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Effect of Supplementation of Probiotic and Beta-Glucan to Antibiotic-Free diets on Growth Performance, Nutrients Digestibility, and Blood Characteristics in Weaned Pigs

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Abstract

The objective of this study was to determine the impact of different supplementation of beta-glucan (β -glucan) and probiotics alone or combined on feed intake, nutrient digestibility, blood characteristics and growth performance in weaned pigs. Three hundred sixty pigs weaned at 25 days of age (average body weight, 6.4kg) were used in a 21 d trial. The treatments consisted of a control (antibiotic-free corn-soy diet), a beta-glucan diet (0.25% β -glucan), a T-probiotics diet (0.2% two-strain probiotics), a M-probiotics diets (0.2% multi-strain probiotics) and a symbiotics diet (0.25% β -glucan and 0.2% multi-strain probiotics), and. All supplementations significantly increased the average daily gain and average daily feed intake compared to the control ($P<0.05$). The apparent digestibility of dry matter, crude protein and ash as well as the apparent absorption of calcium and phosphorus in all treatment groups were higher than those of the control ($P<0.05$). In general, the supplementation of the symbiotics (combining of β -glucan and probiotics) had the greatest effect on nutrient digestibility, and growth. But the effect of symbiotics was not additive than the effects of β -glucan and probiotics added. These results showed that supplementation of prebiotic and probiotics or symbiotics (combination of β -glucan and multi-strain probiotics) to an antibiotic-free diet stimulate beneficial bacteria in the gastrointestinal tract and may also have beneficial effects on nutrient digestibility and growth performance in weaned pigs.

Keywords: Probiotic, Prebiotic, Growth performance, Nutrients digestibility, Blood characteristics.

Introduction

Normally, the intensive farming system of piglets was weaned at 3 to 4 weeks of age and during of post-weaning found that piglets was stressor such as nutritional, environmental social and microbial unbalance [1,2,3]. The stress may result in low feed intake and this is associated with poor weight gain, villous atrophy and malabsorption [4,5]. Growth promoting antibiotics are often used in prophylactic doses to improve animal performance and health and to obtain economic benefits [6]. However, the use of growth promoting antibiotics in pig diets will be banned in the EU from 2006 onwards. Thus, the new development of alternatives to growth promoting antibiotics has a high priority in research. Prebiotics and probiotics are potential alternatives to growth promoting antibiotics and it has been shown that they can affect the gastrointestinal ecosystem in pigs [7].

Beta-glucans are polysaccharides of glucose that can be produced by many prokaryotic and eukaryotic organisms. This group of compounds has several beneficial properties and because of that they have found a wide variety of uses in human and in veterinary medicine, pharmaceutical, cosmetic and chemical industries as well as food and feed production [8,9]. As regard, β -glucans an improvement of growth performance in weanling pigs was suggested [10-12]. The two commercial preparations Bio-Mos® and MTB100® are produced from

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the yeast cell wall of *S. cerevisiae* by Alltech Inc. (Nicholasville, KY). Due to the content of the active polysaccharides β -D-glucan and α -D-mannan, application of these two products as feed supplement to pigs led to the beneficial results such as enhanced weanling piglet's protection from bacterial infections and increased weight gain [13-17, 12].

Probiotics may be candidates as a substitute for antibiotics because they may affect growth in nursery pigs [18, 13]. In several studies a multi-strain probiotics had more effect on growth of the host animal when compared to one-strain probiotics [19]. Rolfe [20] suggested using a combination of pro- and prebiotics (the so called symbiotics) because the prebiotics may stimulate the probiotics. In that respect, Nemcová [21] found an additional effect of supplementing OF and lactobacillus to weaner diet increased beneficial bacteria and decreased harmful bacteria when compared to supplementing lactobacillus only. A review by Vente-Spreeuwenberg and Beynen [4] concluded that gut microflora can be influenced by the feeding of probiotics or prebiotics. It is not known whether the beneficial effect to weanling pigs is a direct effect on gut integrity or on digestion or absorption. Moreover, there is only scarce information on the effect of OF on nutrient digestion and mineral absorption in pigs. Thus, it is interesting to investigate the effect of supplementation of OF and probiotics or the combination of OF and a multi-strain probiotic on fecal microflora, nutrient digestion and performance in weaned pigs.

This experiment was conducted to investigate the effects of a). antibiotic-free diet with beta-glucan as prebiotics, b). two strain probiotics, c). multi-strain probiotics or d). symbiotics (combining β -glucans and multi-strain probiotics) on growth, nutrients digestibility, and blood characteristics in weaned pigs.

Materials and Methods

Animals and Managements

A total of three hundred and sixty pigs (Duroc × Yorkshire) × Landrace weaned at 25 days, with an average initial body weight of 6.17 ± 0.2 kg were used in a 21day trial. The pigs from 10 litters were randomly assigned on the basis of their litter of origin, sex and body weight to one of 4 pens with 18 pigs each. Each pen was assigned to one of 5 treatments with 4 replicates (two pens with gilts and two pens with barrows for each treatment). The piglets were housed with 3 pigs per pen in a galvanized wire mesh pen inside a room. The room temperature was maintained between 26 and 28 °C. Pigs were allowed to consume feed and water *ad libitum* from a two-hole self-feeder and from a nipple waterer.

Experimental Design and Diets

The treatments consisted of T1: control (antibiotic-free corn-soy diet), T2: 2.5% β -glucans, T3: 0.2% multi-strain probiotics, T4: 0.2% two strain probiotics and T5: a combination of 2.5% β -glucans and 0.2% multi-strain probiotics. The β -glucans and probiotics were added to the diet at the expense of corn starch. The experimental diets (Table 1) were formulated to contain 3,340 kcal/kg of ME, 22.0% of CP, 1.50% of lysine, 0.42% of methionine, 0.9% of calcium and 0.8% of phosphorus. The diets were fed in meal form and formulated to meet or exceed the NRC (1998) recommendations for all nutrients. Chromic oxide was added (0.2% in the diet) as an indigestible marker to allow digestibility determinations.

Chemical composition of β -glucans is one part of waste ethanol factory by molasses as yeast substrate from processing and dry matter basis was 95% and 15% of β -glucan as dry matter basis (g/100g) [22]. Multi-strain probiotics and two strain probiotics used in this study were microbial mixtures manufactured by Micro Innovate Co., Ltd, Thailand (Table 2).

Table 1: Diet composition (as-fed basis).

Ingredients (kg)	T1	T2	T3	T4	T5
Corn Extruded	40.5	40.5	40.5	40.5	40.5
Soybean meal (CP 48%)	15.21	15.21	15.21	15.21	15.21
Rice Extruded	25.0	25.0	25.0	25.0	25.0
Cassava Chip	9.55	9.55	9.55	9.55	9.55
Milk powder	2.0	2.0	2.0	2.0	2.0
Fish Meal	2.5	2.5	2.5	2.5	2.5
Coconut oil	2.5	2.5	2.5	2.5	2.5
Tri-calcium Phosphate	1.3	1.3	1.3	1.3	1.3
Limestone	0.15	0.15	0.15	0.15	0.15
Vitamin/mineral premix [a]	0.22	0.22	0.22	0.22	0.22
Salt	0.2	0.2	0.2	0.2	0.2
L-Lysine-HCL	0.15	0.15	0.15	0.15	0.15
DL-Methionine	0.07	0.07	0.07	0.07	0.07
Corn Starch	0.45	0.20	0.25	0.25	0
β -glucans	-	0.25	-	-	0.25
T-Probiotic	-	-	0.2	-	0.2
M-Probiotic	-	-	-	0.2	-
Chromic oxide [b]	0.2	0.2	0.2	0.2	0.2
Chemical composition [b]					
ME, kcal/kg	3,340	3,340	3,340	3,340	3,340
Crude protein, %	22.0	22.0	22.0	22.0	22.0
Lysine, %	1.50	1.50	1.50	1.50	1.50
Methionine, %	0.42	0.42	0.42	0.42	0.42
Calcium, %	0.90	0.90	0.90	0.90	0.90
Phosphorus, %	0.80	0.80	0.80	0.80	0.80

[1]Abbreviated: T1: control; T2: 2% beta-glucan; T3: 0.2% two-strain probiotic; T4: 0.2% multi-strain probiotic; T5: 0.25% beta-glucan + 0.2% multi-strain probiotic.

[a]Provided per kg diet: 10,000 IU of vitamin A, 2,000 IU of vitamin D_3, 42 IU of vitamin E, 5 mg of vitamin K, 9.6 of vitamin B_2, 2.45 of vitamin B_6 40 µg of vitamin B_{12}, 27 mg of pantothenic acid, 49 mg of niacin, 0.05 mg biotin, 140 mg of Cu, 145 mg of Fe, 179 mg of Zn, 12.5 mg of Mn, 0.5 of I, 0.25 of Co and 0.4 mg of Se.

[b]Used as an indigestible marker.

[c]Calculated Values.

Table 2: Specification of multi-strain probiotic and two strain probiotic used in this experimental diets.

Item	Bacteria	Content, cfu/kg
Multi-strain probiotic (M-Probiotic)		
Lactobacillus sp.	*Lactobacillus acidophilus*	8.0×10^{10}
Bacillus sp	*Bacillus subtilis*	9.0×10^{10}
Yeast	*Saccharomyces cerevisieae*	5.0×10^{9}
Two-strain probiotic (T-Probiotic)		
Lactobacillus sp.	*Lactobacillus acidophilus*	8.0×10^{10}
Bacillus sp	*Bacillus subtilis*	9.0×10^{10}

Measurements and Sampling

Body weights and feed consumption were measured on day 7, 14 and 21 to determine daily gain, average daily feed intake and feed conversion ratio. On day 18 of the experiment, fecal samples were collected from the three pigs per pen by rectal massage; the sample was pooled within pen, dried and ground. Laboratory analyses of feed and feces included dry matter (DM), crude protein nitrogen, crude fiber, calcium and phosphorus [23]. The chromium concentration in the feed and in fecal samples was determined using spectrophotometry (Shimadzu, UV-1201, Japan). The red blood cell (RBC) count, white blood cell (WBC) count, hematocrit value, hemoglobin content, platelet count, lymphocyte, neutrophil and monocyte concentrations in whole blood were measured. Blood samples (5mL) were collected via jugular vein into heparanized vacuum tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) from five pigs in each treatment at the termination (chosen as random) of the feeding trial. All blood samples were analyzed with an automated hematology analyzer (ADVIA 120, Bayer, Tarrytown, USA) using commercial reagent kits (lymphocytes, monocytes, neutrophils: ADVIA® 120 PEROX 1, 2 and 3 reagents, Bayer, USA; WBC: ADVIA® 120 BASO reagent; RBC and platelet: ADVIA® 120 BASO reagent; Hemoglobin: ADVIA® 120 HGB reagent).

The parameters were analyzed as a randomized complete block design with analysis of variances performed using the general linear model procedure of SAS (1996), with pen as the experimental unit. Duncan's multiple range test [24] was used to determine significant differences among treatments.

Results

Growth Performance

The results of feed intake, body weight gain and feed efficiency are presented in (Table 3). For days 0 to 7, all four treatments (supplemented with β -glucan or probiotics or symbiotics) significantly improved weight gain compared to the control ($P<0.05$). β -glucan or multi-strain probiotics did improve ($P<0.05$) feed efficiency (gain/feed). For d 7 to 14, body weight gains of pigs fed symbiotics significantly improved ($P<0.05$) compared with either two strain probiotics or control. Average daily feed intake of pigs fed symbiotics was higher than control ($P<0.05$) but not compared to other treatments. For d 14 to 21, daily gain of pigs fed either multi-strain probiotics or symbiotics was higher ($P<0.05$) than that of control animal. There were no significant differences in feed intake and gain/feed ratio between treatments. During the total experimental period, the supplemented animals had similar gain but symbiotics had the highest weight gain.

Nutrients Digestibility

The results on the apparent fecal digestibility of dry matter (DM), crude protein (CP) and crude ash as well as apparent fecal absorption of calcium and phosphorus are presented in (Table 4). Supplementation of the diet with pre- and/or probiotics improved ($P<0.05$) the apparent digestibility of DM, CP, CF and absorption of Ca and P compared to the control. Fecal ammonia concentrations are presented in Table 4. All supplementations significantly reduced the fecal ammonia concentrations compared to the control fed animals ($P<0.05$).

Blood Characteristics

(Table 5) shows the effect of beta-glucan and multi-strain probiotics supplementation on hematological traits. During the experimental period, neutrophil concentration was numerically higher in all treatment groups compared to the control. There were no significant differences in RBC count, WBC count, hematocrit, hemoglobin, lymphocyte, neutrophil and monocyte concentrations of whole blood.

Discussion

We hypothesized that β -glucan, probiotic and symbiotic would help the beneficial growth performance, nutrients digestibility and blood characteristics by stimulating the good microflora or by adding beneficial microbes in the gut. This might improve gut health and in that aspect indirectly cause an increase feed intake. As result animals may have increased growth performance because they eat more. We also investigated whether a specific symbiotic, a combination of β -glucan and multi-strain probiotics, have a higher effect on weight gain and feed intake compared to other treatments in antibiotic-free diets for weaned pigs. Shim [1] have reported about supplementation of oligosaccharide and probiotic alone or combined on feed intake, nutrients digestibility and growth performance in weaned pigs. The result of experiment found that all supplementations increased the average daily gain and average daily feed intake when compared to the control ($p<0.05$). Some researchers (Gibson and Roberfroid, 1995; Kumprecht and Zobac, 1998; Nemcová et al., 1999) have suggested that symbiotic may better for growth performance and health than either prebiotic or probiotic alone. In the present experiment, symbiotics are similar to prebiotic and probiotic alone. The supplementation of symbiotic (combining prebiotic and probiotic) significantly increased growth performance compared to the control ($p<0.05$) and the increase of growth performance for the symbiotic group can be recommended

Table 3: Average daily weight gain (ADG), average daily feed intake (ADFI) and feed efficiency (FE) for weanedpigs[1] fed diets with beta-glucan, multi-strain probiotics, two strain probiotics or synbiotics.

Item	T1[2]	T2[2]	T3[2]	T4[2]	T5[2]	SE[3]
0-7 days						
ADG, g	132^{b}	146^{ab}	141^{ab}	144^{ab}	153^{a}	12
ADFI, g	297	303	291	295	301	20
FE (Gain/feed)	0.44^{b}	0.57^{a}	0.54^{a}	0.55^{a}	0.56^{a}	0.13
7-14 days						
ADG, g	230^{b}	263^{ab}	245^{b}	265^{ab}	285^{a}	18
ADFI, g	290^{b}	335^{ab}	310^{b}	337^{ab}	364^{a}	22
FE (Gain/feed)	0.58	0.59	0.57	0.60	0.61	0.03
14-21 days						
ADG, g	290^{b}	345^{ab}	302^{b}	347^{ab}	356^{a}	24
ADFI, g	303	347	315	345	350	11
FE (Gain/feed)	0.65	0.66	0.66	0.68	0.65	0.14
0-21 days						
ADG, g	217^{b}	251^{ab}	229^{b}	252^{ab}	264^{a}	13
ADFI, g	297^{b}	328^{ab}	305^{b}	325^{ab}	338^{a}	23
FE (Gain/feed)	0.55	0.60	0.59	0.61	0.60	0.06

[1]Three hundred and sixty pigs with an average initial body weight of 6.25 kg.

[2]T1: control; T2: 2.5%β-glucans; T3: 0.2% two strain probiotics; T4: 0.2% multi-strain probiotics and T5: a combination of 2.5% β-glucans and 0.2% multi-strain probiotics.

[3]Pooled standard error.

[ab]Means in the same row with different superscripts differ ($p<0.05$).

Table 4: Nutrients digestibility and ammonia content for weaned pigs[1] fed diets with beta-glucan, multi-strain probiotics, two strain probiotics or symbiotics.

Item (%)	T1[2]	T2[2]	T3[2]	T4[2]	T5[2]	SE[3]
Dry matter	80.2[b]	84.9[ab]	83.8[ab]	85.1[ab]	87.4[a]	0.35
Crude protein	74.3[b]	83.6[ab]	80.7[ab]	83.2[ab]	86.1[a]	0.48
Crude fiber	77.6[b]	84.1[ab]	82.2[ab]	84.3[ab]	87.3[a]	1.24
Calcium	76.5[b]	84.5[ab]	83.3[ab]	84.4[ab]	85.2[a]	0.39
Phosphorus	75.3[b]	80.4[ab]	79.1[ab]	80.3[ab]	82.5[a]	0.47
Ammonia (mg/kg)	310[b]	229[ab]	236[ab]	227[ab]	193[a]	10.2

[1]Fecal samples were collected from one hundred and twenty pigs with an average initial body weight of 6.35 kg.

[2]T1: control; T2: 2.5%β-glucans; T3: 0.2% two strain probiotics; T4: 0.2% multi-strain probiotics and T5: a combination of 2.5% β-glucans and 0.2% multi-strain probiotics.

[3]Pooled standard error.

[ab]Means in the same row with different superscripts differ ($p<0.05$)

Table 5: Blood characteristics for weaned pigs[1] fed diets with beta-glucan, multi-strain probiotics, two strainprobiotics or symbiotics.

Item	T1[2]	T2[2]	T3[2]	T4[2]	T5[2]	SE[3]
Lymphocyte, %	61	57	59	57	56	3.0
Neutrophil, %	25	30	25	29	31	2.5
Monocyte, %	6	4	5	4	3	3.6
Hematocrit, %	33	36	24	36	37	1.8
Hemoglobin, g/dl	10.3	10.8	10.4	10.7	11.2	2.1
WBC, $\times 10^3/mm^3$	27.2	26.8	27.1	26.9	26.5	3.3
RBC, $\times 10^6/mm^3$	5.7	5.5	5.7	5.5	5.3	0.6

[1]Blood samples were taken from eight weaned pigs per treatment.
[2]T1: control; T2: 2.5%β-glucans; T3: 0.2% two strain probiotics; T4: 0.2% multi-strain probiotics and T5: a combination of 2.5% β-glucans and 0.2% multi-strain probiotics.
[3]Pooled standard error.
[ab]Means in the same row with different superscripts differ ($p<0.05$)

by numerically increased average daily feed intake.

The supplementation of a prebiotics did not significantly affect growth. This result was similar to other studies (Olsen and Maribo, 1999; [25]. But other studies in literature reported that β -glucan has clear stimulating effect on growth performance [12,13-17]. The difference may be associated with the different chemical structure (degree of polymerization) of glucose used in the different studies, or length of glucose and presence of other fermentable sources especially non-starch polysaccharides in the diets used in the literature.

We also studied if feeding some multi-strain probiotics to weanling pigs would result in a large effect compared to a two strain probiotics. Supplementation of a multi-strain probiotics tended to result in higher growth performance compared to the two strain probiotics. It has been hypothesized that multi-strain probiotics may also be active at different sites. In this experiment the multi-strain probiotics contained species of microorganisms but it also resulted in a higher number of CFU/g of diet compared to the two strain probiotics. In non-optimal conditions probiotics may improve growth and health [19]. This experiment was conducted under optimal conditions, but animals have been transported. So they experienced some stress. The weaned pigs had been transported (about 30 min. by truck with cover) from a commercial farm to the experimental room at the day of weaning and were not allowed adaptation period before the experiment. Some studies have shown indeed that feeding probiotics improved performances (growth and feed conversion) in young pigs [1,26-28]. Other studies, however, did not find effects of probiotics in weanling piglets [29,30].

Overall, the apparent fecal digestibility of DM and CP was higher in the symbiotic pigs when compared to other treatments ($P<0.05$). It is not known whether the protein digestibility in the ileum is different. Fecal digestibility of CP would be less with prebiotics because there is more fecal biomass. This is considered beneficial. It may be that longer villi and thinner epithelium in the villi of the small intestine [1] may lead to improved digestion and absorption of nutrients in the gastrointestinal tract by stimulate beneficial bacteria. Other studies, however did not effect of prebiotics in weanling piglets [25,31].

Researcher evidence has revealed that non-structure polysaccharide (NSP) can increase the availability of calcium, magnesium, zinc and iron in human [32]. In this experiment, the supplementation of prebiotic and probiotics alone or in combination of both improved the apparent absorption of calcium compared to the control diet ($P<0.05$). Symbiotics significantly increased ($P<0.05$) digestibility of phosphorus compared to other treatment groups. These results coincide with the result [33] that feeding FOS to weaned pigs enhances calcium and phosphorus absorption in rats. Other reports [32, 34] also suggested that prebiotics, such as inulin and FOS may increase bioavailability of minerals by an increased solubilization of minerals by the production of VFA and lower pH resulting from the increased fermentation [35, 36]. So the addition of symbiotics may stimulate the fermentation more in the gut and this may partly contribute the increased digestibility of mineral. However, Houdijk [25] found similar ileal digestibility of calcium and phosphorus by oligo-fructose or TOS and Shim [1] that feeding oligo-fructose (OF) and probiotics alone or combined on nutrient digestibility to weaned pigs increase digestion calcium and phosphor in weaned pigs.

In this experiment the blood characteristics were unaffected by β -glucan, Probiotics and symbiotics supplementation. There were no significant differences in lymphocyte, neutrophil and monocyte, WBC count, RBC count, hematocrit, and hemoglobin concentrations in the whole blood. However, Shim [1] found that hematological traits such as Platelet count was only significantly higher with symbiotics supplementation than that of FOS ($P<0.05$). Herich [37] found that immune parameters such as lymphocytes, leukocyte, neutrophils, $CD4^+$ T cells tended to increase in supplementation of symbiotics (combining lactobacillus and FOS) compared to the single administration of lactobacillus and the control in weaned pigs. Pierre [38] demonstrated that oligo-fructose enhanced the T-lymphocyte function in mice. It has been suggested that prebiotic such as FOS or inulin may be beneficial for the immune system and health of weaned pig.

Conclusion

Each of the supplementations of symbiotics (combining of β -glucan and multi-strain probiotics) prebiotics and probiotics to an antibiotics-free diet resulted in increased growth, increased phosphorus and calcium absorption and a slight increase in nutrient digestibility when compared to the control or other supplementations with prebiotics or probiotics alone.

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