Throughout the whole experimental period, G:F ratio tended to increase as  $\beta$ -mannanase level increased (linear,  $p = 0.06$ ). According to nutrient digestibility, crude fat digestibility increased when the  $\beta$ -mannanase level increased (linear,  $p = 0.04$ ). Accordingly, the total protein and triglyceride concentration increased as  $\beta$ -mannanase level increased in the early weaning phase (linear,  $p = 0.01$ ;  $p = 0.01$ ). During the entire experimental period, the total cholesterol concentration increased significantly (linear,  $p < 0.01$ ), whereas the high-density lipoprotein cholesterol and low-density lipoprotein cholesterol concentration increased with higher levels of  $\beta$ -mannanase (linear,  $p = 0.02$ ;  $p = 0.02$ ). Lastly, diarrhea incidence showed no significant variation during the early and late weaning phases according to  $\beta$ -mannanase levels. As the level of  $\beta$ -mannanase supplementation in the weaning pig diet increased, growth performance, nutrient digestibility, and blood metabolites showed some positive trends. Therefore, supplementing  $\beta$ -mannanase up to 0.15% in the diet of weaning pigs could enhance their productivity.

**Keywords:**  $\beta$ -Mannanase, Growth performance, Nutrient digestibility, Blood metabolites, Diarrhea incidence, Weaning pigs

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Not applicable.

#### Availability of data and material

Upon reasonable request, the datasets of this study can be available from the corresponding author.

#### Authors' contributions

Conceptualization: Jang M.  
Data curation: Jang M, Park M.  
Formal analysis: Jang M.  
Methodology: Jang M.  
Software: Jang M, Park M.  
Validation: Jang M, Kim YY.  
Investigation: Jang M, Park M.  
Writing - original draft: Jang M.  
Writing - review & editing: Jang M, Park M, Kim YY.

#### Ethics approval and consent to participate

All animal-related experimental procedures were carried out in accordance with the Animal Experimental Guidelines provided by the Seoul National University Institutional Animal Care and Use Committee (SNUACUC; SNU-231203-3).

## INTRODUCTION

Hemicellulose, a major component of plant cell walls, is the second most common polysaccharide found in nature, accounting for 20%–35% of the dry weight of lignocellulosic biomass [1]. The predominant type of hemicellulose varies with plant species, and  $\beta$ -mannan contents are relatively high in the major livestock feed ingredients [2].  $\beta$ -Mannan is a non-starch polysaccharide (NSP) characterized by a straight chain of  $\beta$ -1,4-linked d-mannose units, with variations such as glucomannan and galactomannan, which include additional glucose or galactose [3]. The  $\beta$ -mannan content is 0.29% in corn, 1.20% in soybean meal (SBM), and 0.30% in wheat [4,5]. Although these amounts may seem minimal, monogastric animals lack endogenous enzymes to degrade  $\beta$ -mannan, thereby it functions as an anti-nutritional factor (ANF) [6].  $\beta$ -Mannan can induce several issues, notably reduced growth performance and nutrient digestibility, which are especially problematic in weaning pigs with underdeveloped digestive systems [7,8]. Therefore, the inclusion of  $\beta$ -mannanase, an exogenous enzyme that can degrade  $\beta$ -mannan, in the diet of weaning pigs could mitigate these issues.

$\beta$ -Mannanase is an endo-hydrolase that breaks down  $\beta$ -1,4 glycosidic bonds in the  $\beta$ -mannan main chain randomly, cleaving it into mannose and mannan-oligosaccharides (MOS) [9,10]. Currently, commercial  $\beta$ -mannanase products produced by microbes, such as *Paenibacillus lentus* and *Bacillus lentus* are added to various livestock feeds [11,12]. When added to pig feed, these products exhibit positive effects on growth performance, nutrient digestibility, and immune status [8,13,14]. Weaning pigs, in particular, are expected to benefit significantly from  $\beta$ -mannanase owing to various developmental limitations, such as lower digestive enzyme activity compared with growing-finishing pigs [15]. However, previous studies on weaning pigs have reported inconsistent results, with most studies primary concentrating on digestibility and immunity, and there is a notable shortage of studies on blood metabolites and diarrhea incidence.

Therefore, this study was carried out to examine the effects of varying levels of  $\beta$ -mannanase supplementation in corn-SBM-based diet on growth performance, nutrient digestibility, blood metabolites, and diarrhea incidence of weaning pigs.

## MATERIALS AND METHODS

### Experimental animals

A total of 160 pigs with an initial body weight (BW) of  $8.66 \pm 0.060$  kg were used in the experiment. Using a randomized complete block (RCB) design, they were assigned to 4 treatments with 5 replicates and 8 pigs per pen, considering sex and initial BW. The number of replicates was determined based on similar studies in swine nutrition research, where four to six replications are commonly used to obtain statistically significant results and to minimize the effects of individual variability [8,16]. The pigs were raised in pens, each containing a feed trough and a drinking cup. The temperature was initially set to 30°C during the first week of the experiment and decreased by 1°C each following week. The experiment lasted for 5 weeks, divided into the early weaning phase (0–2 weeks) and the late weaning phase (2–5 weeks).

### Experimental diet

Four experimental diets with different levels of  $\beta$ -mannanase for the early and late weaning phases were as follows:  $\beta$ -Man0: corn-SBM based basal diet +  $\beta$ -mannanase 0%;  $\beta$ -Man0.05: basal diet +  $\beta$ -mannanase 0.05%;  $\beta$ -Man0.1: basal diet +  $\beta$ -mannanase 0.1%; and  $\beta$ -Man0.15: basal diet +  $\beta$ -mannanase 0.15%. CTCZYME, a  $\beta$ -mannanase product (800,000 IU/kg) provided by CTCBIO, was supplemented in diets. All diets were designed to fulfill the nutrient requirements

for weaning pigs as recommended by the National Research Council (NRC) [17]. Tables 1 and 2 provide the formulation and chemical composition of the experimental diets.

### Growth performance

Feed intake was recorded daily, and BW and feed remaining in the troughs were measured at the

**Table 1.** Formulation and chemical composition of the experimental diets for the early weaning phase (0–2 weeks)

Criteria	Treatment <sup>1)</sup>			
	$\beta$ -Man0	$\beta$ -Man0.05	$\beta$ -Man0.1	$\beta$ -Man0.15
Ingredient (%)				
Expanded corn	62.27	62.24	62.22	62.19
Soybean meal	2.76	2.78	2.80	2.82
Fermented soybean meal	7.70	7.70	7.70	7.70
Soy oil	0.59	0.62	0.65	0.68
Whey base	4.19	4.12	4.04	3.97
Lactose base	10.00	10.00	10.00	10.00
Fish meal	5.00	5.00	5.00	5.00
Blood plasma	4.00	4.00	4.00	4.00
L-lysine (50%)	0.65	0.65	0.65	0.65
DL-Methionine (98%)	0.09	0.09	0.09	0.09
L-Threonine (98.5%)	0.07	0.07	0.07	0.07
L-Tryptophan (99%)	0.02	0.02	0.02	0.02
DCP	1.37	1.37	1.37	1.37
Limestone	0.69	0.69	0.69	0.69
Vit. Mix <sup>2)</sup>	0.10	0.10	0.10	0.10
Min. Mix <sup>3)</sup>	0.10	0.10	0.10	0.10
Salt	0.30	0.30	0.30	0.30
Zinc oxide	0.10	0.10	0.10	0.10
$\beta$ -mannanase <sup>4)</sup>	0.00	0.05	0.10	0.15
Total	100.00	100.00	100.00	100.00
Chemical composition <sup>5)</sup>				
ME (kcal/kg)	3,400.00	3,400.00	3,400.00	3,400.00
CP (%)	17.50	17.50	17.50	17.50
Lysine (%)	1.35	1.35	1.35	1.35
Methionine (%)	0.39	0.39	0.39	0.39
Threonine (%)	0.79	0.79	0.79	0.79
Tryptophan (%)	0.22	0.22	0.22	0.22
Total Ca (%)	0.80	0.80	0.80	0.80
Total P (%)	0.65	0.65	0.65	0.65
$\beta$ -mannan <sup>6)</sup> (%)	0.31	0.31	0.31	0.31

<sup>1)</sup> $\beta$ -Man0: corn-SBM based basal diet +  $\beta$ -mannanase 0%;  $\beta$ -Man0.05: basal diet +  $\beta$ -mannanase 0.05%;  $\beta$ -Man0.1: basal diet +  $\beta$ -mannanase 0.1%;  $\beta$ -Man0.15: basal diet +  $\beta$ -mannanase 0.15%.

<sup>2)</sup>Supplied at the following levels per kilogram of diet: vitamin A, 8,000 IU; vitamin D3, 1,600IU; vitamin E, 32IU; d-biotin, 64g; riboflavin, 3.2mg; calcium pantothenic acid, 8mg; niacin, 16mg; vitamin B12, 12g; vitamin K, 2.4mg.

<sup>3)</sup>Supplied at the following levels per kilogram of diet: Se, 0.1mg; I, 0.3mg; Mn, 24.8mg; CuSO<sub>4</sub>, 54.1mg; Fe, 127.3 mg; Zn, 84.7mg; Co, 0.3mg.

<sup>4)</sup>CTCZYME,  $\beta$ -mannanase (800,000 IU/kg), provided from CTC Bio.

<sup>5)</sup>Calculated values.

<sup>6)</sup>The  $\beta$ -mannan content in the diet was calculated based on the methods described by Kwon and Kim, and Kiarie et al. [4,5].

CP, crude protein; DCP, dicalcium phosphate; ME, metabolizable energy.

**Table 2.** Formulation and chemical composition of the experimental diets for the late weaning phase (2–5 weeks)

Criteria	Treatment <sup>1)</sup>			
	$\beta$ -Man0	$\beta$ -Man0.05	$\beta$ -Man0.1	$\beta$ -Man0.15
Ingredient (%)				
Expanded corn	71.63	71.71	71.80	71.88
Soybean meal	2.19	2.23	2.26	2.30
Fermented soybean meal	6.00	6.00	6.00	6.00
Wheat bran	1.25	1.09	0.93	0.77
Whey base	3.10	3.09	3.08	3.07
Lactose base	5.00	5.00	5.00	5.00
Fish meal	4.00	4.00	4.00	4.00
Blood plasma	3.50	3.50	3.50	3.50
L-lysine (50%)	0.68	0.68	0.68	0.68
DL-Methionine (98%)	0.08	0.08	0.08	0.08
L-Threonine (98.5%)	0.08	0.08	0.08	0.08
L-Tryptophan (99%)	0.02	0.02	0.02	0.02
DCP	1.23	1.23	1.23	1.23
Limestone	0.64	0.64	0.64	0.64
Vit. Mix <sup>2)</sup>	0.10	0.10	0.10	0.10
Min. Mix <sup>3)</sup>	0.10	0.10	0.10	0.10
Salt	0.30	0.30	0.30	0.30
Zinc oxide	0.10	0.10	0.10	0.10
$\beta$ -mannanase <sup>4)</sup>	0.00	0.05	0.10	0.15
Total	100.00	100.00	100.00	100.00
Chemical composition <sup>5)</sup>				
ME (kcal/kg)	3,350.00	3,350.00	3,350.00	3,350.00
CP (%)	16.00	16.00	16.00	16.00
Lysine (%)	1.23	1.23	1.23	1.23
Methionine (%)	0.36	0.36	0.36	0.36
Threonine (%)	0.73	0.73	0.73	0.73
Tryptophan (%)	0.20	0.20	0.20	0.20
Total Ca (%)	0.70	0.70	0.70	0.70
Total P (%)	0.60	0.60	0.60	0.60
$\beta$ -mannan <sup>6)</sup> (%)	0.31	0.31	0.31	0.31

<sup>1)</sup> $\beta$ -Man0: corn-SBM based basal diet +  $\beta$ -mannanase 0%;  $\beta$ -Man0.05: basal diet +  $\beta$ -mannanase 0.05%;  $\beta$ -Man0.1: basal diet +  $\beta$ -mannanase 0.1%;  $\beta$ -Man0.15: basal diet +  $\beta$ -mannanase 0.15%.

<sup>2)</sup>Supplied at the following levels per kilogram of diet: vitamin A, 8,000 IU; vitamin D3, 1,600 IU; vitamin E, 32 IU; d-biotin, 64 g; riboflavin, 3.2 mg; calcium pantothenic acid, 8 mg; niacin, 16 mg; vitamin B12, 12 g; vitamin K, 2.4 mg.

<sup>3)</sup>Supplied at the following levels per kilogram of diet: Se, 0.1 mg; I, 0.3 mg; Mn, 24.8 mg; CuSO<sub>4</sub>, 54.1 mg; Fe, 127.3 mg; Zn, 84.7 mg; Co, 0.3 mg.

<sup>4)</sup>CTCZYME,  $\beta$ -mannanase (800,000 IU/kg), provided from CTC Bio.

<sup>5)</sup>Calculated values.

<sup>6)</sup>The  $\beta$ -mannan content in the diet was calculated based on the methods described by Kwon and Kim, and Kiarie et al. [4,5].

CP, crude protein; DCP, dicalcium phosphate; ME, metabolizable energy.

end of each phase (weeks 2 and 5). These data were used to calculate average daily gain (ADG), average daily feed intake (ADFI), and the gain to feed (G:F) ratio.

### Blood sampling and analysis

Blood samples were obtained from five selected pigs per treatment, which were representative of the average BW within each treatment, on the initial day and at the end of each phase. Blood was collected from the jugular vein of the pigs after 3 h fast and placed in serum tubes (SST II Advance; BD Vacutainer, Becton Dickinson). After allowing the samples to clot at room temperature for 30 min, they were centrifuged at 1,811×g for 15 min at 4°C (centrifuge 5810R, Eppendorf). Subsequently, the sera were separated and transferred into microtubes (Axygen), then kept at -20°C for the analysis of glucose, blood urea nitrogen (BUN), total protein, triglyceride, total cholesterol, high-density lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL) cholesterol.

### Diarrhea incidence

Diarrhea incidence was monitored daily at 8 AM by a trained researcher for each pen throughout the entire experimental period. The group pen with eight pigs was considered as the experimental unit for assessing diarrhea incidence. This approach is consistent with standard practices in swine nutrition studies, where group-level data provides valuable insights into the overall health status of the animals [13,18]. The evaluation used the following fecal scoring system: point 1 = firm feces, point 2 = soft feces, point 3 = light diarrhea, point 4 = heavy diarrhea, and point 5 = watery diarrhea. After recording the data, the feces were cleared to differentiate new observations from previous ones.

### Nutrient digestibility

12 barrows, averaging  $10.69 \pm 0.68$  kg in BW, were assigned in a completely randomized design with 4 treatments and 3 replicates to measure nutrient digestibility and nitrogen retention. The apparent total tract digestibility (ATTD) of dry matter (DM), crude protein (CP), crude ash, and crude fat was measured using the total collection method. During the whole experimental period, which included a 5-day adaptation phase followed by a 5-day collection phase, the experimental diets were provided at 7 AM and 7 PM to supply three times the maintenance energy requirement, and water was provided *ad libitum*. For the purpose of identifying the initial and final collection points, 8 g of chromium oxide and ferric oxide were incorporated into the experimental diets on the first and last days, respectively, as selection markers. The feces were collected daily and then frozen at -20°C until the collection process was finished. The feces were then dried in a 60°C oven for 72 h and ground into 1 mm particles with a Wiley mill (CT 193 Cyclotec, FOSS). The daily urine samples were diluted by adding 2 L of water and collected in a plastic container with 50 mL of 10% sulfuric acid to prevent nitrogen evaporation. These samples were also frozen at -20°C and subsequently chemically analyzed along with the feces using the protocols of Association of Official Analytical Chemists (AOAC) [19].

### Statistical analysis

The data obtained in this study were analyzed through comparisons of least squares means and assessed using the general linear model (GLM) procedure available in SAS (SAS Institute). The linear and quadratic effects due to increasing levels of  $\beta$ -mannanase supplementation were assessed using orthogonal polynomial contrasts. A pen housing eight pigs was used as the unit for statistical analysis of growth performance and diarrhea incidence, while individual pigs served as units for analyzing nutrient digestibility and blood metabolites. The differences were regarded as statistically significant when  $p < 0.05$ , and highly significant at  $p < 0.01$ , whereas  $0.05 \leq p < 0.10$  was interpreted as indicating a trend in the data.

## RESULTS AND DISCUSSION

### Growth performance

The effects of varying levels of  $\beta$ -mannanase on growth performance are shown in Table 3. During the early weaning phase, ADG tended to increase when  $\beta$ -mannanase level increased (linear,  $p = 0.07$ ). In the late weaning phase, when  $\beta$ -mannanase level increased, ADFI tended to decrease (linear,  $p = 0.08$ ), and G:F ratio increased (linear,  $p = 0.02$ ). Throughout the experimental period, G:F ratio tended to increase as  $\beta$ -mannanase level increased (linear,  $p = 0.06$ ).

Similar to the results of this experiment, Pettey et al. and Balamuralikrishnan et al. reported that adding  $\beta$ -mannanase to weaning pig diets increased ADG and G:F ratio [20,21]. Conversely, some studies reported no changes in growth performance [8,22]. The variations in growth performance results across previous studies are likely a result of differences in the  $\beta$ -mannan and NSP levels, the presence of other ANF, and the amount of  $\beta$ -mannanase added to the experimental diets.

In this experiment, the trend of increased ADG during the early weaning phase is likely a result of improved nutrient digestion and absorption in the diet, facilitated by the addition of  $\beta$ -mannanase.  $\beta$ -mannanase improved the utilization of indigestible NSP, and enhanced the digestibility of crude fat, which will be discussed later. Previous studies have demonstrated that incorporating  $\beta$ -mannanase to feed has a comparable effect with that of supplying extra energy, resulting in increased ADG and G:F ratio [20, 23]. The trend of decreased ADFI during the late weaning phase is likely for the same reason, as pigs could obtain the necessary nutrients from a smaller amount of feed. However, the two different manifestations during each phase are likely because of the developmental stage of the digestive system. In early weaning phase, the developing digestive system, with its limited gut capacity and issues such as diarrhea, makes it difficult to reduce feed intake [15]. By contrast, during the later period, a more stabilized digestive system allows for

**Table 3.** Effects of varying levels of  $\beta$ -mannanase on growth performance in weaning pigs

Criteria	Treatment <sup>1)</sup>				SEM	p-value	
	$\beta$ -Man0	$\beta$ -Man0.05	$\beta$ -Man0.1	$\beta$ -Man0.15		Lin.	Quad.
Body weight (kg)							
Initial		8.66			-	-	-
2 wk	13.82	13.84	14.05	14.58	0.293	0.38	0.96
5 wk	28.22	28.43	28.09	28.27	0.426	0.96	0.80
ADG (g)							
0–2 wk	370.99	370.96	385.34	421.71	10.131	0.07	0.93
2–5 wk	653.32	665.94	668.52	651.57	8.433	0.97	0.91
0–5 wk	559.13	564.74	555.25	559.62	7.408	0.91	0.69
ADFI (g)							
0–2 wk	691.55	692.33	680.11	721.14	17.317	0.65	0.69
2–5 wk	1,264.14	1,253.83	1,229.15	1,134.79	25.199	0.08	0.80
0–5 wk	1,035.10	1,029.23	1,009.54	969.33	17.730	0.20	0.97
G:F ratio							
0–2 wk	0.542	0.534	0.572	0.586	0.012	0.14	0.54
2–5 wk	0.516	0.532	0.546	0.574	0.009	0.02	0.83
0–5 wk	0.540	0.548	0.556	0.578	0.007	0.06	0.82

<sup>1)</sup> $\beta$ -Man0: corn-SBM based basal diet +  $\beta$ -mannanase 0%;  $\beta$ -Man0.05: basal diet +  $\beta$ -mannanase 0.05%;  $\beta$ -Man0.1: basal diet +  $\beta$ -mannanase 0.1%;  $\beta$ -Man0.15: basal diet +  $\beta$ -mannanase 0.15%

ADFI, average daily feed intake; ADG, average daily gain; G:F ratio, gain to feed ratio; Lin, linear; Quad, quadratic.



this reduction.

### Nutrient digestibility

The effects of varying levels of  $\beta$ -mannanase on the ATTD of nutrients are shown in Table 4. Crude fat digestibility increased when  $\beta$ -mannanase level increased (linear,  $p = 0.04$ ). Supplementing  $\beta$ -mannanase did not influence the digestibility of other nutrients and nitrogen retention.

Similar to the results of this experiment, Jang et al. [8] also observed an improvement in the ATTD of crude fat with the inclusion of  $\beta$ -mannanase in the diet of weaning pigs. In most other previous studies, digestibility experiments were conducted on at least growing pigs, and crude fat digestibility was not analyzed. While some studies observed an increase in the digestibility of DM and CP [24], others found no differences in nutrient digestibility despite the addition of  $\beta$ -mannanase [20, 25].

Although  $\beta$ -mannanase is a carbohydrase, its ability to improve crude fat digestibility in this experiment is related to changes in the viscosity of the digestive tract.  $\beta$ -mannan, particularly galactomannan, acts as a soluble NSP (sNSP) that can absorb a considerable amount of water, forming high viscosity digesta in the small intestine [26]. For digestion to occur, free diffusion of substrates and digestive enzymes is necessary, but high viscosity impedes this process [27]. Additionally, NSP itself functions as a physical barrier, obstructing the binding of substrates and enzymes, hindering the breakdown process [28]. The digestion of fats requires the action of various lipases and an emulsification process using bile salts. Therefore, highly viscous digesta caused by  $\beta$ -mannan is likely to have a greater impact on fat digestibility more significantly than other nutrients. Indeed, earlier studies have reported that high viscosity digesta leads to reduced fat digestibility [29]. By adding  $\beta$ -mannanase to the feed,  $\beta$ -mannan can be broken down, reducing the viscosity in the small intestine [14]. This reduction in viscosity mitigates the negative factors that hinder fat digestion and improves digestibility. Furthermore, the breakdown product of  $\beta$ -mannanase, MOS, is a well-known prebiotics that can modify the composition of gut microbiota, and these changes in the microbial population may, influence lipid metabolism through fermentation processes and associated metabolic pathways [30].

**Table 4.** Effects of varying levels of  $\beta$ -mannanase on ATTD of nutrients in weaning pigs

Criteria	Treatment <sup>1)</sup>				SEM	p-value	
	β-Man0	β-Man0.05	β-Man0.1	β-Man0.15		Lin.	Quad.
ATTD of nutrients (%)							
Dry matter	91.76	91.24	91.46	91.15	0.308	0.62	0.69
Crude protein	90.99	89.74	90.02	89.50	0.493	0.41	0.64
Crude ash	72.83	72.95	71.36	72.19	0.331	0.25	0.19
Crude fat	81.23	81.44	81.88	82.16	0.170	0.04	0.78
Nitrogen retention (g/day)							
N-intake	5.16	5.16	5.17	5.16	-	-	-
N-feces	0.55	0.54	0.52	0.56	0.021	0.93	0.74
N-urine	2.16	2.22	2.17	2.21	0.034	0.73	0.61
N-retention <sup>2)</sup>	2.45	2.40	2.48	2.38	0.034	0.70	0.42

<sup>1)</sup> $\beta$ -Man0: corn-SBM based basal diet +  $\beta$ -mannanase 0%;  $\beta$ -Man0.05: basal diet +  $\beta$ -mannanase 0.05%;  $\beta$ -Man0.1: basal diet +  $\beta$ -mannanase 0.1%;  $\beta$ -Man0.15: basal diet +  $\beta$ -mannanase 0.15%

<sup>2)</sup>N-retention (g) = N intake (g) – fecal N (g) – urinary N (g).

ATTD, apparent total tract digestibility; Lin, linear; Quad, quadratic.

### Blood metabolites

The effects of varying levels of  $\beta$ -mannanase on blood metabolites are shown in Table 5. In the early weaning phase, the total protein and triglyceride concentration increased as  $\beta$ -mannanase level increased (linear,  $p = 0.01$ ;  $p = 0.01$ ). During the entire experimental period, the total cholesterol concentration increased significantly (linear,  $p < 0.01$ ), whereas the HDL cholesterol and LDL cholesterol concentration increased with higher levels of  $\beta$ -mannanase (linear,  $p = 0.02$ ;  $p = 0.02$ ). However, no significant differences were found in the glucose and BUN concentration.

In contrast to the outcomes of this experiment, Yoon et al. and Kim et al. found that blood glucose levels increased with higher levels of  $\beta$ -mannanase [16,29]. Except for these studies, as previously mentioned, there is a notable scarcity in research examining various metabolic indicators in the blood of pigs.

The increase in the levels of triglycerides, total cholesterol, HDL cholesterol, and LDL

**Table 5.** Effects of varying levels of  $\beta$ -mannanase on blood metabolites in weaning pigs

Criteria	Treatment <sup>1)</sup>				SEM	p-value	
	$\beta$ -Man0	$\beta$ -Man0.05	$\beta$ -Man0.1	$\beta$ -Man0.15		Lin.	Quad.
Glucose (mg/dL)							
Initial		----- 127.50 -----			-	-	-
2 wk	122.75	119.25	115.25	116.60	1.627	0.14	0.70
5 wk	112.75	109.20	111.00	113.25	1.544	0.83	0.74
BUN (mg/dL)							
Initial		----- 3.43 -----			-	-	-
2 wk	2.98	3.44	2.80	3.08	0.281	0.90	0.46
5 wk	3.18	3.30	1.95	2.45	0.335	0.27	0.29
Total protein (g/dL)							
Initial		----- 4.15 -----			-	-	-
2 wk	4.03	4.06	4.35	4.43	0.063	0.01	0.31
5 wk	5.04	5.13	4.85	5.38	0.080	0.25	0.11
Triglyceride (mg/dL)							
Initial		----- 36.25 -----			-	-	-
2 wk	31.00	27.25	32.80	43.50	2.044	0.01	0.31
5 wk	35.25	26.50	33.50	35.50	1.496	0.52	0.11
Total cholesterol (mg/dL)							
Initial		----- 61.75 -----			-	-	-
2 wk	50.75	56.25	60.40	68.75	2.142	< 0.01	0.70
5 wk	75.00	88.25	85.75	94.40	2.337	< 0.01	0.11
HDL cholesterol (mg/dL)							
Initial		----- 26.75 -----			-	-	-
2 wk	23.50	23.75	26.20	29.25	0.923	0.02	0.83
5 wk	25.50	28.75	27.00	33.40	1.116	0.02	0.13
LDL cholesterol (mg/dL)							
Initial		----- 36.50 -----			-	-	-
2 wk	29.25	34.00	36.20	41.00	1.687	0.02	0.70
5 wk	51.00	61.50	61.00	63.00	1.805	0.02	0.35

<sup>1)</sup> $\beta$ -Man0: corn-SBM based basal diet +  $\beta$ -mannanase 0%;  $\beta$ -Man0.05: basal diet +  $\beta$ -mannanase 0.05%;  $\beta$ -Man0.1: basal diet +  $\beta$ -mannanase 0.1%;  $\beta$ -Man0.15: basal diet +  $\beta$ -mannanase 0.15%

BUN, blood urea nitrogen; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Lin, linear; Quad, quadratic.



cholesterol during the early weaning phase is likely because of the improved crude fat digestibility owing to the addition of  $\beta$ -mannanase. Enhanced fat digestion leads to an increased absorption of the broken down products, fatty acids and glycerol, into the body. The absorbed fatty acids are carried to the liver, where they initiate lipid metabolism processes which synthesize triglycerides and cholesterol, increasing the amount of lipid-related metabolites released into the bloodstream [31]. Elevated levels of total cholesterol, HDL cholesterol, and LDL cholesterol during the late weaning phase are also attributed to the activated lipid metabolism processes. However, the lack of difference in the triglyceride levels at this phase is likely because of the lower metabolizable energy (ME) requirement compared with that in the early weaning phase [17]. As digestive ability improves with growth, the ME requirement is more easily met, reducing the need for triglyceride synthesis as an energy source. At this stage, cholesterol, a major component of cell membranes, is still required in large amounts [32]; therefore, the synthesis of cholesterol from absorbed fatty acids will remain active. Consequently, the concentration of cholesterol-related metabolites in the blood increases with higher  $\beta$ -mannanase levels.

The improvement in crude fat digestibility should have contributed to the increase in blood total protein levels. As CP digestibility did not increase, it can be concluded that it did not influence the total protein levels. Instead, the increase in crude fat digestibility allowed for more efficient energy utilization from dietary fats, thereby exerting a protein-sparing effect, which reduced the need for protein breakdown for energy and resulted in an increase in total protein levels [33].

### Diarrhea incidence

The effects of varying levels of  $\beta$ -mannanase on diarrhea incidence are shown in Table 6. Diarrhea incidence showed no significant variation during the early and late weaning phases according to  $\beta$ -mannanase levels.

Similar to the results of this experiment, Balamuralikrishnan et al. did not observe a difference in diarrhea incidence [21]. By contrast, Vangroenweghe et al. observed a decrease in diarrhea incidence with the addition of  $\beta$ -mannanase [13].

Undigested NSP undergoes abnormal fermentation in the intestines, promoting the growth of pathogenic microorganisms and causing intestinal inflammation, leading to diarrhea [34,35]. Therefore, theoretically,  $\beta$ -mannanase can reduce diarrhea by breaking down NSP. However, the

**Table 6.** Effects of varying levels of  $\beta$ -mannanase on diarrhea incidence in weaning pigs

Criteria	Treatment <sup>1)</sup>				SEM	p-value	
	β-Man0	β-Man0.05	β-Man0.1	β-Man0.15		Lin.	Quad.
Fecal score <sup>2)</sup>							
0–1 wk	1.86	1.94	1.89	1.86	0.045	0.88	0.70
1–2 wk	1.63	1.63	1.51	1.54	0.033	0.24	0.41
2–3 wk	1.49	1.43	1.43	1.46	0.067	0.90	0.97
3–4 wk	1.51	1.37	1.34	1.40	0.063	0.54	0.96
4–5 wk	1.08	1.09	1.08	1.14	0.026	0.49	0.80
0–2 wk	1.74	1.78	1.70	1.70	0.025	0.34	0.38
2–5 wk	1.36	1.29	1.29	1.33	0.043	0.82	0.98
0–5 wk	1.51	1.49	1.45	1.48	0.027	0.59	0.74

<sup>1)</sup> $\beta$ -Man0: corn-SBM based basal diet +  $\beta$ -mannanase 0%;  $\beta$ -Man0.05: basal diet +  $\beta$ -mannanase 0.05%;  $\beta$ -Man0.1: basal diet +  $\beta$ -mannanase 0.1%;  $\beta$ -Man0.15: basal diet +  $\beta$ -mannanase 0.15%.

<sup>2)</sup>The fecal score was measured by scoring the feces as point 1 (firm feces), point 2 (soft feces), point 3 (light diarrhea), point 4 (heavy diarrhea), and point 5 (watery diarrhea).

Lin, linear; Quad, quadratic.

highest fecal score observed in this study was 1.94, indicating a relatively low incidence of diarrhea even during the early weaning phase, which is typically prone to diarrhea. Therefore, it is thought that adding  $\beta$ -mannanase to diets with a higher  $\beta$ -mannan content than the 0.31% calculated in the experimental diets applied in this study could lead to a more significant reduction in diarrhea incidence.

## CONCLUSION

As the level of  $\beta$ -mannanase supplementation in the diet of weaning pigs increased, positive trends were observed in growth performance, nutrient digestibility, and blood metabolites, which are typically influenced by the presence of  $\beta$ -mannan. Therefore, supplementing  $\beta$ -mannanase up to 0.15% in the diet of weaning pigs could enhance their productivity, as it breaks down indigestible  $\beta$ -mannan.

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