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Original Research Article

Effect of dietary supplementation of *Bacillus coagulans* or yeast hydrolysates on growth performance, antioxidant activity, cytokines and intestinal microflora of growing-finishing pigs

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ABSTRACT

This study was to investigate the effects of dietary supplementation of *Bacillus coagulans* (BC) and yeast hydrolysates (YH) on growth performance, antioxidant activity, cytokines and intestinal microflora of growing-finishing pigs. Thirty-six barrows (initial BW = 26.87 ± 2.65 kg) were assigned randomly to 3 treatments with 4 replicates, 3 pigs per replicate. Pigs in the control group (CON) were fed a basal diet, and the diets for the other 2 groups were the basal diet plus BC at 200 mg/kg and the basal diet plus YH at 3,000 mg/kg. The trial lasted for 104 d. Compared with CON, YH treatment significantly increased average daily gain (ADG) and average daily feed intake (ADFI) during the finishing phase ($P < 0.05$), and significantly enhanced ADG during the overall period ($P < 0.05$). Dietary inclusion of BC tended to increase ADFI during the finishing period ($P = 0.08$). Compared with CON, BC treatment improved lysozyme (LZM), complement 3 (C3), complement 4 (C4), interlenkin-10 (IL-10) and total antioxidant capacity (T-AOC) level in serum ($P < 0.05$). Dietary inclusion of YH enhanced the serum IL-10 level ($P < 0.05$) and tended to increase T-AOC level ($P = 0.06$). Dietary inclusion of YH elevated ($P < 0.05$) the number of *Lactobacillus* and *Bacillus* in cecal contents of pigs, promoted the populations of *Bifidobacterium* and *Bacillus* in colonic contents. Moreover, the BC diet increased ($P < 0.05$) the count of *Bifidobacterium* in colonic contents. These results indicated that dietary BC supplementation is beneficial to improve the immunity. Dietary YH supplementation promoted the growth performance and the populations of beneficial bacteria in the hindgut of the growing-finishing pigs.

1. Introduction

In recent years, the adverse effects of the continued use of antibiotics as antibacterial growth regulators in the pig industry on human and animal health have become a major issue (Hu et al., 2013). Thus, the development of alternative strategies or substitutes for antibiotics was continuously demanded after the antibiotics in animal production were completely banned in European Union since 2006 (Hu et al., 2014). The potential substitutes for antibiotics including probiotics, yeast hydrolysates, organic acids and plant extracts have proven to be harmless and friendly.

Probiotics are defined as a living microflora feed supplement, which beneficially affect the host animal by improving growth performance, immune response and intestinal microbial balance (Fuller, 1989). *Bacillus coagulans* (BC) has been regarded as one of the most important probiotics for an appropriate feed additive due to its stability as spore-forming bacteria. It produces a number of enzymes and organic acids, which suppress the colonization of pathogens in intestinal tract through reducing the pH value of contents (Guo et al., 2006). Recently published research has shown

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BC as a potential drug constituent for human diseases (Khadijeh et al., 2016). In addition, previous researches indicated that BC exerted positive effects on growth performance and immunity in fish and chickens (Lin et al., 2012; Zhou et al., 2010). However, the different effects of various *Bacillus* probiotics depend on the characteristics of specific strains (Cutting, 2011). Meanwhile, the effects of BC are still questionable for different animals or the growth period (Liu et al., 2015; Mandel et al., 2010). As reported by Alexopoulos et al. (2004), dietary *Bacillus subtilis* and *Bacillus licheniformis* supplementation enhanced average daily gain (ADG) and average daily feed intake (ADFI) compared with the control group in weaning pigs. Whereas, Giang et al. (2011) documented that no effects on growth performance of pigs for diet supplemented with *Bacillus* in growing-finishing pigs. And little researches so far have dealt with dietary BC supplementation on growing-finishing pigs.

Yeast products such as yeast extract, yeast culture, and yeast hydrolysate (YH) contain different major active substances due to different processing methods, which leads to some controversial results emerged in different animal experiments. (Jung et al., 2012; Molist et al., 2014). Yeast hydrolysate generally includes abundant nucleotides, B-vitamins, amino acids and yeast cell wall polysaccharides (such as β-glucan and mannosan). Nucleotides as an important ingredient of YH have a number of benefits on improving growth performance, regulating immune function, promoting maturation and repairing the gastrointestinal tract of animals (Sauer, 2010; Superchi et al., 2012). Accordingly, dietary nucleotides could satisfy the requirements of physiological function in period of rapid growth, starvation, poorly endogenous synthesis and immune challenge. Also, β-glucan and mannan oligosaccharide were generally used as prebiotics to regulate the immune response by a previous study in animal production (Sauerwein et al., 2007). However, it is not fully clear how YH improves growth performance and modulates the systemic health in growing-finishing pigs. Therefore, the present experiment was conducted to evaluate the effects of dietary BC and YH supplementation on the growth performance, antioxidant, immunity and intestinal microflora in growing-finishing pigs.

2. Materials and methods

In this experiment, the experimental protocol was reviewed and approved by the Animal Experimental Committee of Sichuan Agricultural University. The experiment was conducted at the Animal Experiment Center of Sichuan Agricultural University.

2.1. Feed additives

Enramycin was purchased from Shandong Shengli Bioengineering Co., Ltd (Shandong, China). *Bacillus coagulans* preparation (5.0×10^9 cfu/g) was provided by Sanzheng Business Group (Kunming, China). Yeast hydrolysate (crude protein: 50.80%) was provided by Jiangmen Thealth Bioengineering Co., Ltd (Guangdong, China), and it contains 13.58% nucleotides and 10.00% polysaccharide.

2.2. Animals and experimental design

A total of 36 barrows ([Landrace × Yorkshire] × Duroc; initial BW = 26.87 ± 2.65 kg) were randomly allocated to 3 groups with 4 replicates per group and 3 pigs per replicate (12 pigs per group). Control group (CON) received the basal diet (Table 1) formulated to

Table 1
Ingredient and nutrient composition of the basal diet (%, as-fed basis).

Item	Growing stage		Finishing stage	
	25 to 50 kg	50 to 75 kg	75 to 100 kg	100 to 125 kg
Ingredients				
Maize (CP 7.8%)	73.60	78.00	81.06	82.68
Soybean meanl (CP 43%)	17.37	17.58	14.80	13.85
Fish meal (CP 62.5%)	3.00	0.00	0.00	0.00
Soybean oil	1.50	1.50	1.50	1.40
Sucrose	2.00	0.00	0.00	0.00
L-lysine-HCl (78%)	0.39	0.38	0.37	0.18
DL-methionine (99%)	0.05	0.07	0.03	0.00
L-threonine (98.5%)	0.13	0.12	0.12	0.04
L-tryptophan	0.03	0.03	0.03	0.00
Choline chloride (50%)	0.10	0.15	0.15	0.15
Limestone	0.59	0.71	0.63	0.59
Dicalcium phosphate	0.56	0.83	0.68	0.48
Salt	0.30	0.40	0.40	0.40
Mineral premix [1]	0.35	0.20	0.20	0.20
Vitamin premix [2]	0.03	0.03	0.03	0.03
Total	100.00	100.00	100.00	100.00
Nutrients composition [3]				
Digestible energy, MJ/kg	14.27	14.21	14.24	14.23
Crude protein	15.60	14.15	13.17	12.60
Calcium	0.66	0.59	0.52	0.46
Total phosphorus	0.49	0.46	0.43	0.39
Available phosphorus	0.31	0.27	0.24	0.21
Lysine	0.98	0.85	0.73	0.61
Methionine	0.28	0.27	0.21	0.18
Threonine	0.60	0.52	0.46	0.40
Tryptophan	0.17	0.15	0.13	0.11

[1] Mineral premix provided per kilogram of diet: Cu, 4 mg; Fe, 60 mg; Mn, 2 mg; Zn, 50 mg (25 to 50 kg); Cu, 3.5 mg; Fe, 50 mg; Mn, 2 mg; Zn, 50 mg (50 to 75 kg); Cu, 3 mg; Fe, 40 mg; Mn, 2 mg; Zn, 50 mg (75 to 100 kg and 100 to 125 kg).
[2] Vitamin premix provided per kilogram of diet: vitamin A, 9,000 IU; vitamin D_3, 3,000 IU; vitamin E, 20 IU; vitamin K_3, 3.0 mg; vitamin B_1, 1.5 mg; vitamin B_2, 4.0 mg; vitamin B_6, 3.0 mg; vitamin B_{12}, 0.02 mg; niacin, 30 mg; pantothenic, 15 mg; folic acid, 0.75 mg; biotin, 0.1 mg.
[3] Calculated values.

meet nutrient recommendations according to NRC (2012) and supplemented with enramycin (20 mg/kg). The other 2 groups were fed the same basal diet supplemented with *Bacillus coagulans* (20 mg/kg, BC group) or yeast hydrolysates (3,000 mg/kg, YH group). All pigs were housed in a temperature-controlled room and allowed *ad libitum* access to feed and water. The trial lasted for 104 d. Pigs were weighed at the end of each period. Daily pen feed consumption was recorded during the experimental period. The ADG, ADFI and feed to gain ratio (F:G) were calculated.

2.3. Samples collection

At the end of the experiment, 2 pigs from each pen were selected according to average BW, and blood samples were collected from the anterior vena cava to vacuum tubes. Blood samples were centrifuged at 3,000×*g* for 10 min at 4 °C, serum was separated and stored immediately at −20 °C until analysis. Then, 6 pigs from each treatment were selected according to average BW, electrically stunned, exsanguinated, dehaired, eviscerated and split down the midline according to standard commercial procedures. The contents of the caecum and colon were sampled into sterile cryogenic vials and immediately stored at −80 °C until analysis for microbia.

2.4. Biochemical analysis of serum

Serum innate immune molecules (complement 3, C3; complement 4, C4; lysozyme, LZM) and inflammatory factors (interleukin-1 beta [IL-1β]; tumor necrosis factor-alpha [TNF-α]; interlenkin-10 [IL-10]) were determined by using the commercially available porcine specific ELISA kits (Xinle Biological Technology Co., LTD, Shanghai, China) following the manufacturer's instructions. Serum total antioxidant capacity (T-AOC), activities of glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) and serum malondialdehyde (MDA) level were analyzed using assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions.

2.5. Bacterial DNA isolation and microbial real-time quantitative PCR

Bacteria DNA in the cecal and colonic digesta was extracted using Stool DNA kit (Omega Bio-Tek, Doraville, CA) according to the manufacturer's protocol. For quantitative of total bacteria, *Lactobacillus*, *Escherichia coli*, *Bifidobacterium* and *Bacillus*, primers and probes were described by a previous report (Qi et al., 2011). All the primers and probes were commercially synthesized from Invitrogen (Shanghai, China) (Table 2). Quantification real-time PCR was performed in a CFX-96 real-time PCR Detection System (Bio-Rad Laboratories Inc., Hercules, USA). The SYBR Green PCR reaction system for total bacteria was 25 μL of total volume, including 12.5 μL SYBR Premix Ex Taq II (Tli RNaseh Plus 2 ×), 1 μL each of forward and reverse primers (100 nm), 1 μL template DNA and 9.5 μL distillation–distillation H_2O (ddH_2O). The PCR condition was as follows: one cycle of pre-denaturation at 95 °C for 30 s; 40 cycles of denaturation at 95 °C for 5 s, annealing at 64.5 °C for 30 s and extension at 95 °C for 10 s. The Primer Script PCR kit (Perfect Real Time, TaKaRa) was used for *Lactobacillus*, *E. coli*, *Bifidobacterium* and *Bacillus*. The reaction protocol was composed of one cycle of pre-denaturation at 95 °C for 10 s; 50 cycles of denaturation at 95 °C for 5 s; annealing at different temperature in different bacteria, (*Lactobacillus* at 53.0 °C; *E. coli* at 57.9 °C; *Bifidobacterium* at 55.0 °C; *Bacillus* at 57.9 °C) for 25 s and extension at 95 °C for 10 s. Each reaction was run in a volume of 20 μL with 8 μL Real MasterMix (2.5 ×), 1 μL of forward and 1 μL of reverse primers (100 nmol/L), 1 μL probe enhance solution (20 ×), 0.3 μL probe (100 nmol/L), 1 μL DNA and 7.7 μL ddH_2O. Copies per sample were calculated with Ct-values and standard curve made by previous study (Qi et al., 2011).

2.6. Statistical analysis

Bacterial copies were transformed (log10) before the statistical analysis. To test the effects of dietary BC or YH supplementation, all data were analyzed with *T*-test procedure from SAS statistical software (Version 9.4; S.A.S, Institute Inc., Cary, NC, USA) to compare dietary BC or YH supplementation with CON respectively. Each replicate served as the statistical unit on growth performance, and each pig in sampling served as the statistical unit for other indictors. $P < 0.05$ was considered statistically significant, and $0.05 < P < 0.10$ was considered a tendency. All data were showed as means ± standard error (SE).

3. Results

3.1. Growth performance

During the growing period, no difference was observed on growth performance among treatments (Table 3). However, dietary YH supplementation increased the ADG and ADFI during the finishing phase ($P < 0.05$), and the ADG during the whole experiment period ($P < 0.05$) compared with CON. Dietary inclusion of BC tended to increase ADFI during the finishing period ($P = 0.08$).

Table 2
Primer and probe for real-time PCR.

Item	Primer/probe name and sequence(5′–3′)	Product length, bp	Annealing temperature, °C
Total bacteria	Eub338-F,ACTCCTACGGGAGGCAGCAG Eub518-R,ATTACCGCGGCTGCTGG	200	64.5
Escherichia coli	DC-F,CATGCCGCGTGTATGAAGAA DC-R,CGGGTAACGTCAATGAGCAAA DC-P,AGGTATTAACTTTACTCCCTTCCTC	96	57.9
Lactobacillus	RS-F,GAGGCAGCAGTAGGGAATCTTC RS-R,CAACAGTTACTCTGACACCCGTTCTTC RS-P,AAGAAGGGTTTCGGCTCGTAAAACTCTGTT	126	53.0
Bifidobacterium	SQ-F,CGCGTCCGGTGTGAAAG SQ-R,CTTCCCGATATCTACACATTCCA SQ-P,ATTCCACCGTTACACCGGGAA	121	55.0
Bacillus	YB-F,GCAACGAGCGCAACCCTTGA YB-R,TCATCCCCACCTTCCTCCGGT YB-P,CGGTTTGTCACCGGCAGTCACCT	92	57.9

Table 3
Effects of *Bacillus coagulans* (BC) and yeast hydrolysates (YH) supplementation on growth performance in growing-finishing pigs. [1]

Item	CON	BC	YH	*P*-value	
				CON vs. BC	CON vs. YH
Growing					
ADG, g	792.44 ± 48.41	801.02 ± 34.68	822.10 ± 45.63	0.89	0.67
ADFI, g	1,962.65 ± 128.70	1,969.99 ± 118.72	2,043.92 ± 134.68	0.97	0.68
F:G	2.47 ± 0.02	2.46 ± 0.04	2.49 ± 0.10	0.68	0.91
Finishing					
ADG, g	868.52 ± 17.73	932.41 ± 37.18	971.30 ± 33.35	0.17	0.04
ADFI, g	2,832.61 ± 68.23	3,038.92 ± 67.70	3,044.88 ± 58.38	0.08	0.05
F:G	3.27 ± 0.13	3.28 ± 0.16	3.15 ± 0.12	0.97	0.51
Overall					
ADG, g	825.36 ± 23.80	861.44 ± 18.57	886.66 ± 12.90	0.28	0.05
ADFI, g	2,339.07 ± 101.73	2,432.50 ± 95.28	2,477.03 ± 93.82	0.53	0.36
F:G	2.83 ± 0.10	2.82 ± 0.14	2.79 ± 0.16	0.92	0.69

ADG = average daily gain; ADFI = average daily feed intake; F:G = the ratio of feed intake to gain.
[1] Value are means ± standard error.

3.2. Serum immune response profile

Compared with CON (Table 4), the serum concentrations of C3, C4, LZM and IL-10 of pigs fed BC diet were significantly increased ($P < 0.05$). On the other hand, the serum IL-10 concentration of pigs fed YH supplementation diet was significantly increased ($P < 0.05$).

3.3. Antioxidant activity

Table 5 shows that the concentration of serum T-AOC was significantly enhanced in BC diet compared to CON ($P < 0.05$). Meanwhile, YH supplementation tended to increase serum T-AOC ($P = 0.06$). No significant difference was observed on the activities of serum GSH-Px and SOD, and the content of serum MDA among the treatments ($P > 0.05$).

3.4. Hindgut microflora

Dietary YH supplementation (Table 6) increased *Lactobacillus* and *Bacillus* counts in caecum. Meanwhile, YH supplementation enhanced *Bifidobacterium, Bacillus* counts ($P < 0.05$), and tended to increase *Lactobacillus* counts in colon ($P = 0.07$). The colonic *Bifidobacterium* counts in the BC diet were higher than those of CON ($P < 0.05$), and a tendency of colonic *E. coli* counts was observed to decrease ($P = 0.07$).

4. Discussion

Various studies have documented that the dietary *Bacillus* supplementation improved growth performance of animals (Lee et al., 2013; Meng et al., 2010). In the present study, our data showed that dietary BC supplementation tended to increase ADFI during the finishing period, and ADG increased by 7.36%. These results agree with the previous study conducted by Zhou et al. (2010) in which an enhancement of final weight and daily weight gain on chicken were observed in BC supplemented group. Positive effects also were discovered in dietary BioPlus 2B supplementation on growth performance of growing-finishing pigs (Wang et al., 2009b). One reason for the excellent effectiveness of BC in growth performance may be that the *Bacillus* showed beneficial effects in the animal gut through producing a number of enzymes, such as arabinase, α-amylase, cellulase, maltase and β-glucanase,

Table 4
Effects of *Bacillus coagulans* (BC) and yeast hydrolysates (YH) supplementation on immunity in growing-finishing pigs.[1]

Item	CON	BC	YH	*P*-value	
				CON vs. BC	CON vs. YH
C3, μg/mL	25.80 ± 1.53	36.89 ± 2.24	28.66 ± 0.70	<0.01	0.11
C4, μg/mL	21.85 ± 1.80	35.84 ± 2.90	26.21 ± 1.35	<0.01	0.08
LZM, μg/mL	15.60 ± 0.10	18.87 ± 1.03	16.20 ± 0.63	0.04	0.62
IL-1β, ng/L	16.23 ± 0.91	19.00 ± 1.22	15.98 ± 0.60	0.09	0.83
TNF-α, ng/L	139.28 ± 11.37	178.37 ± 16.12	130.17 ± 9.00	0.06	0.54
IL-10, pg/mL	134.72 ± 9.65	177.50 ± 11.08	165.43 ± 8.66	0.01	0.03

C3 = complement C3; C4 = complement C4; LZM = lysozyme; IL-1β = interleukin-1 beta; TNF-α = tumor necrosis factor-alpha; IL-10 = interleukin-10.
[1] Value are means ± standard error.

Table 5
Effects of *Bacillus coagulans* (BC) and yeast hydrolysates (YH) supplementation on antioxidant activity in growing-finishing pigs (U/mL). [1]

Items	CON	BC	YH	*P*-value	
				CON vs. BC	CON vs. YH
T-AOC	0.98 ± 0.05	1.17 ± 0.06	1.18 ± 0.09	0.03	0.06
GSH-Px	1,301.46 ± 70.08	1,300.85 ± 35.76	1,355.40 ± 83.05	0.99	0.63
SOD	196.86 ± 11.00	191.91 ± 11.89	177.47 ± 5.75	0.68	0.14
MDA, nmol/mL	3.40 ± 0.20	3.35 ± 0.12	3.78 ± 0.24	0.85	0.25

T-AOC = total antioxidant capacity; GSH-Px = glutathione peroxidase; SOD = superoxide dismutase; MDA = malondialdehyde.
[1] Value are means ± standard error.

Table 6
Effects of *Bacillus coagulans* (BC) and yeast hydrolysates (YH) supplementation on hindgut microflora in growing-finishing pigs (lg[copies/g]).[1]

Item	CON	BC	YH	*P*-value	
				CON vs. BC	CON vs. YH
Cecum					
Total bacteria	11.43 ± 0.07	11.48 ± 0.03	11.57 ± 0.04	0.54	0.13
Escherichia coli	7.55 ± 0.19	7.37 ± 0.10	7.82 ± 0.36	0.41	0.52
Lactobacillus	7.70 ± 0.22	7.89 ± 0.08	8.32 ± 0.08	0.46	0.03
Bifidobacterium	8.94 ± 0.43	9.51 ± 0.08	9.77 ± 0.15	0.23	0.10
Bacillus	9.92 ± 0.03	10.03 ± 0.08	10.05 ± 0.02	0.17	0.01
Colon					
Total bacteria	11.61 ± 0.06	11.66 ± 0.04	11.67 ± 0.03	0.48	0.31
Escherichia coli	7.39 ± 0.18	6.72 ± 0.27	7.24 ± 0.27	0.07	0.65
Lactobacillus	8.27 ± 0.19	8.52 ± 0.10	8.68 ± 0.07	0.28	0.07
Bifidobacterium	8.97 ± 0.20	9.57 ± 0.10	9.85 ± 0.09	0.03	<0.01
Bacillus	10.10 ± 0.02	10.11 ± 0.02	10.16 ± 0.02	0.59	0.04

[1] Value are means ± standard error.

resulting in enhanced feed efficiency and weight gain of pigs (Guo et al., 2006). In addition, diets supplemented continually *Bacillus* which had better influence on growth performance compared to that of diets with short-dated administration (Kornegay and Risley, 1996). However, inconsistent results were observed while *Bacillus subtilis* was added alone during the growing-finishing period in growing-finishing pigs (Giang et al., 2011). The inconformity might be due to *Bacillus* has different activities among various strains. On the other hand, it cannot be neglected that the age of pigs, the feeding stages and additive amounts of probiotics were critical factors that influenced the results of different trials.

Yeast hydrolysates can improve the growth performance and health of pigs by stimulating the immune system and beneficially affecting intestinal environment (Superchi et al., 2012). The ingredients of YH were nucleotides, peptides, B-vitamins, amino acids and yeast cell wall polysaccharides (e.g. β-glucan and mannan oligosaccharide), and nucleotides played a key component in YH. In the current experiment, we found that dietary supplementation with YH improved ADG and ADFI during the finishing period of pigs, and increased ADG of overall period. Similarly, positive results were observed on growth performance of weaning pigs fed diets with yeast nucleotides (Carlson et al., 2005). Whereas some others documented that no positive effects on growth performance of pigs fed diet supplemented with nucleotides or other hydrolyzed yeast products was observed (Waititu et al., 2016). This discrepancy might be attributed to the different sources and dosages of yeast hydrolysates. Additionally, glutamate based on YH is a precursor for glutamine, which owns a characteristic flavor contributed to increase voluntary feed intake by palatability promotion (Diehl, 2004). Therefore, a potential reason to the promotion of ADG in this study may be explained for the improvement of ADFI.

Complements 3 and 4 are important components in the organization and function of the complement system that participated in specific and non-specific immune response (Frank and Fries, 1991). Lysozyme could defense against pathogens by integrated with complement and lysed specific Gram-positive bacteria (Yin et al., 2014). Previous researches reported that probiotics could stimulate the immune system through the stimulation of innate immune system and regulation of inflammation, such as complement activity, lysozyme activity and some cytokines levels (Pagnini et al., 2010). In addition, probiotics are immunomodulators by regulating cytokines, including pro- and anti-inflammatory cytokines. Also, cytokines can stimulate innate immunity in response to microbial antigens (Yin et al., 2014). Important pro-inflammatory cytokines TNF-α and IL-1β can activate macrophages that regulate cell death and inflammation (Bradley, 2010). Interlenkin-10, a cytokine with anti-inflammatory property, served as a mainly role in infection by inhibiting the immune response to pathogens and preventing inflammatory diseases (Saraiva and O'Garra, 2010). Therefore, cytokines in serum of animals reflected systemic immune status to a certain extent (Rana, 2009). Our results demonstrated that BC improved immune response of pigs, which was evidenced by the increase in C3, C4, IL-10, TNF-α, IL-1β and LZM levels in serum. This can be explained by previous studies that *Bacillus* species were able to be against pathogenic challenges through producing lactic acid and antimicrobial compounds and altering immune cell numbers (Ou et al., 2011). Our results discovered that YH supplementation just improved serum IL-10 level of growing-finishing pigs. These findings maybe attribute to the immune polysaccharide of yeast cell wall, such as mannan oligosaccharides and β-glucans. Che et al. (2012) suggested that no effect of dietary mannan oligosaccharide on the serum TNF-α concentration in weaning pigs was observed, but serum IL-10 level tended to increase.

Over-generation of reactive oxygen species (ROS) in pigs leads to oxidative stress that induced by pathogen infection, mycotoxin and others, resulting in decreasing growth performance and reducing immune function (Lauridsen et al., 1999). The antioxidative capacity of host was evaluated by measuring some relevant enzymes which prevented the formation of ROS, such as catalases, SOD and GSH-Px. Furthermore, it has been widely used of natural antioxidants, such as vitamin E, vitamin C, tea polyphenols and probiotics to increase the antioxidant activities, relieve stress in livestock production (Zhu et al., 2012). Wang et al. (2009a) reported that diet supplemented with *Lactobacillus fermentum* improved the antioxidative capacity of growing-finishing pigs by enhanced T-AOC, SOD and GSH-Px activities and reduced MDA levels in serum. In the present study, we also observed an increase in serum T-AOC levels when diets were supplemented with BC, but no difference of the activity of SOD, GSH-Px and MDA. A possible reason for the improved T-AOC of dietary BC supplementation is speculated that other various antioxidant enzymes and substances changed. Furthermore, the present results showed a tendency in T-AOC level of pigs fed diets with YH. Previous accumulated researches demonstrated that dietary yeast nucleotides inclusion could ameliorate inflammation and DNA damage caused by oxidative stress (Weaver and Kim, 2014). Frankic et al. (2006) also reported that dietary nucleotides have ability to reduce DNA damage induced by T-2 toxin in immune cells, which was attributed to the antioxidant properties of nucleotides for up-regulation of RNA expression of antioxidative enzymes.

Probiotics are known to benefit gut healthy by suppressing the number of harmful microorganisms and promoting growth of beneficial bacterium (Lee et al., 2013). In this investigation, the

dietary BC inclusion had no significant effects on the populations of microorganism in cecum, but significantly increased the number of *Bifidobacterium* in colon. This was similar to the result of Giang et al. (2011), who suggested faecal *Lactobacillus* and *E. coli* counts were not affected by intaking *Bacillus* in diets of growing-finishing pigs. However, inconsistent results in weaning pigs have been found by another study of Giang et al. (2012), who reported that diets supplemented with *Bacillus* increased intestinal *Lactobacillus* population in stomach, ileum, colon and rectum in weaning pigs, while reduced *E. coli* counts in stomach and ileum. One reason for this inconsistency with several previous researches was that the growth stages of the test animals were different. Compared with weaning pigs, the intestinal flora of growing-finishing pigs is more stable. Therefore, dietary probiotics inclusion of growing-finishing pigs had less dynamic influence than that of weaning pigs in the intestinal microflora (Jensen, 1998). Dietary nucleotides can favor the development of the gastrointestinal tract with an improvement of intestinal microbial in monogastric animals (Sauer, 2010). Li et al. (2015) indicated that increased fecal *Lactobacillus* counts and reduced fecal *E. coli* counts in pigs fed diets with nucleotides were observed. Furthermore, a previous study has reported that other active ingredients of hydrolyzed yeast productions (e.g. mannan oligosaccharide) could increase beneficial microbial populations, such as *Bifidobacterium* spp. and *Lactobobacillus* spp. (Pourabedin et al., 2014). Also, *Bifidobacterium* could lower the pH value of colonic content through hydrolyze sugars to lactic acid, which was able to suppress the proliferation of pathogenic bacteria.

5. Conclusion

Our results revealed that dietary BC supplementation in growing-finishing pigs could improve systemic immune status. Dietary YH supplementation promoted the growth performance and the populations of beneficial bacteria in the hindgut of growing-finishing pigs.

Conflict of interest statement

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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