Impacts of dietary β-glucan (Morus Alba and Curcuma Longa) supplementation on growth performance, apparent total tract digestibility, fecal microbial, fecal characteristics, and blood profiles in weanling pigs

Subin Serpunja, Kathannan Sankara, Jong Keun Kim and In Ho Kim

Department of Animal Resource and Science, Dankook University, Cheonan, South Korea; Toxicological Evaluation Laboratory, Veterinary Drugs and Biologic Division, Animal and Plant Quarantine Agency, Gimcheon-si, Republic of Korea

ABSTRACT

One hundred twenty crossbred (Yorkshire × Landrace) × Duroc weanling pigs (8.36 ± 1.64 kg; 21 d) were used in a 42-day trial. Pigs were randomly distributed (6 replicate pens/treatment; 5 pigs/pen) to 1 of 4 dietary treatments: 1) CON, basal diet; 2) A1, CON + antibiotic (39 ppm tiamulin); 3) BG1, basal diet + 5 g kg⁻¹ (β-glucan); and 4) BG2, basal diet + 10 g kg⁻¹ (β-glucan). On overall, average daily gain of pigs fed with A1(385 g) and BG1(388 g) was significantly higher (P < 0.05) as compared to pigs fed with CON(342 g) and gain to feed ratio of pigs fed with A1(0.74), BG1(0.74) and BG2(0.71) was significantly higher (P < 0.05) as compared to pigs fed with CON(0.63). On d42, apparent total tract digestibility (ATTD) of dry matter (DM) in pigs fed with BG1(83.35%) was significantly higher (P < 0.05) as compared to pigs fed with CON(81.36%). Meanwhile, ATTD of nitrogen (N) in pigs fed with A1(81.84%) and BG1 (81.35%) was significantly higher (P < 0.05) as compared to pigs fed with CON(78.54%) diet. Pigs fed with A1(8.21 log10cfu/g) and BG2(8.11 log10cfu/g) had significant higher (P < 0.05) Lactobacillus population as compared to pigs fed with CON(7.43 log10cfu/g). In conclusion, β-glucan shows a beneficial effect on growth, nutrient digestibility of DM and N, and beneficial microbial Lactobacillus of weanling pig.

1. Introduction

Piglets are subjected to a lot of nutritional, environmental and immunological stresses during weaning. These stressors lead to decreased feed intake and growth depression. There are also cases of the inflammatory responses of piglets (Campbell et al. 2013). Besides these, the balance of microflora in the gut is disturbed. So, there is a high possibility of susceptibility of the piglet to the enteric pathogens (Hampson 1986). In order to combat this problem, antibiotic have been used in both the therapeutic and sub-therapeutic levels (Williams et al. 2001).

The use of antibiotic helps to reduce mortality and morbidity of weaning pig which subsequently increase the profit of swine producers (Cromwell 2001). But the frequent use of antibiotic resulted in the development of resistant genes by the bacteria in both animals and humans (Aarestrup and Carstensen 1998). Due to this, the European Union banned the use of antibiotic in 2006 on the livestock industry. This led to the need for investigation in alternatives of antibiotic in livestock industries (Simon et al. 2003). Among several alternatives, β-glucan which is a naturally available bioactive compound has attracted the deep attention of the researchers as an alternative to feed antibiotic (Smith et al. 2011).

β-glucan are polysaccharides of D-glucose monomers that are interconnected by β-glycosidic bonds. Yeast and fungal cell walls, plants, and some bacteria have β-1,3 and β-1,6 glycosidic linkages (β-glucan) (Jung et al. 2004). β-glucan has been found to possess antitumor, antiviral, antibacterial, anticoagulatory, antimicrobial and wound healing activities by enhancing the immune system of the host (Ohno et al. 1987; John et al. 1995; Shin et al. 2005). In the research of Stuyven et al. (2008), the pigs fed with β-glucan 0.5 g kg⁻¹ (Saccharomyces cerevisiae) for two weeks after weaning were found to be less susceptible to the infection by F4+ enterotoxigenic E. Coli (ETEC). The use of β-glucan derived from oat, fungi and mushroom in the diet of weaning pig has shown beneficial effects on gastrointestinal functions, feed efficiency, growth performance, and the immune system (Newman et al. 1980; Hahn et al. 2006; Lee et al. 2017). β-glucan with different structure has different bioactivities. It can improve animal health by enhancing the resistance of host to bacterial, viral, parasitic and fungal infections (Diluzio 1983; Bohn and Bemiller 1995).

β-glucan used in our experiment was extracted from Morus Alba and Curcuma Longa. Morus Alba was found to possess antimicrobial activity in the research of Salem et al. (2013) whereas, Curcuma Longa is found to possess anti-parasitic, antimicrobial, anti-oxidant, anti-inflammatory potency, when they are parenteral and oral, applied in animal models (Araújo and Leon 2001). There are limited studies of β-glucan as feed additives in weaning pigs. We investigated 2 levels of β-glucan (5 and 10 g kg⁻¹) as a replacer to antibiotic in weaning pigs. We...
hypothesized that the use of β-glucan may improve the gut health status by acting as a novel source of prebiotics and have beneficial effects on the performance of weaned pig (Lam and Cheung 2013). The purpose of our research was to ascertain the impacts of β-glucans in influencing growth performance, apparent total tract digestibility, fecal microbial, fecal characteristics, and blood profiles in weaning pigs.

2. Materials and methods

The protocol used for the current experiment was approved by the Animal Care and Use Committee of Dankook University.

2.1. Source of β-glucan and antibiotic

β-glucan was provided by STR Biotech. Co., Ltd., Chunchon city, Kangwon-do, South Korea. The β-glucan products were extracted from *Morus Alba* and *Curcuma Longa*. The company quoted the product having 86.1% (1 → 3 β-glucan / 1 → 6 β-glucan), 1.3% lipids, and 4.2% protein. The antibiotic used in our experiment was provided by Novartis AG, Basel, Switzerland.

2.2. Experimental design, animals, housing, and diets

A total of 120 crossbred ([Yorkshire × Landrace] × Duroc) weanling pigs having an initial body weight (BW) of 8.36 ± 1.64 kg (21 d) were used in a 42 day trial. Pigs were randomly allotted to any 1 of 4 experimental diets (6 replicate pens/treatment, 5 pigs/pen). Experimental diets were: 1) CON, basal diet; 2) A1, basal diet + antibiotic (39 ppm tiamulin); 3) BG1, basal diet + 5 g kg⁻¹ (β-glucan); and 4) BG2, basal diet + 10 g kg⁻¹ (β-glucan). The diet composition of phase I (0 to 21 d) and phase II (22 to 42 d) is presented in Table 1. The diets were formulated as per the recommendation of NRC (1998). β-glucan and antibiotic were supplied by mixing the specified quantity in the experimental diet. All the pigs were enclosed in an environmentally controlled room (30°C) with a slatted plastic floor. The room temperature was decreased by 1°C for 5 wk. Stainless steel feeder and a nipple water drinker were provided in each pen for easy access to feed and water. Ventilation was provided by a mechanical system. Twelve hour of automatic artificial lighting was provided each day.

2.3. Sampling and measurement

The BW of piglet was assessed at the beginning of the trial and at the end of the d 21 and d 42. Feed intake was registered on a pen basis to calculate average daily feed intake (ADFI). Apparent total tract digestibility (ATTD) of dry matter (DM), nitrogen (N) and gross energy (GE) were determined by adding 0.2% of chromic oxide as the digestibility marker (Fenton and Fenton 1979). Pigs were fed with chromium mixed diet 7 d before the fecal samples collection. Fresh fecal samples were collected (2 pigs/replicate pens) via rectal massage at the completion of the d 21 and d 42. All the feed and fecal samples were placed on defreeze at −20°C until further analysis. Before chemical analysis, feed and fecal samples were dried in an electrical oven (model FC-610, Advantec, Toyo Seisakusho Co. Ltd., Tokyo, Japan) at 70°C for 72 hr and were ground in a 1-mm sieve. All feed and fecal samples were analyzed for DM (method 934.01, AOAC 2000) and N (method 968.0, AOAC 2000) by N analyzer (Kjtec 2300 N Analyzer; Foss Tecator AB, Höganäs, Sweden). The GE was calculated by measuring the heat generated due

Table 1. Feed composition of experimental diet (as-fed basis).

| Item                        | Phase I (0 to 21 d) | Phase II (22 to 42 d) |
|-----------------------------|--------------------|----------------------|
|                             | CON    | A1     | BG1    | BG2    | CON    | A1     | BG1    | BG2    |
| Extruded corn               | 44.49  | 44.49  | 44.49  | 44.49  | 61.97  | 61.97  | 61.97  | 61.97  |
| Soybean meal (48% CP)       | 21.20  | 21.20  | 21.20  | 21.20  | 27.80  | 27.80  | 27.80  | 27.80  |
| Fish meal (66% CP)          | 3.50   | 3.50   | 3.50   | 3.50   | 0.00   | 0.00   | 0.00   | 0.00   |
| Soy oil                     | 2.55   | 2.55   | 2.55   | 2.55   | 1.05   | 1.05   | 1.05   | 1.05   |
| Lactose                     | 8.30   | 8.30   | 8.30   | 8.30   | 0.00   | 0.00   | 0.00   | 0.00   |
| Whey                        | 10.00  | 10.00  | 10.00  | 10.00  | 5.00   | 5.00   | 5.00   | 5.00   |
| Di-calcium phosphate        | 1.50   | 1.50   | 1.50   | 1.50   | 1.50   | 1.50   | 1.50   | 1.50   |
| Sugar                       | 3.00   | 3.00   | 3.00   | 3.00   | 0.00   | 0.00   | 0.00   | 0.00   |
| Plasma powder (AP 920)      | 3.00   | 3.00   | 3.00   | 3.00   | 0.00   | 0.00   | 0.00   | 0.00   |
| L-Lys HCl (78%)             | 0.39   | 0.39   | 0.39   | 0.39   | 0.46   | 0.46   | 0.46   | 0.46   |
| DL-Met (50%)                | 0.30   | 0.30   | 0.30   | 0.30   | 0.20   | 0.20   | 0.20   | 0.20   |
| L-Thr (89%)                 | 0.19   | 0.19   | 0.19   | 0.19   | 0.20   | 0.20   | 0.20   | 0.20   |
| Choline chloride (25%)      | 0.10   | 0.10   | 0.10   | 0.10   | 0.10   | 0.10   | 0.10   | 0.10   |
| Vitamin premix              | 0.10   | 0.10   | 0.10   | 0.10   | 0.10   | 0.10   | 0.10   | 0.10   |
| Trace mineral premixb       | 0.20   | 0.20   | 0.20   | 0.20   | 0.20   | 0.20   | 0.20   | 0.20   |
| Lysine                      | 0.98   | 0.98   | 0.98   | 0.98   | 1.13   | 1.13   | 1.13   | 1.13   |
| Salt                        | 0.20   | 0.20   | 0.20   | 0.20   | 0.25   | 0.25   | 0.25   | 0.25   |
| Total                       | 100    | 100    | 100    | 100    | 100    | 100    | 100    | 100    |
| Calculated Nutritional Content: | 3,540 | 3,540  | 3,540  | 3,540  | 3,410  | 3,410  | 3,410  | 3,410  |
| ME, kcal/kg                 | 20.00  | 20.00  | 20.00  | 20.00  | 19.00  | 19.00  | 19.00  | 19.00  |
| CP, %                       | 1.50   | 1.50   | 1.50   | 1.50   | 1.35   | 1.35   | 1.35   | 1.35   |
| Lys, %                      | 0.62   | 0.62   | 0.62   | 0.62   | 0.53   | 0.53   | 0.53   | 0.53   |
| Met, %                      | 0.97   | 0.97   | 0.97   | 0.97   | 0.84   | 0.84   | 0.84   | 0.84   |
| Met + Cys %                 | 0.95   | 0.95   | 0.95   | 0.95   | 0.90   | 0.90   | 0.90   | 0.90   |

*Provided per kg of complete diet: vitamin A, 11,025 IU; vitamin D3, 1,103 IU; vitamin E, 44 IU; vitamin K, 4.4 mg; riboflavin, 8.3 mg; niacin, 50 mg; thiamine, 4 mg; d-pantothenic, 29 mg; choline, 166 mg; and vitamin B12, 33 μg.

bProvided per kg of complete diet: Cu (as CuSO₄·5H₂O), 12 mg; Zn (as ZnSO₄), 85 mg; Mn (as MnO₂), 8 mg; I (as KI), 0.28 mg; and Se (as Na₂SeO₃·5H₂O), 0.15 mg. AP920 is a spray-dried plasma powder obtained from American Protein Corporation, Ames, IA, USA.
to combustion in the samples by the use of oxygen bomb calorimeter (Parr 6100 Parr Instrument Co., Moline, IL). Chromium was analyzed by using UV absorption spectrophotometer (Shimadzu UV-12061, Shimadzu, and Kyoto, Japan).

### 2.4. Fecal microbial, fecal characteristics, and blood profile analysis

For fecal microbial analysis, fecal samples were grabbed from 2 pigs per pen via rectal massage at d 42. The samples were placed on ice and taken to the laboratory, where analysis was immediately carried out. One gram of composite fecal sample was moderated with 9 ml of 1% peptone broth (Becton, Dickinson and Co., Franklin Lakes, NJ) and then stirred for homogenization. The *Lactobacilli* medium III agar plates were used for isolation of *Lactobacillus*, and Mac-Conkey agar plates were used for isolation of *E.Coli*. The fecal samples were undergone through 10-fold dilutions (in 1% peptone solution). The sample was placed on *Lactobacilli* medium III agar plates and incubated for 48 hr at 39°C in an anaerobic environment. Similarly, the sample was placed on Mac-Conkey agar plates and placed in an incubator for 24 hr at 37°C. The viable counts of *E. Coli* and *Lactobacillus* colonies were noted immediately after removal from the incubator.

For fecal pH and moisture content, fecal samples were collected from pigs on different pens. The fecal pH and moisture content were analyzed on randomly collected fecal of 5 pigs per treatment at d 7, d 21 and d 42. Fecal moisture content was detected by placing the fecal samples in an electric oven at 60°C for 72 hr. pH values of fecal were measured by diluting 10 g of fecal using pH metre (Istek, Model 77p).

Blood samples (10 ml) were collected by randomly selecting 2 pigs from each pen (12 pigs per treatment) at d 7, d 21 and d 42 via anterior vena cava puncture. Blood samples were collected into both clot activator vacuum tubes and K3 EDTA vacuum tubes manufactured by Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA. White blood cells (WBC), lymphocyte (%), and Red blood cells (RBC) present in blood were detected by using an automatic blood analyzer manufactured by ADVIA120; Bayer, Tarrytown, NY.

### 2.5. Statistical analysis

All the experimental data were analyzed as a complete randomized block design by using General Linear Model (GLM) Procedure of SAS (SAS Inst. Inc., Cary, NC 1996). Repeated measures ANOVA was used to analyze the parameters which were measured over the course of the experiment. The pen was taken as the experimental unit. Duncan’s multiple range test was used for evaluation of difference among the treatments. Results were presented as the least squares means SEM, and statistically significant was considered at $P < 0.05$.

### 3. Result and discussion

#### 3.1. Growth performance

The effect of β-glucan on growth performance is shown in Table 2. In our study, the treatment diets A1, BG1 and BG2 did not show any effect ($P > 0.05$) on ADG (364 g, 364 g, 333 g) and G:F (0.74, 0.72, 0.68) in pigs during phase I (d 0 to d 21) as compared to CON (334 g, 0.67) diet. In phase II (d 22 to d 42), treatment diets A1, BG1, and BG2 that were fed to pigs significantly increased ($P < 0.05$) ADG (457 g, 463 g, 453 g) and G:F ratio (0.70, 0.68, 0.68) as compared to pigs fed with CON (393 g, 0.57) diet. The pigs fed A1 (385 g) and BG1 (388 g) diets had significantly higher ($P < 0.05$) ADG as compared to CON (242 g) during the overall experiment period.

### Table 2. The effect of β-glucan supplementation on growth performance in weanling pigs.

| Items     | CON   | A1    | BG1   | BG2   | SEM  | P-value |
|-----------|-------|-------|-------|-------|------|---------|
| Phase I (d 0 to d 21) |       |       |       |       |      |         |
| ADG, g    | 343   | 364   | 364   | 333   | 16   | NS      |
| ADFI, g   | 497   | 487   | 500   | 483   | 12   | NS      |
| G:F       | 0.67  | 0.74  | 0.72  | 0.68  | 0.03 | NS      |
| Phase II (d 22 to d 42) |       |       |       |       |      |         |
| ADG, g    | 393   | 457   | 463   | 453   | 17   | *       |
| ADFI, g   | 681   | 652   | 680   | 658   | 23   | NS      |
| G:F       | 0.57  | 0.70  | 0.68  | 0.68  | 0.02 | *       |
| Overall (d 0 to d 42) |       |       |       |       |      |         |
| ADG, g    | 342   | 385   | 388   | 371   | 11   | *       |
| ADFI, g   | 536   | 517   | 524   | 519   | 11   | NS      |
| G:F       | 0.63  | 0.74  | 0.74  | 0.71  | 0.02 | *       |

Abbreviation: CON: basal diet; A1: CON + antibiotic (39 ppm tiamulin); BG1: CON + 5 g kg−1 β-glucan; BG2: CON + 10 g kg−1 β-glucan; ADG: average daily gain; ADFI: average daily feed intake; G:F: gain feed ratio; SEM: Standard error of the means; NS: No significance; *: Significance.

$a$Means in the same row with different superscript differ significantly ($P < 0.05$).

In our study, the treatment diets A1, BG1 and BG2 did not show any effect ($P > 0.05$) on ADG (364 g, 364 g, 333 g) and G:F (0.74, 0.72, 0.68) in pigs during phase I (d 0 to d 21) as compared to CON (334 g, 0.67) diet. In phase II (d 22 to d 42), treatment diets A1, BG1, and BG2 that were fed to pigs significantly increased ($P < 0.05$) ADG (457 g, 463 g, 453 g) and G:F ratio (0.70, 0.68, 0.68) as compared to pigs fed with CON (393 g, 0.57) diet. The pigs fed A1 (385 g) and BG1 (388 g) diets had significantly higher ($P < 0.05$) ADG as compared to CON. In contrast, a decrease in growth (1st wk) was observed when 0.10% β-glucan was supplemented on weanling pigs but the addition of 0.025% or 0.05% β-glucan improved ADG and ADFI without affecting G:F (Dritz et al. 1995). Likewise, the supplementation of 0, 0.05, 0.075, 0.1 and 0.125% β-glucan on weanling pig (19 d) showed no improvement in growth performance until 2 wk, but overall ADG was improved when β-glucan was added at less than 0.1% in diet (d 0 to d 34 after weaning) (Schoenherr et al. 1994).

In case of β-glucan supplemented diet BG1 (388 g, 0.74) was significantly higher as compared to pigs fed with CON (342 g, 0.63) diet and consistent with pigs fed with antibiotic treatment diet A1 (385 g, 0.74) significantly increased ($P < 0.05$) ADG as compared to pigs fed with CON (242 g) during the overall experiment period.
which indicated that β-glucan had a positive effect on weaning pigs. The exact mechanism through which β-glucan supplementation improves pig growth performance is unclear (Li et al. 2006). The inclusion of β-glucan can increase interleukin-1 secretion in monocytes which activate immune system and may limit the growth performance (Poutsiaka et al. 1993; Dritz et al. 1995). β-glucan not only augment the secretion of interleukin-1, it can preferentially stimulate the secretion of interleukin-1 receptor antagonist. Interleukin-1 receptor antagonist is a macrophase cytokine which binds to type I interleukin-1 receptor and limits the cell activation (Hannum et al. 1990; Poutsiaka et al. 1993). If feeding β-glucan alters the balance of interleukin-1 and interleukin-1 receptor antagonist such that the antagonist is preferentially secreted, then one would expect less activation of the immune system during the feeding period, which could result in improved growth performance. Although these parameters are not measured in our study, based on the previous studies of Poutsiaka et al. (1993) and Hannum et al. (1990) we can assume that these might be the cause of increase in growth performance in our study. In a study by Gauldie and Baumann (1991), there was a decrease in haptoglobin (an acute-phase protein) production with the supplementation of 0.025 and 0.05% β-glucan. The decrease in haptoglobin indicates lower inflammatory production that leads to increase in weight gain of the pig, which might be another reason for increase in weight of the pigs fed with β-glucan (Eurell et al. 1992). In previous studies and our study, we found variation in the performance of β-glucan in terms of growth performance. A potential reason for this divergence may be due to the difference in molecular weight, cleanliness, and use of β-glucan that is extracted from different sources. The chemicals used in extraction could vary structure and chemical composition of β-glucan which would influence its bioactivity (Noppawat et al. 2017). Also, different doses included in the diet may produce different results.

3.2. Nutrient digestibility

The result for the effects of β-glucan on ATTD of DM, N and GE is shown in Table 3. The treatment diets A1, BG1 and BG2 had no significant effect ($P > 0.05$) on ATTD of DM (84.97, 84.37, 84.11%), N (82.79, 82.23, 82.93%) and GE (84.8, 84.54, 84.05%) as compared to CON (83.41, 81.58, 85.7%) in phase I (d 0 to d 21). However, digestibility of DM in pigs fed with BG1 (83.35%) and N content in pigs fed with A1 (81.84%) and BG1 (81.35%) was significantly higher ($P < 0.05$) than CON (81.36%, 78.54%) in phase II (d 22 to d 42). Gross energy in pigs fed with A1 (83.37%), BG1 (83.97%) and GB2 (81.79%) was not affected ($P > 0.05$) by any treatment diets in our experiment as compared to CON (82.02%). This indicated that weaning pig can be benefited with the supplementation of β-glucan on digestibility of nutrient. The beneficial effect on growth might have been attained by the improved digestibility of DM and N. Dry matter digestibility in pigs fed with 0.1% β-glucan (mulberry leaves and Curcuma) was higher than the control diet formulated with corn-soybean (Lee et al. 2017). But it was inconsistent with previous studies, as no effect on nutrient digestibility in growing-finishing pig was observed with the supplementation of β-glucan (0.2%) (Ko et al. 2000). A similar result was obtained by Bae et al. (1999), who did not observe any effect in nutrient digestibility with increasing the concentration of β-glucan. Yoo et al. (1985) concluded that there was no effect of antibiotic supplementation on nutrient digestibility of growing-finishing pigs. But antibiotics were found helpful in enhancing the nutrient digestibility and growth of weaned pig that were supplied with β-glucan (Hahn et al. 2006). In our current research, β-glucan supplemented diet BG1 showed a positive effect on DM digestibility in pigs, which is consistent with the previous study of Lee et al. (2017). Likewise, antibiotic supplemented diet A1 and β-glucan supplemented diet BG1 showed a positive effect on N digestibility in pigs than the pigs fed with CON diet on phase II. In a research by Li et al. (2006), plasma concentration of IL-10 was increased while decreasing the plasma concentration of TNF-α and IL-6 with the addition of 50 ppm of β-glucan. TNF-α and IL-6 are pro-inflammatory cytokines that modulates immunity as well as play a major role in nutrient metabolism (Spurlock 1997). If feeding β-glucan promotes secretion of anti-inflammatory cytokines, such as IL-10, and decreases secretion of pro-inflammatory cytokines, such as TNF-α and IL-6, then less activation of the immune system during the feeding period would be achieved, which could result in increased growth performance as observed in our study. This might be the possible reason for the increase in nutrient digestibility of our experiment.

3.3. Fecal microbial, fecal pH and fecal moisture content

The effect of fecal microbial counts in pigs fed with β-glucan supplemented diet is shown in Table 4. In our study, the fecal Lactobacillus counts in pigs fed with treatment diet A1 (8.21 log10 cfu/g) and BG2 (8.11 log10 cfu/g) increased significantly.

---

### Table 3. The effect of β-glucan supplementation on nutrient digestibility in weanling pigs.

| Items, %   | CON  | A1  | BG1  | BG2  | SEM  | P-value |
|-----------|------|-----|------|------|------|---------|
| Phase I (d 21) |      |     |      |      |      |         |
| Dry matter | 83.41 | 84.97 | 84.37 | 84.11 | 0.67  | NS      |
| Nitrogen   | 81.58 | 82.79 | 82.23 | 82.93 | 0.96  | NS      |
| Gross energy | 83.57 | 84.8 | 84.54 | 84.05 | 0.68  | NS      |
| Phase II (d 42) |      |     |      |      |      |         |
| Dry matter | 81.36$^a$b | 82.94$^{ab}$ | 83.35$^a$ | 81.91$^{ab}$ | 0.51  | $^*$     |
| Nitrogen   | 78.54$^{ab}$ | 81.84$^a$ | 81.35$^a$ | 79.66$^{ab}$ | 0.80  | $^*$     |
| Gross energy | 82.02 | 83.37 | 82.97 | 81.79 | 0.70  | NS      |

Abbreviation: CON: basal diet; A1: CON + antibiotic (39 ppm tiamulin); BG1: CON + 5 g kg$^{-1}$ β-glucan; BG2: CON + 10 g kg$^{-1}$ β-glucan; SEM: Standard error of the means. NS: No significance; $^*$: Significance.

$^{a,b}$Means in the same row with different superscript differ significantly ($P < 0.05$).

### Table 4. The effect of β-glucan supplementation on fecal microbial in weanling pigs.

| Items | CON  | A1  | BG1  | BG2  | SEM  | P-value |
|-------|------|-----|------|------|------|---------|
| d 42 |      |     |      |      |      |         |
| Lactobacillus | 7.43$^b$ | 8.21$^a$ | 7.86$^{ab}$ | 8.11$^a$ | 0.17  | $^*$     |
| E. coli | 5.51  | 4.99 | 5.32 | 5.28 | 0.18  | NS      |
| Salmonella | 2.19  | 2.08 | 2.13 | 2.11 | 0.04  | NS      |

Abbreviation: CON: basal diet; A1: CON + antibiotic (39 ppm tiamulin); BG1: CON + 5 g kg$^{-1}$ β-glucan; BG2: CON + 10 g kg$^{-1}$ β-glucan; SEM: Standard error of the means. NS: No significance; $^*$: Significance.

$^{a,b}$Means in the same row with different superscript differ significantly ($P < 0.05$).
<p>(P < 0.05) as compared to pigs fed with CON (7.43 log10cfu/g) diet. A numerical reduction of E. coli (5.51 log10cfu/g vs 4.99 log10cfu/g, 5.32 log10cfu/g, 5.28 log10cfu/g) and Salmonella (2.19 log10cfu/g vs 2.08 log10cfu/g, 2.13 log10cfu/g, 2.11 log10cfu/g) was observed by the antibiotic and β-glucan supplementation, but the effect was not significant (P > 0.05) as compared to CON. The antibiotic used in our experiment was a broad-spectrum antibiotic which acts against a wide range of disease caused by the bacteria. Lynch et al. (2007) reported that the addition of antibiotic could decrease fecal E. coli counts and increase the fecal Lactobacillus counts in weanling pig. There was a reduction in Enterobacteriaceae population (P < 0.05) when β-glucan (250 mg/kg) extracted from L. Hyperborea, L. Digitata, and S. Cerevisiae was added to the diet. But the addition of β-glucan did not have any effect (P > 0.05) on Lactobacilli and Bifidobacteria populations in the colon and ileum (Sweeney et al. 2012). The previous study of Lee et al. (2017) also found no effect on the microbial population when the pigs were fed with 0.1% β-glucan (mulberry leaves and Curcuma). But, our study result shows a significant increase (P < 0.05) in Lactobacillus population with the addition of 10 g kg<sup>−1</sup> β-glucan. However, no such significant effect was observed with supplementation of 5 g kg<sup>−1</sup> β-glucan. The previous study of O’Shea et al. (2010) have detected that the use of β-glucan (barley and oat-based) could influence to uplift the population of Lactobacillus. Lactobacillus are beneficial in competing and exclusion of pathogenic microorganisms that are present in the gastrointestinal tract epithelium of pigs and improve the host microbial balance along with nutrient digestibility. By stimulating phagocytosis, β-glucan helps to kill pathogenic bacteria (Stuyven et al. 2008). The use of β-glucan can enhance lactic acid production and growth of beneficial microbes (Arena et al. 2014). The results of the studies on broilers, pigs and rats have shown that lactic acid production in ceca could enhance the beneficial bacteria like Bifidobacteria spp. and Lactobacillus spp. by dominating pathogenic bacteria like Salmonella spp. and E.Coli (Bielecka et al. 2002; Patterson and Burkholer 2003; Yan et al. 2012; Hossain et al. 2016). This could be the reason for the proliferation of beneficial bacteria, Lactobacillus in our study. Therefore, the increase in Lactobacillus might be another reason for the better growth performance and nutrient digestibility of weanling pigs. However, dietary supplementation of β-glucans did not affect the fecal pH and fecal moisture content of weanling pigs (Table 5). The result of fecal characteristics corroborates with the findings of Lee et al. (2017), as they also did not find any effect with the supplementation of β-glucan.  

### Table 6. The effect of β-glucan supplementation on blood profiles in weanling pigs.

| Item (%) | CON | A1 | BG1 | BG2 | SEM | P-value |
|----------|-----|----|-----|-----|-----|---------|
| White blood cell (×10<sup>3</sup>/μl) |     |    |     |     |     |         |
| d 7      | 12.60 | 12.50 | 12.30 | 12.15 | 1.60 | NS      |
| d 21     | 14.19<sup>a</sup> | 19.55<sup>b</sup> | 21.18<sup>b</sup> | 18.12<sup>ab</sup> | 1.57<sup>a</sup> | *       |
| d 42     | 18.50 | 20.30 | 18.75 | 19.21 | 2.95<sup>NS</sup> |         |
| Red blood cell (×10<sup>6</sup>/μl) |     |    |     |     |     |         |
| d 7      | 43.50 | 44.10 | 46.40 | 43.40 | 3.90 | NS      |
| d 21     | 46.55 | 47.26 | 48.12 | 47.29 | 2.45 | NS      |
| d 42     | 60.50 | 62.30 | 60.60 | 55.32 | 2.53<sup>NS</sup> |         |

Abbreviation: CON: basal diet; A1: CON + antibiotic (39 ppm tiamulin); BG1: CON + 5 g kg<sup>−1</sup> β-glucan; BG2: CON + 10 g kg<sup>−1</sup> β-glucan; SEM: Standard error of the means. NS: No significance; *: Significance.

**Means in the same row with different superscript differ significantly (P < 0.05).**

### 3.4. Blood profiles

The effects of β-glucan supplementation on blood profile are shown in Table 6. In our study, supplementation of β-glucan resulted in a significant increase (P < 0.05) of white blood cell in pigs fed with A1 (19.55 × 10<sup>3</sup>/μl), BG1 (21.18 × 10<sup>3</sup>/μl) and BG2 (18.12 × 10<sup>3</sup>/μl) respectively at d 21 (P < 0.05) as compared to pigs fed with CON (14.19 × 10<sup>3</sup>/μl) diet. But no such effect was observed in d 7 and d 42 of the experiment. No significant difference (P > 0.05) was noted in the concentrations of red blood cell and lymphocyte percentage among treatment diets. In previous studies, Ewaschuk et al. (2012) found that dietary β-glucan (barley derived) supplementation on weaning pig increased the concentration of peripheral erythrocytes, leukocytes, and lymphocytes in the blood. However, in the study of Cho et al. (2013) on broiler chickens, the addition of β-glucan (0.1%) had no such effect on WBC, RBC, lymphocyte, and IgG concentrations. The results were supported by the results of Chae et al. (2006), An et al. (2008) and Cox et al. (2010), who did not observe any effect on the blood parameters being measured. In a recent study of Lee et al. (2017), no effect was observed in the RBC, WBC and lymphocyte % with the supplementation of 0.1% β-glucans (mulberry leaves and Curcuma). It supports that supplementing pig diet with β-glucans had no adverse effect on the health of weaned pigs.

### 4. Conclusion

Taken together, β-glucan supplemented diet (BG1) improved the growth performance, nutrient digestibility of DM and N, fecal Lactobacillus counts and WBC concentrations in the blood compared with control. Furthermore, β-glucan supplementation showed comparable effects as antibiotic indicating β-glucan can be used an alternative feed additive in the diet of weaning pig.

### Disclosure statement

No potential conflict of interest was reported by the authors.
References

Aarestrup FM, Carstensen B. 1998. Effect of tylosin used as a growth promoter on the occurrence of macrolide resistant enterococci and staphylococci in pigs. Microb Drug Resist. 4:307–312.

An BK, Cho BL, You SJ, Paik HD, Chang HI, Kim SW, Yun CW, Kang CW. 2008. Growth performance and antibody response of broiler chicks fed yeast derived glucans and single-strain probiotics. Asian-Australas J Anim Sci. 21:1027–1032.

AOAC. 2000. Official method of analysis. 17th ed. Gaithersburg (MD): Association of Official Analytical Chemists.

Araújo CAC, Leon LL. 2001. Memórias do Instituto Oswaldo Cruz biological activities of Curcum a longa L. Mem Inst Oswaldo Cruz. CRIA, Brazil. 96:723–728.

Arena MP, Caggianello G, Fiocco D, Russo P, Torelli M, Spono G, Capozzi V. 2014. Barley β-glucans-containing food enhances probiotic performances of beneficial bacteria. Int J Mol Sci. 15(2):3025–3039.

Bae KH, Ko TG, Kim JH, Cho WT, Han YK, Han IK. 1999. Use of metabolically active substances to substitute for antibiotics in finishing pigs. Korean J Anim Sci. 41:23–30.

Bieklecka M, Biedrzycka E, Majkowska A, Juskiewicz J, Wroblewska M. 2002. Effect of non-digestible oligosaccharides on gut microecosystems in rats. Food Res Int. 35:139–144.

Bohn JA, BeMiller JN. 1995. (1–3)-β-D-Glucans as biological response modifiers: a review of structure-functional activity relationships. Carbohydr Polym. 28:3–14.

Campbell JM, Crenshaw JD, Javier P. 2013. The biological stress of early weaned piglets. J Anim Sci Biotech. 4:19.

Chae BJ, Lohakare JD, Moon WK, Lee SL, Park YH, Hanh TW. 2006. Influence of dietary β-glucan on growth performance and immunity in broilers. Res Vet Sci. 80:291–298.

Cho JH, Zhang ZF, Kim IH. 2013. Effects of single or combined dietary supplementation of β-glucan and kafr on growth performance, blood characteristics and meat quality in broilers. Br Poult Sci. 54:216–221.

Cox CM, Summers LH, Kim S, Mcelroy AP, Bedford MR, Dalloul RA. 2010. Immune responses to dietary β-glucan in broiler chicks during an Eimeria challenge. Poult Sci. 89:2597–2607.

Cromwell GL. 2001. Antimicrobial and promicrobial agents, wine nutrition. CRC Press LLC; p. 401–426.

Decuyper J, Dierick NJ, Boddez S. 1998. The potentials for immunomodulatory substances (beta-1,3/1,6 glucans) in pig nutrition. Anim Feed Sci Technol. 72:259–265.

DiLuzio NR. 1983. Immunopharmacology of glucan: a broad spectrum enhancer of host defense mechanisms. Trends Pharmacol Sci. 4:344–347.

Dritz SS, Shi J, Kielian TL, Goodband RD, Nelssen JL, Tokach MD, Chengappa LM. 1983. Immunopharmacology of glucan: a broad spectrum antagonist activity of a human interleukin-1 inhibitor. Nature (Lond). 343:336–340.

Hiss S, Sauerwein H. 2003. Influence of dietary β-glucan on growth performance, lymphocyte proliferation, specific immune response, and haptoglobin plasma concentrations in pigs. J Anim Physiol Anim Nutr (Berl). 87:2–11.

Hossain MM, Begum M, Kim IH. 2016. Effect of Leuconostoc mesenteroides KCCM35046 fermented aged garlic extract on egg production, egg quality, odour gas emissions, targeted E. coli colony, haematological characteristics and fatty acids composition of egg yolk in laying hens. J Appl Anim Res. 44:458–465.

John A, Bohn J, BeMiller N. 1995. (1-4)-β-D-Glucans as biological response modifiers: a review of structure-functional activity relationships. Carbohydr Polym. 28:3–14.

Jung K, Ha Y, Ha SK, Han DU, Kim DW, Moon WK, Chae C. 2004. Antiviral effect of Saccharomycetes cerevisiae β-glucan to swine influenza virus by increased production of interferon-α and nitric oxide. J Vet Med B. 51:72–76.

Ko TG, Kim JD, Han YK, Han IK. 2000. Study for the development of antibiotics-free diet for growing pigs. Korean J Anim Sci. 42:45–54.

Lam KL, Cheung PCK. 2013. Non-digestible long chain beta-glucans as novel prebiotics. Bioact Carbohydr Dietary Fibre. 2:1–45–64.

Lee SI, Kim JK, Hancock JD, Kim IH. 2017. β-glucan from mulberry leaves and curcuma can improve growth performance and nutrient digestibility in early weaned pigs. J Appl Anim Res. 45:209–214.

Li J, Li DF, Xing JJ, Cheng ZB, Lai CH. 2006. Effects of beta-glucan extracted from Saccharomycetes cerevisiae on growth performance, and immunological and somatotropic responses of pigs challenged with Escherichia coli lipopolysaccharide. J Anim Sci. 84:2374–2381.

Lynch MB, Sweeney T, Callan JJ, O’Doherty JV. 2007. Effects of increasing the intake of dietary β-glucans by exchanging wheat for barley on nutrient digestibility, nitrogen excretion, intestinal microflora, volatile fatty acid concentration and manure emissions in finishing pigs. Animal. 1:812–819.

Newman RK, Eislick RF, Peppar JW, El-Negoumy AM. 1980. Performance of pigs fed hulled and covered barleys supplemented with or without bacterial diastase. Nutr Reports Int. 22:833–837.

Noppawat P, Bhagavathi SS, Sasithorn S, Sartjin P, Periyanaina K, Khontaroski C, Chaiyavat C. 2017. Extraction of β-glucan from Saccharomycetes cerevisiae: comparison of different extraction methods and in vivo assessment of immunomodulatory effect in mice. Food Sci Technol Campinas. 37(1):124–130.

NRC. 1998. Nutrient requirements of swine. 10th Rev. National Academy Press, editor. Washington (DC): National Academy Press.

Ohno N, Kurachi K, Yadamoe T. 1987. Anti-tumor activity of highly branched (1, 3)-β-D-glucan, SSG, obtained from Schizotinia sierotium IFO 9395. J Pharmacobio-dynam. 10:478–486.

O’Shea CJ, Sweeney T, Lynch MB, Gahan DA, Callan JJ, O’Doherty JV. 2010. Effect of β-Glucans contained in barley- and oat-based diets and exogenous enzyme supplementation on gastrointestinal fermentation of finisher pigs and subsequent manure odor and ammonia emissions. J Anim Sci. 88(4):1141–1140.

Patterson JA, Burkholder KM. 2003. Application of prebiotics and probiotics in poultry production, Poult Sci. 82:627–631.

Poutsiaka DD, Mengozzi M, Sinha B, Dinarello CA. 1993. Linking of the β-glucan receptor on human monocytes results in interleukin-1 receptor antagonist but not interleukin-1 production. Blood. 82:3695.

Salem MZM, Gohar HAY, El-Sayed AW. 2013. Biological activity of extracts from Morus alba L., Albizzia lebbek (L.) Benth. and Casuarina glauca Sieber against the growth of some pathogenic bacteria. Int J Agr Food Sci. 2:1.

SAS. 1996. SAS user’s guide. Release 6.12 ed. Cary (NC): SAS Inst. Inc.

Schoenherr WD, Pollmann DS, Coalson JA. 1994. Titration of macro gard-S on measurements of gut health: selected bacterial characteristics and fatty acids composition of egg yolk in laying hens. J Appl Anim Res. 44:458–465.

Shin MS, Lee S, Lee KY, Lee HJ. 2005. Structural and biological characterization of aminated-derivatized oat beta-glucan. J Agric Food Chem. 53(14):5554–5558.

Simon O, Vilfried V, Scharek L. 2003. Micro-organisms as feed additives – probiotics. In Proc: 9th Intern. Symp. on Digestive Physiology in Pigs; Banff, AB, Canada. p. 295–318.

Smith AG, O’Doherty JV, Reilly P. 2011. The effects of laminarin derived from Laminaria digitata on measurements of gut health: selected bacterial
populations, intestinal fermentation, mucin gene expression and cytokine gene expression in the pig. Br J Nutr. 105:669–677.

Spurlock ME. 1997. Regulation of metabolism and growth during immune challenge: an overview of cytokine function. J Anim Sci. 75:1773–1783.

Stuyven E, Cox E, Vancaeneghem S, Arnouts S, Deprez S, Goddeeris BM. 2008. Effect of B-glucans on an ETEC infection in piglets. Vet Immunol Immunopathol. 128:60–66.

Sweeney T, Collins CB, Reilly P, Pierce KM, Ryan M, O’Doherty JV. 2012. Effect of purified b-glucans derived from *Laminaria digitata*, *Laminaria hyperborea* and *Saccharomyces cerevisiae* on piglet performance, selected bacterial populations, volatile fatty acids and pro-inflammatory cytokines in the gastrointestinal tract of pigs. Br J Nutr. 108:1226–1234.

Williams BA, Verstegen MWA, Tamminga S. 2001. Fermentation in the large intestine of single-stomached animals and its relationship to human health. Nut Res Rev. 14:207–227.

Yan L, Meng QW, Kim IH. 2012. Effect of an herb extract mixture on growth performance, nutrient digestibility, blood characteristics, and fecal microbial shedding in weanling pigs. Livest Sci. 145:189–195.

Yoo MI, Han IK, Won KK, Sohn KS, Kang SW. 1985. Growth stimulating effect of Virginia mycin for growing-finishing swine. Korean J Anim Sci. 27:284–290.