β-glucan from mulberry leaves and curcuma can improve growth performance and nutrient digestibility in early weaned pigs

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**ABSTRACT**

We investigate the effects of dietary supplementation of β-glucans from mulberry leaves and curcuma on growth performance, nutrient digestibility, blood characteristics, and characteristics of faeces in weaned pigs. A total of 75 crossbred weaned pigs [(Yorkshire × Landrace) × Duroc] with an average body weight (BW) of 8.48 ± 1.65 kg was used in a 5 wk trial. The pigs were sorted into pens with five pigs per pens and five pens per treatments. Treatments were (1) a corn-soybean meal-based control, (2) 0.1% β-glucans from mulberry leaves, and (3) 0.1% β-glucans from curcuma. Pigs fed β-glucans from mulberry leaves and curcuma had higher average daily gain (ADG) and gain/feed intake ratio (G/F) than control. On digestibility, pigs fed β-glucans from mulberry leaves and curcuma had higher on digestibility of dry matter (DM) and energy than corn-soybean meal-based control during 2 weeks. No significant differences were observed on blood characteristics and faecal microflora, score, moisture, and pH among treatments. Difference of mulberry leaves and curcuma was not observed on growth performance, nutrient digestibility, blood characteristics, and characteristics of faeces. In conclusion, dietary supplementation of β-glucan from mulberry leaves and curcuma can improve ADG, G/F, and dry matter and energy of nutrient digestibility in weaned pigs. Therefore, β-glucans can use as antibiotics alternatives, improving the productivity.

1. Introduction

The mechanism of antibiotics was involved with the fat and protein content of the feed and use of antibiotics in animal feed can moderate carcass quality (Humphrey et al. 2002; Walker et al. 2005; Pedroso et al. 2006; Yan & Kim 2012; Li & Kim 2013; Zhao et al. 2013a; Zhou et al. 2013a). Because of recent limitation of antibiotics addition, new feed additives such as herbs, spices, prebiotics, and probiotics have received increased attention as possible alternatives to antibiotics (Windsch et al. 2008; Chu et al. 2011; Huang et al. 2012; Yan et al. 2012; Zhang et al. 2012; Liu et al. 2013; Wang et al. 2013; Zhang & Kim 2013; Zhao et al. 2013b; Cho & Kim 2014; Park & Kim 2014; Zhang & Kim 2014).

β-glucan, a polysaccharide of D-glucose monomers linked by β-glycosidic bonds, is present in cellulose in plants, the bran of cereal grains, the cell wall of yeast, fungi, and bacteria. β-glucan can activate the immune system and stimulates a cascade of pathways that enhance both innate and adaptive immune responses (Vannucci et al. 2013). It is well studied that dietary supplementation of β-glucan impacted several gastrointestinal events and growth performance in pigs. β-glucans in oat-based diets resulted in lower digestibility of protein and fat in the small intestine of young pigs (Knudsen et al. 1993). In other report, dietary supplementation of β-glucans in young pigs showed a detrimental effect on digestibility, feed efficiency, and subsequent growth in young pigs (Newman et al. 1980; Graham et al. 1989).

β-glucans from different sources such as oat, fungi, and mushrooms result in variable effects on growth and immune parameters (Dritz et al. 1995; Decuyper et al. 1998; Fortin et al. 2003; His & Sauerwein 2003). Thus, the source of dietary β-glucan used in animal nutrition may be an important factor that can influence its efficacy. In the present study, we used water-soluble β-glucan from mulberry leaves and curcuma. It has been reported that mulberry leaves have anti-inflammatory effects (Lim et al. 2013). Additionally, curcuma is an Indian spice derived from the rhizomes of the plant that has pharmacological activities, mainly anti-inflammatory and anti-proliferative (Dulbecco & Savarino 2013). Thus, we used β-glucan from mulberry leaves and curcuma to evaluate and compare the effect of β-glucans from two different sources as prebiotics.

Thus, the objective of the present study was to determine the effect of β-glucans from mulberry leaves and curcuma on growth performance, nutrient digestibility, blood characteristics, and characterization of feces (score, moisture, pH, and microflora) in weaned pigs.

2. Materials and methods

The Animal Care and Use Committee of Dankook University approved all experimental protocols used in the current study.
2.1. Preparation of β-glucan

β-glucan products were provided by a commercial company (STR Biotech. Co., Ltd., Chuncheon city, Kangwon-do, South Korea). The β-glucan products were from Morus alba and Curcuma longa, and were guaranteed to contain 86.1% β-1, 3/1, 6-glucan, 4.2% protein, and 1.3% lipids.

2.2. Experimental design, animals, and diets

A total of 75 crossbred weaned pigs ([Yorkshire × Landrace] × Duroc) with an average BW of 8.48 ± 1.65 kg was used in a 5 wk trial. The pigs were sorted into pens with five pigs per pens and five pens per treatments. Treatments were (1) a corn-soybean meal-based control, (2) 0.1% β-glucan from mulberry leaves, and (3) 0.1% β-glucan from curcuma. All diets were formulated to meet or exceed the NRC (2012) nutrition requirement (Table 1). Dietary calcium (Ca), phosphorus (P), and crude protein (CP) were analysed according to the procedures described by the AOAC (2012). Dietary Ca was assayed by atomic absorption spectrophotometry after wet ash procedures and P was determined by colourimetry. Amino acids contents were measured using an amino acid analyzer (Beckman 6300, Beckman Coulter, Inc., Fullerton, California, USA) after 24 h N-HCl hydrolysis at 110°C (AOAC, 2012). Energy was determined by using a Parr 6100 oxygen bomb calorimeter (Parr Instrument Co., Moline, Illinois, USA). All pigs were housed in an environmentally controlled room with a slatted plastic floor. Each pen was equipped with a self-feeder and nipple waterer to allow ad libitum access to feed and water throughout the experimental period. Temperature during 1 week was maintained at 32°C and was lowered to 2.5°C each week thereafter.

2.3. Sampling and measurements

Individual pig BW was recorded at the beginning, d14, and d35 of the experimental period, and feed consumption was recorded on a pen basis during the experiment to calculate average daily gain (ADG), average daily feed intake (ADFI), and G:F. During the experimental period, pigs were fed diets mixed with 2% Cr2O3 (chromic oxide) as an indigestible marker for the determination of apparent total tract digestibility (ATTD) for DM and nitrogen (N). On d14 and d35, faecal samples were collected from at least two pigs in each pen via rectal massage. All feed and fecal samples were stored at −20°C until analysis. Faeces samples were thawed at 57°C for 72 h, ground to pass through a 1-mm screen, and analysed for DM and nitrogen (N). Faecal moisture contents were determined by randomly collecting faeces from each pen at week 2 and the end of the experiment via massaging the rectum. The collected faecal samples were dried at 60°C for 72 h to allow the determination of faecal moisture content. Faecal pH was determined with pH-meter by diluting 10 g of faeces collected at weeks 2 and 5 (Istek, Model 77p).

Faecal samples were collected via rectal massage from two pigs in each pen and pooled, placed on ice transported to the laboratory, and analysed for microfloral counts. Viable counts of bacteria in the faecal samples were determined by plating serial 10-fold dilutions (in 1% peptone solution) onto MacConkey agar plates (Difco Laboratories, Detroit, MI) and Lactobacilli medium III agar plates (Medium 638, DSMZ, Braunschweig, Germany) to isolate Escherichia coli and Lactobacillus, respectively. The lactobacilli medium III agar plates were then incubated for 48 h at 39°C under anaerobic conditions. The MacConkey agar plates were incubated for 24 h at 37°C. The Escherichia coli and Lactobacillus colonies were counted immediately after removal from the incubator.

2.4. Statistical analysis

All data were subjected to statistical analyses as a randomized complete block design using the GLM procedure of the SAS software, with the pen as the experimental unit. Orthogonal contrasts used to separate treatments means were (1) control vs. β-glucans from mulberry leaves and curcuma and

| Item                         | Phase I (d1–d14) | Phase II (d15–d35) |
|------------------------------|------------------|--------------------|
| Ingredient, %                |                  |                    |
| Extruded corn                | 44.49            | 61.97              |
| Soybean meal (48% CP)        | 21.20            | 27.80              |
| Fish meal (66% CP)           | 3.50             | –                  |
| Soy oil                      | 2.55             | 1.05               |
| Lactose                      | 8.30             | –                  |
| Whey                         | 10.00            | 5.00               |
| Monocalcium phosphate        | –                | –                  |
| Decalcium phosphate          | 1.50             | 1.50               |
| Sugar                        | 3.00             | –                  |
| Plasma powder (AP 920)       | 3.00             | –                  |
| L-Lysine HCl                 | 0.39             | 0.46               |
| DL-Methionine                | 0.30             | 0.24               |
| L-Threonine                  | 0.19             | 0.20               |
| Choline chloride             | 0.10             | 0.10               |
| Vitamina                     | 0.10             | 0.10               |
| Mineralsb                    | 0.20             | 0.20               |
| Limestone                    | 0.98             | 1.13               |
| Salt                         | 0.20             | 0.25               |
| Total                        | 100              | 100                |
| Calculated content, %        |                  |                    |
| ME, kcal/kg                  | 3540             | 3410               |
| CP                           | 20.00            | 19.00              |
| Lys                          | 1.50             | 1.35               |
| Met                          | 0.62             | 0.53               |
| Met + Cys                    | 0.97             | 0.84               |
| Ca                           | 0.95             | 0.90               |
| Total P                      | 0.75             | 0.70               |
| Avail P                      | 0.55             | 0.43               |
| Crude fat                    | 5.02             | 3.98               |
| Crude fibre                  | 1.87             | 2.45               |

*aProvided per kg of complete diet: vitamin A, 11,025 IU; vitamin D3, 1103 IU; vitamin E, 44 IU; vitamin K, 4.4 mg; riboflavin, 8.3 mg; niacin, 50 mg; thiamine, 4 mg; pantothenic acid, 29 mg; choline, 166 mg; and vitamin B12, 33 μg.

*bProvided per kg of complete diet: Cu, 12 mg; Zn, 85 mg; Mn, 8 mg; I, 0.28 mg; and Se, 0.15 mg.
Table 2. The effects of β-glucan from mulberry leaves and curcuma on growth performance in weaning pigs.

| Items       | Control | Mulberry leaves | Curcuma | SEM | Control vs. β-glucans | Mulberry leaves vs. curcuma |
|-------------|---------|----------------|---------|-----|-----------------------|---------------------------|
| Phase 1 (d1–d14) |         |                |         |     |                       |                           |
| ADG, g      | 267     | 286            | 307     | 10.18 | 0.041                 | 0.170                     |
| ADFI, g     | 330     | 334            | 340     | 7.81  | 0.481                 | 0.604                     |
| G/F         | 0.809   | 0.856          | 0.903   | 0.02  | 0.007                 | 0.057                     |
| Phase 2 (d15–d35) |       |                |         |     |                       |                           |
| ADG, g      | 444     | 493            | 510     | 34.01 | 0.199                 | 0.730                     |
| ADFI, g     | 865     | 905            | 917     | 48.58 | 0.457                 | 0.868                     |
| G/F         | 0.513   | 0.545          | 0.556   | 0.03  | 0.293                 | 0.919                     |
| Overall (d1–d35) |      |                |         |     |                       |                           |
| ADG, g      | 373     | 410            | 429     | 23.01 | 0.133                 | 0.576                     |
| ADFI, g     | 651     | 677            | 686     | 30.97 | 0.441                 | 0.834                     |
| G/F         | 0.573   | 0.606          | 0.625   | 0.03  | 0.176                 | 0.693                     |

Table 3. The effects of mulberry leaves and curcuma on nutrient digestibility in weaning pigs.

| Items       | Control | Mulberry leaves | Curcuma | SEM | Control vs. β-glucans | Mulberry leaves vs. curcuma |
|-------------|---------|----------------|---------|-----|-----------------------|---------------------------|
| 2 weeks     |         |                |         |     |                       |                           |
| DM, %       | 80.5    | 81.7           | 83.4    | 0.65 | 0.026                 | 0.090                     |
| N, %        | 79.7    | 80.2           | 82.9    | 1.22 | 0.246                 | 0.145                     |
| Energy, %   | 80.9    | 81.9           | 83.2    | 0.61 | 0.046                 | 0.183                     |
| 5 weeks     |         |                |         |     |                       |                           |
| DM, %       | 80.1    | 81.9           | 83.0    | 0.11 | 0.098                 | 0.493                     |
| N, %        | 79.7    | 81.7           | 83.1    | 1.58 | 0.174                 | 0.543                     |
| Energy, %   | 80.2    | 82.3           | 83.4    | 1.12 | 0.071                 | 0.496                     |

No significant differences were observed on lymphocyte content among all treatments during the experimental period. No significant differences were observed on RBS content among all treatments during the experimental period. No significant differences were observed on WBC content among all treatments during the experimental period.

No significant differences of β-glucans effect on lymphocyte, RBC, and WBC in the experimental period were found as compared to mulberry leaves with curcuma (Table 4). Pigs fed β-glucans from mulberry leaves and curcuma had no difference on faecal characteristics (score, moisture, pH, microflora contents compared to control (Table 5)).

4. Discussion

It is increasing limitation for livestock producers to minimize the use of antibiotics. Prebiotics or probiotics have been the subject of much research as potential replacements for antibiotic growth promoters in livestock. Prebiotics, primarily derived from non-digestible oligosaccharides, are non-digestible food substances that selectively stimulate the growth of favourable species of bacteria in the gut, benefitting the host. It is well reported that Oligo-fructose, fructo-oligosaccharide, inulin, and β-glucan have been used as prebiotics (Kaplan & Hutkins 2000; Smiricky-Tjardes et al. 2003; Loh et al. 2006; Arena et al. 2014). It is well known that β-glucan acts as an immunostimulant to activate immune cells by binding to its specific receptor dectin-1, a c-type lectin receptor expressed on the surface of macrophages (Brown et al. 2002; Vos et al. 2007). Yadav and Schorey (2006) demonstrated that the β-glucan/dectin-1 complex activates macrophages in combination with TLR2.
Faecal micro

ora

Table 4. The effects of β-glucan from mulberry leaves and curcuma on blood characteristics in weaning pigs.

| Items                | Control | Mulberry leaves | Curcuma | SEM  | Control vs. β-glucans | Mulberry leaves vs. curcuma |
|----------------------|---------|-----------------|---------|------|-----------------------|-----------------------------|
| Lymphocyte, %        |         |                 |         |      |                       |                             |
| 0 week               | 46.8    | 45.8            | 46.1    | 4.34 | 0.875                 | 0.972                       |
| 2 weeks              | 58.0    | 58.6            | 55.4    | 3.46 | 0.819                 | 0.524                       |
| 5 week               | 60.8    | 62.6            | 60.0    | 2.54 | 0.633                 | 0.866                       |
| RBC, 10^6/ul         |         |                 |         |      |                       |                             |
| 0 week               | 6.3     | 6.2             | 6.2     | 0.13 | 0.747                 | 0.873                       |
| 2 weeks              | 6.0     | 5.9             | 6.1     | 0.13 | 0.964                 | 0.490                       |
| 5 weeks              | 6.2     | 6.2             | 6.2     | 0.12 | 0.878                 | 0.701                       |
| WBC, 10^3/ul         |         |                 |         |      |                       |                             |
| 0 week               | 12.7    | 12.6            | 12.2    | 0.90 | 0.810                 | 0.765                       |
| 2 weeks              | 16.0    | 14.1            | 14.4    | 1.52 | 0.380                 | 0.896                       |
| 5 weeks              | 18.5    | 20.6            | 20.2    | 0.91 | 0.129                 | 0.762                       |

Table 5. The effects of β-glucan from mulberry leaves and curcuma on characterization of feces (score, moisture, pH, and microflora) in weaning pigs.

| Items          | Control | Mulberry leaves | Curcuma | SEM  | Control vs. β-glucans | Mulberry leaves vs. curcuma |
|----------------|---------|-----------------|---------|------|-----------------------|-----------------------------|
| Faecal score a| 3.2     | 3.1             | 3.1     | 0.04 | 0.351                 | 0.216                       |
| Moisture, %    |         |                 |         |      |                       |                             |
| 2 weeks        | 69.7    | 69.7            | 70.3    | 1.47 | 0.882                 | 0.810                       |
| 5 weeks        | 68.6    | 68.1            | 70.0    | 1.02 | 0.738                 | 0.229                       |
| pH             |         |                 |         |      |                       |                             |
| 2 weeks        | 6.1     | 6.1             | 6.1     | 0.03 | 0.751                 | 0.312                       |
| 5 weeks        | 5.9     | 5.8             | 5.8     | 0.05 | 0.312                 | 0.218                       |
| Faecal microflora |       |                 |         |      |                       |                             |
| 2 weeks        | Lactobacillus | 7.5 | 7.6            | 7.5    | 0.06 | 0.290                 | 0.375                       |
|                | E. coli  | 6.7             | 6.9     | 6.8  | 0.05 | 0.107                 | 0.755                       |
| 5 weeks        | Lactobacillus | 7.6 | 7.7            | 7.6    | 0.05 | 0.272                 | 0.386                       |
|                | E. coli  | 6.7             | 6.8     | 6.8  | 0.05 | 0.106                 | 0.769                       |

* Faecal score ranges from 1 to 5, with 1 = hard and dry pellet but small mass, 2 = hard and formed stool, 3 = soft and formed stool but moist, 4 = soft and unformed stool, and 5 = watery and liquid stool.

and its signaling pathway, which causes a pro-inflammatory response by secreting TNF-α. In pig diets, β-glucan could benefit growth performance and immune function. Zhou et al. (2013b) demonstrated that dietary supplementation with β-glucan increased plasma leucocytes counts, increased lymphocyte proliferation activity and decreased TNF-α concentration and faecal E. coli numbers, whereas the growth performance and faecal Lactobacillus spp. counts were unaffected in weaned pigs.

In the present study, pigs fed the β-glucans had higher growth performance than the control. Many studies reported that β-glucan supplementation enhanced growth performance in pigs (Dritz et al. 1995; Li et al. 2006). Dritz et al. (1995) reported that supplementing nursery-pig diets with 0.025% β-glucan increases growth performance. They suggested that a complex interaction exists between growth performance and disease susceptibility in pigs fed β-glucan. Li et al. (2006) indicated that the addition of β-glucan to weaned pig diets is able to offer some benefits on growth performance and immune response to a lipopolysaccharide challenge. However, Hahn et al. (2006) reported that increasing the dietary concentrations of β-glucan did not improve ADG without antibiotic in weaning pigs. β-glucans produced by different production methods may have different effects on growth performance and immune function in weaned piglets. Source of β-glucan produced by different methods may vary in their structure, chemical composition, or both, which may influence its activity and the amount that should be added to get a growth response (Dritz et al. 1995; Fortin et al. 2003; Hiss & Sauerwein 2003; Li et al. 2006; Dulbecco & Savarino 2013).

In the present study, pigs fed the β-glucans had higher digestibility of DM. The effects of the β-glucan diets on nutrient digestibility in pigs were inconsistent with previous studies. Hahn et al. (2006) reported that increasing the dietary concentrations of β-glucan did not improve nutrient digestibility without antibiotic, and in weaned pigs antibiotics seem to be more effective in improving nutrient digestibility and growth performance than β-glucan (Hahn et al. 2006). Brennan and Cleary (2005) demonstrated that cereal mixed-linked β-(1,3)–(1,4)-d-glucan is regarded as potentially detrimental in pig production because of their negative effects on nutrient digestibility and pig performance. Metzler-Zebeli et al. (2011) reported that dietary inclusion of oat β-glucan can benefit the composition and metabolic activity of gastric microbiota, caecal, and colonic microbiota. In other studies, mixed-linked β-glucan, supplemented either in the form of cereals or as a concentrate, was readily fermented, reduced the intestinal number of enterobacteria and increased intestinal butyrate concentrations in growing pigs (Lynch et al. 2007; Metzler-Zebeli et al. 2010).

5. Conclusion

The present study indicated that dietary supplementation of β-glucans from mulberry leaves and curcuma can improve growth performance and nutrient digestibility in weaned pigs.
Therefore, we can expect that β-glucans have antibiotic alternative effects, also improve the productivity.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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