Inclusion of yeast-derived protein in weanling diet improves growth performance, intestinal health, and anti-oxidative capability of piglets

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ABSTRACT: The effects of yeast-derived protein (YP) on growth performance, intestinal health, and oxidative status of weanling piglets were investigated. A total of 80 weaned piglets (PIC 327 × 1050, 26 ± 2 days old, 6.20 ± 0.10 kg) were randomly allocated into 2 groups, 5 pens per each group and 8 piglets per each pen, receiving control diet and diet with inclusion of 4% YP at the expenses of fish meal (YP diet) for a period of 28 days. The diets were formulated to contain similar nutrient levels. Compared with control, piglets fed YP diet had markedly higher overall average daily growth (+14%, $P < 0.05$) and lower final feed conversion ratio (–8%, $P < 0.01$). Concentrations of serum serine, cystathionine, histidine, hydroxyproline, and urea were decreased in piglets fed YP diet ($P < 0.05$), whereas alanine and aspartate were increased ($P < 0.01$). Moreover, serum antioxidant enzyme activity (glutathione peroxidase) was markedly increased (+19%, $P < 0.01$) in piglets fed YP diet relative to piglets fed control diet. In addition, feeding YP diet considerably ($P < 0.05$) increased the copy numbers of lactobacilli and total bacteria in the colon of piglets at the end of the experiment. Furthermore, the mRNA abundance of innate immunity-related genes (*TLR4*, *NF-κB1*, and *IL-6*) was increased ($P < 0.06$) in the ileum of piglets fed YP diet. Collectively, results of this study indicated that diet with the inclusion of YP improved growth performance and partially enhanced anti-oxidative capability as well as intestinal innate immunity of weaning piglets.

Keywords: yeast; swine; nucleotide; immune; oxidative stress

INTRODUCTION

Piglets are subjected to various stresses at weaning due to a range of factors including separation from the sow, post-weaning diarrhea, and reduced utilization of nutrients as a result of dietary changes, which can cause severe damages to intestinal health. High quality feed ingredients have been used to alleviate weaning stress, improve growth performance, and immune function of piglets (Che et al. 2012). Animal proteins such as spray-dried plasma protein (SDPP) have a well-balanced amino acid profile and are highly digestible for piglets. However, animal nutritionists are facing challenge for the cost and potential bio-security risk by using SDPP, moreover, the ban of some of the animal proteins renders nutritionists to seek novel non-animal protein ingredients. Yeast-derived protein (YP), enriched with free amino acids, short chain peptides, nucleotides, and inositol (Pereira et al. 2012) has been proposed to be a superior protein ingredient as an alternative to SDPP for weaning pigs. As a mixture of yeast cell contents by removing the cell wall from yeast cells (Chae et al. 2001), YP possesses similar apparent (AID) and standardized (SID) ileal digestibility of amino acids

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with SDPP for weanling piglets (Mateo and Stein 2007; Moehn et al. 2010). In addition, the bioactive components of YP (nucleotides, etc.) may be beneficial for animal growth and intestinal health by enhancing gut integrity and body immunity (Owens and McCracken 2007). For instance, dietary nucleotide supplementation has been shown to improve growth performance, immune response, and anti-oxidative capability of piglets (Salobir et al. 2005; Superchi et al. 2012; Sauer et al. 2012b).

Although YP has been demonstrated to improve growth performance, the explanation of the underlying mechanism is still lacking. This study, therefore, was conducted to determine the effects of YP on growth performance, oxidative status, and intestinal health in weanling piglets.

MATERIAL AND METHODS

The experiment followed the actual law of animal protection and was approved by the Animal Care and Use Committee of the Sichuan Agricultural University, and was performed in accordance with the National Research Council's Guideline for the Care and Use of Laboratory Animals.

Animals and diets. The pig experiment was conducted at the Giastar Pig Experimental base, Hongqi, Sichuan Province, P.R. China. A total of 80 weaned piglets (PIC 327 × 1050, 26 ± 2 days) with initial live weight of 6.20 ± 0.10 kg were used in the experiment. The piglets were assigned to one of the two dietary treatments for a period of 28 days. The dietary treatments consisted of basal diet (control diet, n = 40) and basal diet with inclusion of 4% YP at the expense of fish meal (YP diet, n = 40). Each treatment included 5 pens and 8 piglets per each pen. Piglets were housed in fully slatted pens (2.65 × 2.05 m). The YP (AB Yestex™; Associated British Agriculture (Shanghai) Co., Ltd., Shanghai, China) was derived from baker's yeast, containing hydrolyzed proteins, nucleic acid, and nucleotides. The nutrients composition of YP is presented in Table 1. Room temperature was maintained at 30°C in the first week after weaning and then reduced by 2°C per week. The piglets were fed *ad libitum* from a four-space feeder (1.25 × 0.25 × 0.10 m) with trays placed underneath the feeders in order to avoid wastage of feed. Water was available *ad libitum* from nipple drinkers. Diets were formulated to meet nutrient requirements of piglets proposed by the National Research Council (1998). The nutritive values of individual ingredients used for dietary formation were taken from tabulated values (Feed Database in China 2013), while crude protein (CP) and amino acids of YP were analyzed, digestible energy was calculated from digestible nutrient contents (Spiehs et al. 2002). The ingredient composition and nutrient levels of the dietary treatments are presented in Table 2.

Table 1. Measured composition of yeast-derived protein (YP)[1]

Ingredients	Concentration
Dry matter (%)	93.15
Crude protein (%)	41.30
Amino acids (%)	
Glutamic acid	4.16
Aspartic acid	3.45
Leucine	2.66
Alanine	2.07
Lysine	2.71
Valine	2.04
Arginine	1.63
Proline	1.66
Serine	1.73
Phenylalanine	1.69
Isoleucine	1.87
Threonine	1.96
Glycine	1.50
Tyrosine	1.16
Histidine	0.80
Methionine	0.57
Tryptophan	0.58
Cystine	0.26
Nucleic acid (%)	12.20
Nucleotides (mg/100 g)	
GMP	113.80
AMP	7.60

GMP = guanosine monophosphate, AMP = adenosine monophosphate

[1]amino acids of YP, and contents of GMP and AMP were analyzed (PONY Testing International Group, Reports ID No. E10254004301D-4 and No. E10254004301D-1, respectively)

Sample collecting. Throughout the study, feed intake per pen was recorded daily and piglets were weighed weekly for calculating average daily feed intake (ADFI), average daily growth (ADG), and feed conversion ratio (FCR). At day 28 blood samples were collected from the cervical vein into vacuum tubes (Shandong Chengwu Medical Products Factory, Shandong, China) from one piglet per each pen. Blood samples were stored

Table 2. Composition and nutrient contents of the experimental diets

Item	Control diet	YP diet
Ingredients (%)		
Maize	40.70	37.90
Puffing corn	20.00	20.00
Soybean meal (46% CP)	10.00	10.00
Fermented soybean meal	5.00	6.00
Soy protein concentrate (65% CP)	8.00	8.00
Whey powder (3% CP)	6.00	6.00
Fish meal (65% CP)	5.00	2.00
Soybean oil	2.00	2.30
YP	–	4.00
Limestone	1.00	1.10
Calcium dihydrogen phosphate	0.40	0.80
Zinc oxide	0.30	0.30
Choline chloride (50%)	0.10	0.12
NaCl	0.10	0.17
Lysine (98.5%)	0.20	0.22
Methionine (84%)	0.05	0.10
Threonine (98.5%)	0.05	0.04
Tryptophan (10%)	0.10	–
Compound premix[1]	1.00	1.00
Nutrient levels		
Digestible energy[2] (Mcal/kg)	14.13	14.13
Crude protein (%)	20.60	20.60
Calcium (%)	0.80	0.80
Total phosphorus (%)	0.60	0.60
Available phosphorus (%)	0.40	0.40
Salt (%)	0.40	0.40
Crude fat (%)	4.90	4.80
Choline (mg/kg)	1500	1500
Lysine (%)	1.40	1.40
Methionine and cysteine (%)	0.76	0.78
Threonine (%)	0.87	0.86
Tryptophan (%)	0.24	0.24

YP = yeast-derived protein, CP = crude protein

[1]contents per kg of diet: Fe 150 mg, Cu 195 mg, Zn 150 mg, Mn 30 mg, I 0.3 mg, Se 0.3 mg, vitamin A 12 000 I.U., vitamin D_3 3200 I.U., vitamin E 80 I.U., vitamin K 32.50 mg, vitamin B_1 2.50 mg, vitamin B_2 6.50 mg, vitamin B_6 5 mg, vitamin B_{12} 50 mg, nicotinic acid 45 mg, pantothenic acid 20 mg, folic acid 1.50 mg, biotin 0.15 mg, enzyme preparation and preservatives

[2]calculated according to digestible nutrient contents

at room temperature for 40 min before centrifuging (3000 *g*, 15 min, 4°C) for serum collection (Centrifuge 5810R; Eppendorf AG, Hamburg, Germany). Serum samples were stored at –80°C for biochemical assays. After serum collection, the same piglet/each pen/each treatment was slaughtered by severing the carotid arteries under deep barbiturate anesthesia with intravenous injection of sodium pentobarbital (30 mg/kg body weight). The liver, spleen, and kidney of each piglet were removed and weighed immediately. The length and weight of small intestine were measured after the removal of luminal contents. Duodenum, jejunum, and ileum tissue samples were rinsed by saline water (0.9% NaCl), pieces of liver and intestinal (duodenum, jejunum, and ileium) tissues were immediately frozen in liquid nitrogen, and then stored at –80°C for gene expressions. Another portion of intestinal tissues in duodenum, jejunum, and ileum (approximately 2 cm long, respectively) was preserved in 4% methanol solution for histological analyses. In addition, the chyme of the ileum and colon were removed immediately and stored at –80°C for microbial analyses.

Small-intestinal morphology measurement. The duodenal, jejunal, and ileal samples were embedded in paraffin. As in our previous study (Che et al. 2012), each sample was used to prepare 5 slides and each slide had 3 sections (5 μm thick), which were stained with eosin and hematoxylin for intestinal morphology measurement by 20 well-oriented villus-crypt units per each section (Scion Image software, Version 4.02, 2004) and villus height to crypt depth ratio (VCR) was calculated.

Serum amino acid analyses. Serum samples stored at –80°C were defrosted. Serum (50 μl) was dispensed into a vial and supplemented with 50 μl of 3% sulfosalicylic acid and preserved at 4°C for 1 h. Protein was removed immediately by centrifuging at 3000 *g* at 4°C for 20 min (Thermo Scientific Sorvall Legend Micro 17R; Thermo Fisher Scientific, Waltham, USA). The supernatant was transferred to sample vials, and amino acid concentrations were analyzed with an automatic amino acid analyzer (L-8800 Amino Acid Analyzer; Hitachi, Tokyo, Japan) using the ninhydrin colourimetry method with a multi-segment tandem column in High Performance Liquid Chromatography. All chromatographic procedures were performed at room temperature, and the samples and standards were evaluated in duplicate.

Antioxidant parameters determination. Serum antioxidant parameters were analyzed by assay kits

from Nanjing Jiancheng Institute of Bioengineering (Nanjing, Jiangsu, China), the assay was conducted following the instructions of the kits producers. All samples were measured in triplicate. After thawing serum samples in ice-cold buffers, the activities of superoxide dismutase (SOD), catalase (CAT), nitric oxide synthase (NOS), glutathione peroxidase (GP_X), total antioxidative capability (T-AOC), and malondialdehyde (MDA) level were determined using colourimetric methods with Beckman DU-800 UV-visible spectrophotometer (Beckman Coulter Inc., Fullerton, USA).

Microbial analyses. According to previously established methods (Han et al. 2012), bacterial DNA was isolated from the chyme samples in ileum and colon by stool DNA Kit (Omega Bio-Tek, Doraville, USA) according to the manufacturer's instruction. Quantitative RT-PCR of total bacteria was performed with SYBR® Green PCR reagents (TaKaRa, Kyoto, Japan), whereas quantitative RT-PCR for bifidobacterium, lactobacillus, and *Escherichia coli* were performed with Taq Primers and fluorescent oligonucleotide probes were commercially synthesized (Life Technologies Ltd., Beijing, China). The RT-PCR primers and probes combination used for total bacteria, bifidobacterium, lactobacillus, and *E. coli* were presented in Supplementary **Table S1**. A 10-fold serial dilution series of the copies, ranging from 1×10^1 to 1×10^{12} copies/µl, was used to construct the standard curves for total bacteria, bifidobacterium, lactobacillus, and *E. coli*. The copy numbers (copies/µl) were calculated via measuring the concentration of the plasmid using the spectrophotometer DU-800 (Beckman Coulter Inc.) according to the equation:

DNA copy numbers = (DNA concentration (µg/µl) × 6.0233×10^{23} copies/mol)/(DNA size (bp) × 660×10^6).

The standard curves of bacteria were generated by the cycle threshold (Ct) values in each dilution that was measured in duplicate using a quantitative RT-PCR. Each standard curve was constructed by a linear regression of the plotted points, and the Ct values were plotted against the logarithm of the template copy numbers. From the slope of each standard curve, PCR amplification efficiency (E) was calculated following the equation $E = 10^{(-1/slope-1)}$.

Total RNA extraction and real-time RT-PCR. Total RNA was extracted from frozen distal intestinal tissues and liver using Trizol Reagent (Invitrogen, Carlsbad, USA) according to the manufacturer's instructions, the quality and purity of RNA samples were assessed by electrophoresis on 1.0% agarose gel and nucleic acid analyzer DU-800 (Beckman Coulter Inc.). Reverse transcription-polymerase chain reaction was performed in triplicate to amplify the target gene and the reference gene (*β-actin* and *GAPDH*) using One Step SYBR® Prime Script™ RT-PCR Kit II (Catalog No. DRR086A; TaKaRa, Kyoto, Japan) using reverse transcription-polymerase chain reaction (ABI Prism® 7900HT Sequence Detection System; Applied Biosystems, Carlsbad, USA). The sequences of primers and length of the products are given in Supplementary **Table S2**. The reaction mixture (10.0 µl) contained 5.6 µl of freshly premixed one step SYBR® Green RT-PCR Master Mix and PrimeScript™ Enzyme Mix, 0.8 µl of the primers, and 150 ng of RNA template. The RT-PCR program was designed with 1 cycle of 42°C for 5 min, 1 cycle of 95°C for 10 s, 40 cycles of 95°C for 5 s, and 60°C for 34 s, followed by the dissociation step at 95°C for 15 s, 60°C for 60 s, and 95°C for 15 s. At the end of amplification, melting curve analysis was performed to identify amplification specificity. For each of the target genes, the Ct values of all the samples were then calculated by subtracting the average Ct of the corresponding normal body weight group or normal body weight group with formula feed. The Ct values were converted to fold differences by raising 2 to the power –Ct (2^{-Ct}) according to Livak and Schmittgen (2001).

Statistical analyses. Values are given as means ± SEM. All data were subjected to One-Way Analysis of Variance (ANOVA) by using SPSS software (Version 20, 2011). The data analyzed included ADG, ADFI, and FCR with each pen as statistic unit, while organ indices, serum amino acids, serum antioxidant enzyme activities, intestinal bacteria, gene expressions, and morphology variables (villus height, crypt depth, and VCR) with one piglet per each pen as statistic unit and 5 piglets per each treatment group. A probability level of $P < 0.05$ was considered significant, whereas $0.05 < P < 0.10$ was considered a tendency.

RESULTS

Growth performance and organ indices. During weeks 1 and 3, ADG tended to be greater (+14~17%, $P < 0.09$) in piglets fed YP diet than in piglets fed control diet, but the overall ADG was markedly higher in piglets fed YP diet ($P < 0.05$) (Table 3). Consequently, piglets fed YP diet had greater final

Table 3. Effect of diet containing yeast-derived protein (YP) on growth performance of weaning piglets

Production variables	Control diet	YP diet	*P*-value
BW (kg)			
Initial	6.18 ± 0.04	6.21 ± 0.06	0.707
Day 7	7.53 ± 0.08	7.99 ± 0.24	0.105
Day 14	9.80 ± 0.30	10.67 ± 0.41	0.126
Day 21	13.58 ± 0.33	14.70 ± 0.51	0.102
Day 28	17.72 ± 0.36	19.38 ± 0.52	0.032
ADG (g/day)			
Day 1–7	193 ± 9	255 ± 28	0.068
Day 1–14	259 ± 20	319 ± 26	0.108
Day 1–21	353 ± 15	405 ± 23	0.087
Day 1–28	412 ± 12	470 ± 18	0.027
ADFI (g/day)			
Day 1–7	309 ± 10	327 ± 28	0.569
Day 1–14	397 ± 27	426 ± 30	0.490
Day 1–21	522 ± 23	548 ± 30	0.520
Day 1–28	617 ± 21	651 ± 27	0.345
FCR			
Day 1–7	1.62 ± 0.08	1.31 ± 0.09	0.034
Day 1–14	1.54 ± 0.02	1.34 ± 0.03	0.001
Day 1–21	1.48 ± 0.01	1.35 ± 0.02	0.001
Day 1–28	1.50 ± 0.01	1.38 ± 0.02	0.001

BW = body weight, ADG = average daily growth, ADFI = average daily feed intake, FCR = feed conversion ratio
values are means ± SEM (n = 5)

Table 4. Effect of diet containing yeast-derived protein (YP) on organ indices of weaning piglets

Item	Control diet	YP diet	*P*-value
Small intestine weight (g)	942 ± 33	1070 ± 68	0.169
Small intestine length (cm)	1199 ± 56	1345 ± 57	0.105
Liver weight (g)	486 ± 8	532 ± 20	0.069
Spleen weight (g)	36 ± 4	46 ± 4	0.115
Kidney weight (g)	106 ± 4	102 ± 5	0.545
Small intestine weight : BW (%)	5.38 ± 0.43	5.43 ± 0.55	0.897
Small intestine length : BW (cm/kg)	69.18 ± 9.41	68.43 ± 4.83	0.878
Liver weight : BW (%)	2.80 ± 0.15	2.71 ± 0.16	0.385
Spleen weight : BW (%)	0.21 ± 0.04	0.26 ± 0.02	0.077
Kidney weight : BW (%)	0.61 ± 0.03	0.52 ± 0.05	0.008

BW = body weight
values are means ± SEM (n = 5)

body weight ($P < 0.05$) than piglets fed control diet. Accordingly, due to the similar ADFI, piglets fed YP diet had significantly lower final FCR ($P < 0.01$) than piglets fed control diet. Furthermore, the relative weight of the kidney of piglets fed YP diet was significantly lower than that of piglets fed control diet ($P < 0.01$). The relative intestinal weight, intestinal length, liver and spleen weight were not significantly different between two groups (Table 4).

Amino acids concentrations. Serum concentrations of cystathionine, serine, histidine, and hydroxyproline were markedly decreased (–16~44%, $P < 0.05$), while those of alanine and aspartate were markedly increased (+16 and 21%, respectively, $P < 0.05$) in piglets fed YP diet compared with piglets fed control diet (Table 5). In addition, serum concentration of urea was significantly decreased (–46%, $P < 0.01$) in piglets fed YP diet relative to control diet.

Activities and gene expression of antioxidant enzymes. Serum GP_X activity was significantly higher (+19%, $P < 0.01$) in piglets fed YP diet relative to piglets fed control diet, but the serum activities of SOD, CAT, NOS, T-AOC, and MDA did not markedly differ between the two groups (Table 6). Likewise, liver and jejunum gene expressions of GP_X, SOD, CAT, and NOS were not significantly different between the two groups (data not shown).

Intestinal morphology and microbial analyses. There were no significant effects on villus heights, crypt depths, and VCR by feeding YP diet (data not shown). The copy numbers of lactobacilli and total bacteria in the colon were increased ($P < 0.05$) by feeding YP diet (Table 7).

The mRNA expression of innate immunity-related genes. Expression levels of genes encoding for *NF-κB1* and *IL-6* were markedly increased ($P < 0.05$) in the ileum of piglets fed YP diet compared with piglets fed control diet. In addition, feeding YP diet tended to increase ($P = 0.057$) mRNA abundance of toll-like receptor 4 (*TLR4*). However, no significant difference was observed for ileum gene expression of *IL-1*, *NF-κB2*, *TLR9*, and *TLR2* between the two groups (Table 8).

DISCUSSION

Performance. In the present study, piglets fed YP diet grew faster than piglets fed the control diet, accordingly the overall FCR was improved though there was no significant difference for ADFI among piglets. Consistently, Carlson et al. (2005)

Table 5. Effect of diet containing yeast-derived protein (YP) on serum concentrations of amino acids and urea of weaning piglets

Item	Control diet	YP diet	*P*-value
Sulfur metabolism amino acids (μM)			
Methionine	5.95 ± 0.45	6.84 ± 1.00	0.517
Taurine	11.94 ± 1.16	10.38 ± 1.73	0.481
Serine	18.15 ± 0.65	15.15 ± 0.89	0.044
Glycine	75.98 ± 7.47	81.92 ± 6.86	0.572
Cystathionine	2.25 ± 0.29	1.28 ± 0.16	0.031
Cystine	2.58 ± 0.30	2.94 ± 0.49	0.663
P-Serine	2.43 ± 0.03	2.24 ± 0.19	0.090
Indispensable amino acids (μM)			
Arginine	20.57 ± 2.27	17.45 ± 1.33	0.267
Histidine	8.55 ± 0.57	6.71 ± 0.25	0.029
Isoleucine	21.29 ± 1.24	18.61 ± 2.31	0.345
Leucine	31.44 ± 1.98	26.47 ± 3.35	0.237
Lysine	23.65 ± 2.24	23.40 ± 3.15	0.952
Phenylalanine	18.47 ± 0.98	16.87 ± 0.40	0.171
Threonine	23.31 ± 2.67	21.70 ± 1.03	0.544
3-Methylhistidine	1.45 ± 0.12	1.19 ± 0.07	0.210
1-Methylhistidine	8.01 ± 1.70	6.37 ± 1.28	0.461
Valine	36.24 ± 1.76	32.07 ± 3.43	0.311
Dispensable amino acids (μM)			
Alanine	38.82 ± 2.96	47.07 ± 1.63	0.040
Aspartate	2.87 ± 0.09	3.32 ± 0.07	0.004
Citrulline	9.92 ± 1.19	10.01 ± 0.83	0.950
Glutamate	22.7 ± 1.58	25.03 ± 2.40	0.443
Hydroxyproline	20.55 ± 0.68	14.42 ± 1.17	0.010
Ornithine	7.29 ± 0.88	6.37 ± 0.43	0.365
Proline	104.96 ± 11.07	111.55 ± 10.20	0.670
Tyrosine	18.06 ± 1.65	16.41 ± 0.75	0.394
β-Alanine	1.36 ± 0.20	1.21 ± 0.10	0.552
Others (μM)			
Urea	306.34 ± 26.89	166.58 ± 9.57	0.003
Carnosine	6.92 ± 1.22	7.22 ± 1.83	0.901

values are means ± SEM (n = 5)

suggested that the addition of YP to nursery diet improved piglets' growth performance, allowing them to reach slaughter weight faster. In this study, the better growth performance in piglets fed YP diet could be partially explained by the lower concentrations of serum amino acids and urea, which suggested that protein deposition was greater in piglets receiving YP diet. Particularly, urea is the main nitrogenous end product arising from the amino acids catabolism in mammals, systematic concentration of urea is inversely related to protein deposition and FCR (Coma et al. 1995), the inadequacy or poor quality of dietary protein can increase serum concentration of urea (Shen et al. 2011). In addition, urea concentration has been classically recognized as an indicator of renal function, the lower concentration of serum urea would alleviate the metabolic load of kidney (White et al. 1991), which may explain why relative kidney weight in piglets fed YP diet was lower. In contrast, there were higher serum concentrations of alanine and aspartate in piglets fed YP diet, in-

Table 6. Effect of diet containing yeast-derived protein (YP) on oxidative status of weaning piglets

Item	Control diet	YP diet	*P*-value
GP_X (U/ml)	255.54 ± 8.53	303.31 ± 7.22	0.003
SOD (U/ml)	31.542 ± 0.978	34.291 ± 0.965	0.544
MDA (nmol/ml)	1.46 ± 0.32	2.14 ± 0.36	0.230
CAT (U/ml)	4.403 ± 0.72	3.779 ± 0.55	0.512
NOS (U/ml)	16.550 ± 2.474	21.494 ± 2.032	0.210
T-AOC (U/ml)	0.712 ± 0.221	0.664 ± 0.194	0.884

GP_X = glutathione peroxidase, SOD = superoxide dismutase, MDA = malondialdehyde, CAT = catalase, NOS = nitric oxide synthase, T-AOC = total antioxidative capability
values are means ± SEM (n = 5)

dicating the increased availability of amino acids to peripheral tissue (Glade 1991; Wu 2009).

Likewise, YP appeared to have beneficial effects on feed intake, live weight gain, and feed conversion in birds. It has been suggested that the positive effect of YP on growth performance is ascribed to the nucleotides inside (Owens and McCracken 2007), which are contained in mammalian milk. As the substrate for synthesis of DNA, RNA, and enzyme co-factors, nucleotides are required for the development of gastrointestinal tract and modulation of the immune system (Mateo et al. 2004). During the weaning period, dietary change from milk to solid diet leads to the insufficiency of nucleotides (Domeneghini et al. 2004), which is however highly required for intestinal epithelial turnover and development of immune system in weaning piglets (Maldonado et al. 2001). As a consequence, nucleotides can be easily exhausted in piglets under weaning stress (Mateo et al. 2004). Therefore, exogenous supply of nucleotides contributed to growth performance of piglets with nutritional role for maturation of some tissues such as lymphoid tissue and intestine in periods of intensive development (Sanchez-Pozo and Gil 2002). Exogenous nucleotides have been demonstrated to maintain the function of rapidly dividing tissues, stimulate innate immunity, and reduce the incidence of enteric diseases (Uauy 1994; Sauer et al. 2011). Nutritionally, furthermore, it has been reported that YP had comparable nutrients digestibility as SDPP (Mateo and Stein 2007).

Intestinal morphology analyses. In this study, the histological analysis showed feeding nucleotides-enriched YP did not markedly affect intestinal morphology, as an indicator to reflect the renewal rate of intestinal epithelial cells. Previous studies by Van der Peet-Schwering et al. (2007) and also Sauer et al. (2012b) indicated that dietary nucleotides failed to improve intestinal morphology, but some other studies showed there was a positive effect of dietary nucleotides on intestinal repair under

Table 7. Effect of diet containing yeast-derived protein (YP) on microbial copy numbers (log^{10} Cfu/g of digesta) in the ileum and colon of weaning piglets

Segment	Item	Control diet	YP diet	*P*-value
Ileum	*E. coli*	5.38 ± 0.13	5.37 ± 0.09	0.939
	lactobacilli	6.92 ± 0.23	7.42 ± 0.17	0.121
	bifidobacterium	7.45 ± 0.20	7.62 ± 0.09	0.482
	total bacteria	10.21 ± 0.06	10.23 ± 0.04	0.801
Colon	*E. coli*	5.43 ± 0.13	5.67 ± 0.18	0.332
	lactobacilli	7.20 ± 0.17	8.27 ± 0.13	0.021
	bifidobacterium	7.97 ± 0.11	8.17 ± 0.07	0.143
	total bacteria	10.26 ± 0.04	10.44 ± 0.06	0.044

values are means ± SEM (n = 5)

Table 8. Effect of diet containing yeast-derived protein (YP) on mRNA abundance of innate immunity-related genes in the ileum of weaning piglets

Gene	Control diet	YP diet	*P*-value
IL-1	1.00 ± 0.24	1.31 ± 0.16	0.308
NF-κB2	1.00 ± 0.13	1.09 ± 0.14	0.676
TLR4	1.00 ± 0.07	1.33 ± 0.11	0.057
TLR9	1.00 ± 0.18	1.24 ± 0.03	0.287
TLR2	1.00 ± 0.06	1.13 ± 0.12	0.380
IL-6	1.00 ± 0.15	1.45 ± 0.10	0.041
NF-κB1	1.00 ± 0.21	1.92 ± 0.15	0.024

TLR = toll-like receptor, *IL* = interleukin, *NF-κB* = nuclear factor kappa B
values are means ± SEM (n = 5)

certain stress conditions (Bueno et al. 1994). It is generally accepted that the intestinal response to nucleotides may vary depending on the health status of animals.

Microbial analyses. Considering the importance of intestinal microflora for animal health and nutrients utilization, we determined the microbial copy numbers of *E. coli*, lactobacilli, bifidobacterium, and total bacteria in ileum and colon. The results suggest that inclusion of YP in weaning diet led to a significant increase of lactobacilli and total bacteria in colon but not in ileum. These findings are similar as the results from Gil et al. (1986), who reported that the addition of nucleotides to infant formula resulted in increased percentages of bifidobacteria and enterobacteria in the stool. *In vitro* study also showed that dietary nucleotides might stimulate the proliferation of beneficial bacteria (Sauer et al. 2010). Microbial composition in the gastrointestinal tract can be affected by dietary differences (Collins and Gibson 1999). Several studies demonstrated that some other yeast products (brewer's yeast cells, etc.) can affect the composition of intestinal microflora (Naughton et al. 2001; White et al. 2002).

Serum antioxidant enzyme activities, gene expressions in jejunum and liver. Oxidative stress is one of the major factors impairing gastrointestinal integrity, causing intestinal inflammation. Antioxidant enzymes are an important part of the antioxidant system protecting the cells against oxidative stress. The antioxidant capability can be assessed indirectly by measuring antioxidant enzyme activities such as GP_X, SOD, CAT, and NOS as well as oxidative metabolites (MDA, etc.) (Buonocore and Groenendaal 2007). In this study, there was a markedly higher activity of serum GP_X in piglets fed YP diet, suggesting YP could improve the anti-oxidative capability of piglets. This improvement on anti-oxidative capability could be ascribed to the YP-containing nucleotides, which was reported to alleviate oxidative stress of piglets induced by high dietary polyunsaturated fatty acids (Salobir et al. 2005). In addition, the YP used in this experiment contained a relatively higher (by 4.47%) content of glutamine. As a precursor for biosynthesis of cellular antioxidant glutathione, glutamine has been demonstrated to attenuate oxidative stress and related gene expression in mammals (Mates et al. 2002; Tsai et al. 2012).

Toll-like receptors are typical pattern recognition receptors signalling the luminal antigens and mediating mucosal innate host defense, maintaining mucosal and commensal homeostasis. It has been demonstrated that the TLR4-Myd88-NF-κB signal pathway is involved in inflammation (Kawai and Akira 2009). In the present study, mRNA expressions of *TLR4*, *NF-κB1*, and *IL-6* were greater in the ileum of piglets fed YP diet compared with piglets in the control group. It was recognized that the nucleic acid and nucleotides in YP contributed to the innate immune response. Consistently, Sauer et al. (2011) found that weanling piglets fed nucleic acids for 2–4 weeks improved lymphocyte function and *in vitro* proliferative responses to a non-specific T-cell mitogen. Moreover, intestinal lymphocyte maturation and mRNA abundance of *IL-6* and *IL-8* were also enhanced by dietary nucleotides (Gil 2002). In addition, exogenous nucleotides stimulated humoral immune response and production of immunoglobulins in neonates and weaned piglets (Maldonado et al. 2001; Lee et al. 2007; Sauer et al. 2012a). In contrast, the restriction of dietary nucleotides resulted in a decreased production of IL-2, natural killer cell cytotoxicity, and macrophage activation (Carver 1994).

In conclusion, results of the current study indicated that inclusion of YP in diet improved growth performance of piglets, and partially enhanced anti-oxidative capability as well as intestinal innate immunity.

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