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7 Abstract

8 This study was carried out to examine the effects of varying levels of β -mannanase supplementation in corn-9 soybean meal (SBM)-based diet on growth performance, nutrient digestibility, blood metabolites, and diarrhea 10 incidence of weaning pigs. A total of 160 pigs with an initial body weight (BW) of 8.66 ± 0.060 kg were used in 11 the experiment. Using a randomized complete block (RCB) design, they were assigned to 4 treatments with 5 12 replicates and 8 pigs per pen, considering sex and initial BW. Treatments for early (0 - 2 weeks) and late 13 weaning phases (2 - 5 weeks) were as follows: β -Man0: corn-SBM-based basal diet + β -mannanase 0%; β -14 Man0.05: basal diet + β -mannanase 0.05%; β -Man0.1: basal diet + β -mannanase 0.1%; and β -Man0.15: basal 15 diet + β -mannanase 0.15%. During the early weaning phase, average daily gain tended to increase when β -16 mannanase level increased (linear, p = 0.07). When β -mannanase level increased in the late weaning phase, the 17 average daily feed intake tended to decrease (linear, p = 0.08), and gain to feed ratio (G:F ratio) increased (linear, 18 p = 0.02). Throughout the whole experimental period, G:F ratio tended to increase as β -mannanase level 19 increased (linear, p = 0.06). According to nutrient digestibility, crude fat digestibility increased when the β -20 mannanase level increased (linear, p = 0.04). Accordingly, the total protein and triglyceride concentration 21 increased as β -mannanase level increased in the early weaning phase (linear, p = 0.01; p = 0.01). During the 22 entire experimental period, the total cholesterol concentration increased significantly (linear, p < 0.01), whereas 23 the high-density lipoprotein cholesterol and low-density lipoprotein cholesterol concentration increased with 24 higher levels of β -mannanase (linear, p = 0.02; p = 0.02). Lastly, diarrhea incidence showed no significant 25 variation during the early and late weaning phases according to β -mannanase levels. As the level of β -26 mannanase supplementation in the wearing pig diet increased, growth performance, nutrient digestibility, and 27 blood metabolites showed some positive trends. Therefore, supplementing β -mannanase up to 0.15% in the diet 28 of weaning pigs could enhance their productivity.

29

30 Keywords: β-mannanase, Growth performance, Nutrient digestibility, Blood metabolites, Diarrhea
 31 incidence, Weaning pigs

32

34 Introduction

35 Hemicellulose, a major component of plant cell walls, is the second most common polysaccharide found in 36 nature, accounting for 20 - 35% of the dry weight of lignocellulosic biomass [1]. The predominant type of 37 hemicellulose varies with plant species, and β -mannan contents are relatively high in the major livestock feed 38 ingredients [2]. β -mannan is a non-starch polysaccharide (NSP) characterized by a straight chain of β -1,4-linked 39 d-mannose units, with variations such as glucomannan and galactomannan, which include additional glucose or 40 galactose [3]. The β -mannan content is 0.29% in corn, 1.20% in soybean meal (SBM), and 0.30% in wheat [4, 41 5]. Although these amounts may seem minimal, monogastric animals lack endogenous enzymes to degrade β -42 mannan, thereby it functions as an anti-nutritional factor (ANF) [6]. β-mannan can induce several issues, 43 notably reduced growth performance and nutrient digestibility, which are especially problematic in weaning 44 pigs with underdeveloped digestive systems [7, 8]. Therefore, the inclusion of β -mannanase, an exogenous 45 enzyme that can degrade β -mannan, in the diet of weaning pigs could mitigate these issues.

46 β -mannanase is an endo-hydrolase that breaks down β -1,4 glycosidic bonds in the β -mannan main chain 47 randomly, cleaving it into mannose and mannan-oligosaccharides (MOS) [9, 10]. Currently, commercial β -48 mannanase products produced by microbes, such as Paenibacillus lentus and Bacillus lentus are added to 49 various livestock feeds [11, 12]. When added to pig feed, these products exhibit positive effects on growth 50 performance, nutrient digestibility, and immune status [13-15]. Weaning pigs, in particular, are expected to 51 benefit significantly from β -mannanase owing to various developmental limitations, such as lower digestive 52 enzyme activity compared with growing-finishing pigs [16]. However, previous studies on weaning pigs have 53 reported inconsistent results, with most studies primary concentrating on digestibility and immunity, and there is 54 a notable shortage of studies on blood metabolites and diarrhea incidence.

55 Therefore, this study was carried out to examine the effects of varying levels of β -mannanase 56 supplementation in corn-SBM-based diet on growth performance, nutrient digestibility, blood metabolites, and 57 diarrhea incidence of weaning pigs.

58

59 Materials and Methods

60 Experimental animals

A total of 160 pigs with an initial body weight (BW) of 8.66 ± 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 [13, 17]. 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). 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 (SNUIACUC; SNU-231203-3).

71

72 **Experimental diet**

Four experimental diets with different levels of β -mannanase for the early and late weaning phases were as follows: β -Man0: corn-SBM based basal diet + β -mannanase 0%; β -Man0.05: basal diet + β -mannanase 0.05%; β -Man0.1: basal diet + β -mannanase 0.1%; and β -Man0.15: basal diet + β -mannanase 0.15%. CTCZYME, a β mannanase product (800,000 IU/kg) provided by CTCBIO (Hwaseong, South Korea), was supplemented in diets. All diets were designed to fulfill the nutrient requirements for weaning pigs as recommended by the National Research Council (NRC) [18]. Tables 1 and 2 provide the formulation and chemical composition of the experimental diets.

80

81 Growth performance

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

85

86 Blood sampling and analysis

87 Blood samples were obtained from five selected pigs per treatment, which were representative of the average 88 BW within each treatment, on the initial day and at the end of each phase. Blood was collected from the jugular 89 vein of the pigs after 3 hours fast and placed in serum tubes (SST II Advance; BD Vacutainer, Becton Dickinson, 90 Plymouth, UK). After allowing the samples to clot at room temperature for 30 minutes, they were centrifuged at 91 3,000 rpm and 4°C for 15 minutes (5810R; Eppendorf, centrifuge 5810R, Hamburg, Germany). Subsequently, 92 the sera were separated and transferred into microtubes (Axygen, Union City, CA, USA), then kept at -20°C for 93 the analysis of glucose, blood urea nitrogen (BUN), total protein, triglyceride, total cholesterol, high-density 94 lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL) cholesterol.

96 Diarrhea incidence

Diarrhea incidence was monitored daily at 8:00 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 grouplevel data provides valuable insights into the overall health status of the animals [14, 19]. 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.

104

105 Nutrient digestibility

106 12 barrows, averaging 10.69 ± 0.68 kg in BW, were assigned in a completely randomized design with 4 107 treatments and 3 replicates to measure nutrient digestibility and nitrogen retention. The apparent total tract 108 digestibility (ATTD) of dry matter (DM), crude protein (CP), crude ash, and crude fat was measured using the 109 total collection method. During the whole experimental period, which included a 5-day adaptation phase 110 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 111 112 the initial and final collection points, 8 g of chromium oxide and ferric oxide were incorporated into the 113 experimental diets on the first and last days, respectively, as selection markers. The feces were collected daily 114 and then frozen at -20 °C until the collection process was finished. The feces were then dried in a 60°C oven for 115 72 hours and ground into 1 mm particles with a Wiley mill (CT 193 Cyclotec, FOSS, Höganäs, Sweden). The 116 daily urine samples were diluted by adding 2 L of water and collected in a plastic container with 50 mL of 10% 117 sulfuric acid to prevent nitrogen evaporation. These samples were also frozen at -20° C and subsequently 118 chemically analyzed along with the feces using the protocols of Association of Official Analytical Chemists 119 (AOAC) [20].

120

121 Statistical analysis

122 The data obtained in this study were analyzed through comparisons of least squares means and assessed using 123 the general linear model (GLM) procedure available in SAS (SAS Institute Inc., Cary, NC, USA). The linear 124 and quadratic effects due to increasing levels of β -mannanase supplementation were assessed using orthogonal 125 polynomial contrasts. A pen housing eight pigs was used as the unit for statistical analysis of growth 126 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 $0.05 \le p < 0.10$ was interpreted as indicating a trend in the data.

129

130 **Results & Discussion**

131 Growth performance

The effects of varying levels of β-mannanase on growth performance are shown in Table 3. During the early weaning phase, ADG tended to increase when β-mannanase level increased (linear, p = 0.07). In the late weaning phase, when β-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 β-mannanase level increased (linear, p = 0.02).

137 Similar to the results of this experiment, Pettey et al. and Balamuralikrishnan et al. reported that adding β-138 mannanase to weaning pig diets increased ADG and G:F ratio [21, 22]. Conversely, some studies reported no 139 changes in growth performance [8, 23]. The variations in growth performance results across previous studies are 140 likely a result of differences in the β-mannan and NSP levels, the presence of other ANF, and the amount of β-141 mannanase added to the experimental diets.

142 In this experiment, the trend of increased ADG during the early weaning phase is likely a result of improved 143 nutrient digestion and absorption in the diet, facilitated by the addition of β-mannanase. β-mannanase improved 144 the utilization of indigestible NSP, and enhanced the digestibility of crude fat, which will be discussed later. 145 Previous studies have demonstrated that incorporating β -mannanase to feed has a comparable effect with that of 146 supplying extra energy, resulting in increased ADG and G:F ratio [21, 24]. The trend of decreased ADFI during 147 the late weaning phase is likely for the same reason, as pigs could obtain the necessary nutrients from a smaller 148 amount of feed. However, the two different manifestations during each phase are likely because of the 149 developmental stage of the digestive system. In early weaning phase, the developing digestive system, with its 150 limited gut capacity and issues such as diarrhea, makes it difficult to reduce feed intake [16]. By contrast, during 151 the later period, a more stabilized digestive system allows for this reduction.

152

153 Nutrient digestibility

154 The effects of varying levels of β -mannanase on the ATTD of nutrients are shown in Table 4. Crude fat 155 digestibility increased when β -mannanase level increased (linear, p = 0.04). Supplementing β -mannanase did not 156 influence the digestibility of other nutrients and nitrogen retention. Similar to the results of this experiment, Jang et al. [13] also observed an improvement in the ATTD of crude fat with the inclusion of β-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 [25], others found no differences in nutrient digestibility despite the addition of β-mannanase [21, 26].

162 Although β -mannanase is a carbohydrase, its ability to improve crude fat digestibility in this experiment is 163 related to changes in the viscosity of the digestive tract. β -mannan, particularly galactomannan, acts as a soluble 164 NSP (sNSP) that can absorb a considerable amount of water, forming high viscosity digesta in the small 165 intestine [27]. For digestion to occur, free diffusion of substrates and digestive enzymes is necessary, but high 166 viscosity impedes this process [28]. Additionally, NSP itself functions as a physical barrier, obstructing the 167 binding of substrates and enzymes, hindering the breakdown process [29]. The digestion of fats requires the 168 action of various lipases and an emulsification process using bile salts. Therefore, highly viscous digesta caused 169 by β -mannan is likely to have a greater impact on fat digestibility more significantly than other nutrients. Indeed, 170 earlier studies have reported that high viscosity digesta leads to reduced fat digestibility [30]. By adding β -171 mannanase to the feed, β -mannan can be broken down, reducing the viscosity in the small intestine [15]. This 172 reduction in viscosity mitigates the negative factors that hinder fat digestion and improves digestibility. 173 Furthermore, the breakdown product of β-mannanase, MOS, is a well-known prebiotics that can modify the 174 composition of gut microbiota, and these changes in the microbial population may, influence lipid metabolism 175 through fermentation processes and associated metabolic pathways [31].

176 **Blood metabolites**

177 The effects of varying levels of β -mannanase on blood metabolites are shown in Table 5. In the early weaning 178 phase, the total protein and triglyceride concentration increased as β -mannanase level increased (linear, p =179 0.01; p = 0.01). During the entire experimental period, the total cholesterol concentration increased significantly 180 (linear, p < 0.01), whereas the HDL cholesterol and LDL cholesterol concentration increased with higher levels 181 of β -mannanase (linear, p = 0.02; p = 0.02). However, no significant differences were found in the glucose and 182 BUN concentration.

183 In contrast to the outcomes of this experiment, Yoon et al. and Kim et al. found that blood glucose levels 184 increased with higher levels of β -mannanase [17, 30]. Except for these studies, as previously mentioned, there is 185 a notable scarcity in research examining various metabolic indicators in the blood of pigs.

186 The increase in the levels of triglycerides, total cholesterol, HDL cholesterol, and LDL cholesterol during the 187 early weaning phase is likely because of the improved crude fat digestibility owing to the addition of β -188 mannanase. Enhanced fat digestion leads to an increased absorption of the broken down products, fatty acids 189 and glycerol, into the body. The absorbed fatty acids are carried to the liver, where they initiate lipid metabolism 190 processes which synthesize triglycerides and cholesterol, increasing the amount of lipid-related metabolites 191 released into the bloodstream [32]. Elevated levels of total cholesterol, HDL cholesterol, and LDL cholesterol 192 during the late weaning phase are also attributed to the activated lipid metabolism processes. However, the lack 193 of difference in the triglyceride levels at this phase is likely because of the lower metabolizable energy (ME) 194 requirement compared with that in the early weaning phase [18]. As digestive ability improves with growth, the 195 ME requirement is more easily met, reducing the need for triglyceride synthesis as an energy source. At this 196 stage, cholesterol, a major component of cell membranes, is still required in large amounts [33]; therefore, the 197 synthesis of cholesterol from absorbed fatty acids will remain active. Consequently, the concentration of 198 cholesterol-related metabolites in the blood increases with higher β-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 [34].

204

205 Diarrhea incidence

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

Similar to the results of this experiment, Balamuralikrishnan et al. did not observe a difference in diarrhea
 incidence [22]. By contrast, Vangroenweghe et al. observed a decrease in diarrhea incidence with the addition of
 β-mannanase [14].

211 Undigested NSP undergoes abnormal fermentation in the intestines, promoting the growth of pathogenic 212 microorganisms and causing intestinal inflammation, leading to diarrhea [35, 36]. Therefore, theoretically, β-213 mannanase can reduce diarrhea by breaking down NSP. However, the highest fecal score observed in this study 214 was 1.94, indicating a relatively low incidence of diarrhea even during the early weaning phase, which is 215 typically prone to diarrhea. Therefore, it is thought that adding β-mannanase to diets with a higher β-mannan 216 content than the 0.31% calculated in the experimental diets applied in this study could lead to a more significant 217 reduction in diarrhea incidence.

218

219 Conclusion

- 220 As the level of β-mannanase supplementation in the diet of weaning pigs increased, positive trends were 221 observed in growth performance, nutrient digestibility, and blood metabolites, which are typically influenced by 222 the presence of β-mannan. Therefore, supplementing β-mannanase up to 0.15% in the diet of weaning pigs 223 could enhance their productivity, as it breaks down indigestible β-mannan.
- 224

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231 **References**

- Saha BC. Hemicellulose bioconversion. J Ind Microbiol Biotechnol. 2003;30(5):279-91.
 https://doi.org/10.1007/s10295-003-0049-x
- Moreira LRS, Filho EXF. An overview of mannan structure and mannan-degrading enzyme systems. Appl Microbiol Biotechnol. 2008;79(2):165-78. https://doi.org/10.1007/s00253-008-1423-4
- Casillo A, Fabozzi A, Russo Krauss I, Parrilli E, Biggs CI, Gibson MI, et al. Physicochemical Approach to Understanding the Structure, Conformation, and Activity of Mannan Polysaccharides. Biomacromolecules. 2021;22(4):1445-57. https://doi.org/10.1021/acs.biomac.0c01659
- 4. Kiarie EG, Steelman S, Martinez, Marco, Livingston K. Significance of single β-mannanase supplementation on performance and energy utilization in broiler chickens, laying hens, turkeys, sows, and nursery-finish pigs: a meta-analysis and systematic review. Transl Anim Sci. 2021;5(4).
 242 https://doi.org/10.1093/tas/txab160
- 5. Kwon WB, Kim BG. Effects of Supplemental Beta-mannanase on Digestible Energy and Metabolizable
 Energy Contents of Copra Expellers and Palm Kernel Expellers Fed to Pigs. Asian-Australas J Anim Sci.
 2015;28(7):1014-9. https://doi.org/10.5713/ajas.15.0275
- Tiwari UP, Fleming SA, Abdul Rasheed MS, Jha R, Dilger RN. The role of oligosaccharides and polysaccharides of xylan and mannan in gut health of monogastric animals. J Nutr Sci. 2020;9:e21. https://doi.org/10.1017/jns.2020.14
- Dégen L, Halas V, Babinszky L. Effect of dietary fibre on protein and fat digestibility and its consequences on diet formulation for growing and fattening pigs: A review. Acta Agric Scand A Anim Sci. 2007;57(1):1-9. https://doi.org/10.1080/09064700701372038
- 252 8. Jang J-C, Kim KH, Jang YD, Kim YY. Effects of Dietary β-Mannanase Supplementation on Growth
 253 Performance, Apparent Total Tract Digestibility, Intestinal Integrity, and Immune Responses in Weaning
 254 Pigs. Animals (Basel). 2020;10(4):703. https://doi.org/10.3390/ani10040703
- McCleary BV, Matheson NK. Enzymic Analysis of Polysaccharide Structure. In: Tipson RS, Horton D, editors. Advances in Carbohydrate Chemistry and Biochemistry. 44: Academic Press; 1987. p. 147-276. https://doi.org/10.1016/S0065-2318(08)60079-7
- Dhawan S, Kaur J. Microbial Mannanases: An Overview of Production and Applications. Crit Rev Biotechnol. 2007;27(4):197-216. https://doi.org/10.1080/07388550701775919
- Soni H, Kango N. Microbial Mannanases: Properties and Applications. In: Shukla P, Pletschke BI, editors.
 Advances in Enzyme Biotechnology. New Delhi: Springer India; 2013. p. 41-56. https://doi.org/10.1007/978-81-322-1094-8_4
- 263 12. Dawood A, Ma K. Applications of Microbial β-Mannanases. Front Bioeng Biotechnol. 2020;8:598630.
 264 https://doi.org/10.3389/fbioe.2020.598630
- 265 13. Jang JC, Kim KH, Jang YD, Kim YY. Effects of Dietary β-Mannanase Supplementation on Growth

- Performance, Apparent Total Tract Digestibility, Intestinal Integrity, and Immune Responses in Weaning
 Pigs. Animals (Basel). 2020;10(4). https://doi.org/10.3390/ani10040703
- Vangroenweghe F, Poulsen K, Thas O. Supplementation of a β-mannanase enzyme reduces post-weaning diarrhea and antibiotic use in piglets on an alternative diet with additional soybean meal. Porcine Health Manag. 2021;7(1):8. https://doi.org/10.1186/s40813-021-00191-5
- Jang KB, Kim YI, Duarte ME, Kim SW. Effects of β-mannanase supplementation on intestinal health and growth of nursery pigs. J Anim Sci. 2024;102:skae052. https://doi.org/10.1093/jas/skae052
- 16. Nyachoti C, Zijlstra R, de Lange C, Patience J. Voluntary feed intake in growing-finishing pigs: A review of the main determining factors and potential approaches for accurate predictions. Can J Anim Sci. 2004;84:549-66. https://doi.org/10.4141/A04-001
- Kim JS, Ingale SL, Hosseindoust AR, Lee SH, Lee JH, Chae BJ. Effects of mannan level and β-mannanase
 supplementation on growth performance, apparent total tract digestibility and blood metabolites of growing
 Animal. 2017;11(2):202-8. https://doi.org/10.1017/S1751731116001385
- 279 18. Committee on Nutrient Requirements of Swine, National Research Council. Nutrient requirements of swine. 11th ed. Washington, DC, USA: National Academies Press; 2012.
- 19. Kim H, Shin H, Kim YY. Effects of different levels of dietary crude protein on growth performance, blood profiles, diarrhea incidence, nutrient digestibility, and odor emission in weaning pigs. Anim Biosci. 2023;36(8):1228-40. https://doi.org/10.5713/ab.22.0440
- 284 20. AOAC. Official Methods of Analysis. 16th Edition. Association of Official Analytical Chemists.
 285 Washingtons, D.C., U.S.A. 1995.
- 286 21. Pettey LA, Carter SD, Senne BW, Shriver JA. Effects of beta-mannanase addition to corn-soybean meal diets on growth performance, carcass traits, and nutrient digestibility of weanling and growing-finishing pigs2. J Anim Sci. 2002;80(4):1012-9. https://doi.org/10.2527/2002.8041012x
- 289 22. Balamuralikrishnan B., Lee J. H., Kim I. H. (2018). Effects of dietary β-mannanase supplementation of soybean meal on the performance of weanling pigs. Anim Nutr Feed Technol. 18 (1), 13–23. https://doi.org/10.5958/0974-181X.2018.00002.1
- 23. Carr SN, Allee GL, Rincker PJ, Fry RS, Boler DD. Effects of endo-1,4-β-d-mannanase enzyme (Hemicell HT 1.5 ×) on the growth performance of nursery pigs. Prof Anim Sci. 2014;30(4):393-9. https://doi.org/10.15232/pas.2014-01326
- 24. Bass B, Frank J, Johnson Z, Maxwell C, Lee J. Effect of dietary mannanase supplementation on pig growth
 performance. University of Arkansas Animal Science Department Report 2010. 2010:80-81.
- 297 25. Lv JN, Chen YQ, Guo XJ, Piao XS, Cao YH, Dong B. Effects of Supplementation of β-Mannanase in 298 Corn-soybean Meal Diets on Performance and Nutrient Digestibility in Growing Pigs. Asian-Australas J 299 Anim Sci. 2013;26(4):579-87. https://doi.org/10.5713/ajas.2012.12612
- 300
 26. Mok CH, Kong C, Kim BG. Combination of phytase and β-mannanase supplementation on energy and nutrient digestibility in pig diets containing palm kernel expellers. Anim Feed Sci Technol. 2015;205:116-

- 302 21. https://doi.org/10.1016/j.anifeedsci.2015.04.012
- 303
 27. Shastak Y, Ader P, Feuerstein D, Ruehle R, Matuschek M. β-Mannan and mannanase in poultry nutrition.
 304 Worlds Poult Sci J. 2015;71(1):161-74. https://doi.org/10.1017/S0043933915000136
- 305 28. Passos AA, Park I, Ferket P, von Heimendahl E, Kim SW. Effect of dietary supplementation of xylanase 306 on apparent ileal digestibility of nutrients, viscosity of digesta, and intestinal morphology of growing pigs 307 fed corn and soybean meal based diet. Anim Nutr. 2015;1(1):19-23. 308 https://doi.org/10.1016/j.aninu.2015.02.006
- 29. Căpriță R, Căpriță A, Julean C. Biochemical aspects of non-starch polysaccharides. Sci Pap Anim Sci Biotechnol. 2010;43(1):368-375.
- 30. Yoon SY, Yang YX, Shinde PL, Choi JY, Kim JS, Kim YW, et al. Effects of mannanase and distillers
 dried grain with solubles on growth performance, nutrient digestibility, and carcass characteristics of
 grower-finisher pigs1. J Anim Sci. 2010;88(1):181-91. https://doi.org/10.2527/jas.2008-1741
- 314 31. Halas V, Nochta I. Mannan Oligosaccharides in Nursery Pig Nutrition and Their Potential Mode of Action.
 315 Animals (Basel). 2012;2(2):261-74. https://doi.org/10.3390/ani2020261
- 316
 32. Alves-Bezerra M, Cohen DE. Triglyceride Metabolism in the Liver. Compr Physiol. 2017;8(1):1-8. https://doi.org/10.1002/cphy.c170012
- 318 33. Romsos DR, Allee GL, Leveille GA. In Vivo Cholesterol and Fatty Acid Synthesis in the Pig Intestine.
 319 Proc Soc Exp Biol Med. 1971;137(2):570-3. https://doi.org/10.3181/00379727-137-35623
- 320 34. Li S, Sauer WC. The effect of dietary fat content on amino acid digestibility in young pigs. J Anim Sci. 1994;72(7):1737-43. https://doi.org/10.2527/1994.7271737x
- 322 35. Choct M, Hughes RJ, Wang J, Bedford MR, Morgan AJ, Annison G. Increased small intestinal fermentation is partly responsible for the anti-nutritive activity of non-starch polysaccharides in chickens. Br Poult Sci. 1996;37(3):609-21. https://doi.org/10.1080/00071669608417891
- 36. Pluske JR, Durmic Z, Pethick DW, Mullan BP, Hampson DJ. Confirmation of the Role of Rapidly
 Fermentable Carbohydrates in the Expression of Swine Dysentery in Pigs after Experimental Infection123.
 J Nutr. 1998;128(10):1737-44. https://doi.org/10.1093/jn/128.10.1737

Tables and Figures 1. 329

330 Table 1. Formulation and chemical composition of the experimental diets for the early weaning phase 331 (0 - 2 weeks)

Criteria	Treatment ¹							
Cinteria	β-Man0	β-Man0.05	β-Man0.1	β-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 ²	0.10	0.10	0.10	0.10				
Min. Mix ³	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				
β-mannanase ⁴	0.00	0.05	0.10	0.15				
Total	100.00	100.00	100.00	100.00				
Chemical composition ⁵								
ME, kcal/kg	3400.00	3400.00	3400.00	3400.00				
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				
β -mannan ⁶ , %	0.31	0.31	0.31	0.31				

¹ β -Man0: corn-SBM based basal diet + β -mannanase 0%; β -Man0.05: basal diet + β -mannanase 0.05%; β -Man0.1: basal diet + β mannanase 0.1%; β -Man0.15: basal diet + β -mannanase 0.15%

² 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. ³ Supplied at the following levels per kilogram of diet: Se, 0.1mg; I, 0.3mg; Mn, 24.8mg; CuSO4, 54.1mg; Fe, 127.3mg; Zn, 84.7mg; Co,

0.3mg.

⁴ CTCZYME, β-mannanase (800,000 IU/kg), provided from CTC Bio (Seoul, South Korea).

⁵ Calculated values.

 6 The β -mannan content in the diet was calculated based on the methods described by Kwon and Kim, and Kiarie et al. [4, 5].

333 Table 2. Formulation and chemical composition of the experimental diets for the late weaning phase (2

334 - 5 weeks)

Criteria		Treat	ment ¹	
Criteria	β-Man0	β-Man0.05	β-Man0.1	β-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 ²	0.10	0.10	0.10	0.10
Min. Mix ³	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
β-mannanase ⁴	0.00	0.05	0.10	0.15
Total	100.00	100.00	100.00	100.00
Chemical composition ⁵				
ME, kcal/kg	3350.00	3350.00	3350.00	3350.00
СР, %	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
β-mannan ⁶ , %	0.31	0.31	0.31	0.31

¹ β -Man0: corn-SBM based basal diet + β -mannanase 0%; β -Man0.05: basal diet + β -mannanase 0.05%; β -Man0.1: basal diet + β -

mannanase 0.1%; β -Man0.15: basal diet + β -mannanase 0.15% ² 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.

³ Supplied at the following levels per kilogram of diet: Se, 0.1mg; I, 0.3mg; Mn, 24.8mg; CuSO4, 54.1mg; Fe, 127.3mg; Zn, 84.7mg; Co, 0.3mg. ⁴ CTCZYME, β-mannanase (800,000 IU/kg), provided from CTC Bio (Seoul, South Korea).

 6 The β -mannan content in the diet was calculated based on the methods described by Kwon and Kim, and Kiarie et al. [4, 5].

336	Table 3. Effects of varyin	g levels of β -mannanase on	growth performance in weaning pigs
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Criteria β-Man0		Treatment ¹			SEM ²	p-v:	alue ³
	β-Man0.05	β-Man0.1	β-Man0.15	SEM-	Lin.	Quad	
Body weight,	, kg						
Initial		8.	66		-	-	-
2 week	13.82	13.84	14.05	14.58	0.293	0.38	0.96
5 week	28.22	28.43	28.09	28.27	0.426	0.96	0.80
ADG, g							
0-2 weeks	370.99	370.96	385.34	421.71	10.131	0.07	0.93
2-5 weeks	653.32	665.94	668.52	651.57	8.433	0.97	0.91
0-5 weeks	559.13	564.74	555.25	559.62	7.408	0.91	0.69
ADFI, g							
0-2 weeks	691.55	692.33	680.11	721.14	17.317	0.65	0.69
2-5 weeks	1264.14	1253.83	1229.15	1134.79	25.199	0.08	0.80
0-5 weeks	1035.10	1029.23	1009.54	969.33	17.730	0.20	0.97
G:F ratio							
0-2 weeks	0.542	0.534	0.572	0.586	0.012	0.14	0.54
2-5 weeks	0.516	0.532	0.546	0.574	0.009	0.02	0.83
0-5 weeks	0.540	0.548	0.556	0.578	0.007	0.06	0.82

 $\frac{1}{\beta}$ -Man0: corn-SBM based basal diet + β -mannanase 0%; β -Man0.05: basal diet + β -mannanase 0.05%; β -Man0.1: basal diet + β -mannanase 0.1%; β -Man0.1: basal diet + β -mannanase 0.15% ² Standard error of the mean ³ Abbreviation: Lin. (linear) and Quad. (quadratic)

338 **Table 4**. Effects of varying levels of β -mannanase on ATTD of nutrients in weaning pigs

Criteria	Treatment ¹				SEM ²	<i>p</i> -value ³	
Criteria	β-Man0	β-Man0.05	β-Man0.1	β-Man0.15	SEM-	Lin.	Quad.
ATTD of nutr	ients, %						
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 reter	ntion, 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 ⁴	2.45	2.40	2.48	2.38	0.034	0.70	0.42

¹ β -Man0: corn-SBM based basal diet + β -mannanase 0%; β -Man0.05: basal diet + β -mannanase 0.05%; β -Man0.1: basal diet + β -mannanase 0.1%; β -Man0.1: basal diet + β -mannanase 0.15% ² Standard error of the mean ³ Abbreviation: Lin. (linear) and Quad. (quadratic) ⁴ N-retention (g) = N intake (g) – fecal N (g) – urinary N (g)

Table 5. Effects of varying levels of β -mannanase on blood metabolites in weaning pigs 341

Criteria		Treat	ment ¹		SEM ²	p-va	alue ³
Criteria	β-Man0	β-Man0.05	β-Man0.1	β-Man0.15	SEM-	Lin.	Quad.
Glucose, md/d							
Initial	-	127	.50		-	-	-
		119.25			1.627	0.14	0.70
5 week	112.75	109.20	111.00	113.25	1.544	0.83	0.74
BUN, mg/dL							
Initial		3.4	43		-	-	-
2 week	2.98	3.44	2.80	3.08	0.281	0.90	0.46
5 week	3.18	3.30	1.95	2.45	0.335	0.27	0.29
Total protein,	g/dL						
Initial		4.06	15		-	-	-
2 week	4.03	4.06	4.35	4.43	0.063	0.01	0.31
5 week	5.04	5.13	4.85	5.38	0.080	0.25	0.11
Triglyceride, n	ng/dL						
Initial	-		25		-	-	-
2 week		27.25			2.044		0.31
5 week	35.25	26.50	33.50	35.50	1.496	0.52	0.11
Total cholester							
		61.			-	-	-
2 week		56.25	60.40	68.75	2.142	< 0.01	0.70
5 week		88.25	85.75	94.40	2.337	< 0.01	0.11
HDL cholester							
		26.			-	-	-
2 week		23.75			0.923	0.02	0.83
5 week		28.75	27.00	33.40	1.116	0.02	0.13
LDL cholester							
Initial	-	36.	.50	-	-	-	-
	29.25		36.20		1.687	0.02	0.70
5 week		61.50				0.02	0.35

 5 Week
 51.00
 61.00
 61.00
 63.00
 1.005
 6.02
 6.05

 ¹ β-Man0: corn-SBM based basal diet + β-mannanase 0%; β-Man0.05: basal diet + β-mannanase 0.1%; β-Man0.15: basal diet + β-mannanase 0.15%
 2
 3
 3

 ² Standard error of the mean
 3
 4
 4
 4
 4

Table 6. Effects of varying levels of β -mannanase on diarrhea incidence in weaning pigs 343

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

¹ β-Man0: corn-SBM based basal diet + β-mannanase 0%; β-Man0.05: basal diet + β-mannanase 0.05%; β-Man0.1: basal diet + β-mannanase 0.15%

² Standard error of the mean

³ Abbreviation: Lin. (linear) and Quad. (quadratic)
⁴ 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)

344

-0