Effects of yeast culture supplementation on growth performance, intestinal health, and immune response of nursery pigs

Y. B. Shen, X. S. Piao, S. W. Kim, L. Wang, P. Liu, I. Yoon and Y. G. Zhen

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Effects of yeast culture supplementation on growth performance, intestinal health, and immune response of nursery pigs[1]

Y. B. Shen,* X. S. Piao,*[2] S. W. Kim,† L. Wang,* P. Liu,* I. Yoon,‡ and Y. G. Zhen‡

*State Key Laboratory of Animal Nutrition, China Agricultural University, Beijing 100094, China;
†Department of Animal Science, North Carolina State University, Raleigh 27695;
and ‡Diamond V Mills, Cedar Rapids, IA 52403

ABSTRACT: A total of 216 weaning pigs were used in 2 experiments to determine the effects of dietary supplementation of yeast culture (YC) at different dose levels on the growth performance, nutrient digestibility, intestinal morphology, intestinal microflora, and immune response in weanling pigs and to determine whether YC can be a candidate to replace antibiotic growth promoters (AGP). In Exp. 1, 192 pigs (7.5 ± 0.2 kg of BW) weaned at 28 d of age were randomly allotted to 6 treatments: 1) control (without AGP or YC); 2) AGP (chlortetracycline, 80 mg/kg); 3) 2.5 g/kg of YC (Diamond V XP Yeast Culture); 4) 5 g/kg of YC; 5) 10 g/kg of YC; and 6) 20 g/kg of YC. Each treatment had 8 replicated pens with 4 pigs per pen. Pigs were fed the experimental diets for 21 d. Average daily gain of pigs fed 5 g/kg of YC was greater ($P < 0.05$) than that of pigs in the control and other YC groups. However, there was no difference between the YC and AGP group. Pigs supplemented with 5 g/kg of YC, 10 g/kg of YC, and AGP had a greater ($P < 0.01$) ADFI than the control; however, G:F was not affected by treatment. Thus, 5 g/kg of YC supplementation level was chosen for Exp. 2. In Exp. 2, to elucidate the mode of action of YC, 24 nursery pigs (5.8 ± 0.1 kg of BW; 21 d of age) were randomly allotted into 3 treatments for a 21-d trial. Treatments consisted of 1) control (without AGP or YC), 2) AGP, and 3) 5 g/kg of YC. Blood samples were collected weekly to measure $CD4^+$, $CD8^+$ percentage, and blood cytokine content. All pigs were harvested to determine treatment effects on gut microbiota, morphology, and immune function. Dietary supplementation of 5 g/kg of YC improved ($P < 0.05$) ADG of pigs compared with the control group, but performance of pigs fed YC was similar to those fed AGP. Pigs receiving 5 g/kg of YC had greater ($P < 0.05$) digestibility of DM, CP, GE, and jejunal villus height and villus height:crypt depth ratio ($P < 0.05$) compared with pigs fed the control diet. However, no differences in performance, digestibility, or gut morphology were observed between pigs fed YC and AGP. Gut interferon (IFN)-γ concentrations were greater ($P < 0.01$) for pigs supplemented with YC compared with control pigs and pigs supplemented with AGP on d 21. However, plasma IFN-γ concentrations were decreased ($P < 0.01$) in pigs supplemented with YC and AGP compared with control pigs on d 7, and $CD4^+$ was decreased ($P < 0.01$) in pigs supplemented with YC and AGP on d 14. Results indicate that dietary YC supplementation at 5 g/kg had a positive effect on growth performance of nursery pigs by improving jejunal villus height and villus height:crypt depth ratio and by modulating gut immune response. The comparable effect of 5 g/kg of YC supplementation and AGP on the growth performance of nursery pigs indicates that YC may be a good candidate as an antibiotic alternative.

Key words: growth performance, immunity, microflora, morphology, yeast culture

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INTRODUCTION

Young pigs often face postweaning challenges including diarrhea, low feed intake, BW loss, and these can cause severe damages to intestinal health and function (Pluske et al., 1996). Subtherapeutic use of antibiotics has widely been applied to nursery pigs to solve postweaning problems (Barton, 2000). However, recent issues with bacterial resistance to these antibiotics urged scientists to find alternatives to the use of antibiotics (Cassel, 1995; Mathew et al., 1998; Bach Knudsen, 2001; Smith et al., 2002).

Fully fermented yeast culture (**YC**) is a dried product containing yeast and various metabolites of yeast

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[2]Corresponding author: piaoxsh@mafic.ac.cn
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fermentation. Research has indicated that YC supplementation is beneficial to ruminants (Harrison et al., 1988; Dawson et al., 1990; Cole et al., 1992) and pigs (Kornegay et al., 1995; van der Peet-Schwering et al., 2007) by enhancing growth performance, milk production, nitrogen balance, and nutrient digestion. However, other studies reported no benefits of dietary YC supplementation to pig diets (Veum and Bowman, 1973; Jurgens, 1995).

Enzymes, vitamins, saccharides, and other metabolites produced from yeast fermentation may benefit growth, metabolism, and health of pigs. Several studies have investigated the action of yeast cell wall components on immune function of weaning pigs (Seljelid et al., 1987; Muchmore et al., 1990; Podzorski et al., 1990; Davis et al., 2004) showing that yeast cell wall components (e.g., mannans) may modulate immune response of pigs to maintain animal health and improve growth performance by reducing pathogenic bacteria and improving gut health (Anderson et al., 1999). van der Peet-Schwering et al. (2007) report YC could improve the performance of weanling pigs. However, studies showing the effects of YC on gut health and immunity of nursery pigs are limited. The present study was conducted to determine the effects of dietary supplementation of YC on the growth performance, nutrient digestibility, intestinal morphology, intestinal ecology, and immune function changes of nursery pigs compared with an antibiotic growth promoter (**AGP**).

MATERIALS AND METHODS

Experimental protocols were approved by the Institutional Animal Care and Use Committee of China Agricultural University.

Animals and Experimental Design

Exp. 1. A total of 192 pigs (Large White × Landrace; barrows; 7.5 ± 0.2 kg of BW) weaned at 28 d of age were randomly assigned to 6 treatments: 1) control (without AGP or YC), 2) AGP (chlortetracycline, 80 mg/kg, Jinhe Biotechnology Co. Ltd., Tuoketuo, China), 3) 2.5 g/kg of YC (Diamond V XP Yeast Culture, Diamond V Mills, Cedar Rapids, IA), 4) 5 g/kg of YC, 5) 10 g/kg of YC, and 6) 20 g/kg of YC. Each treatment had 8 replicated pens with 4 pigs per pen. During the 3-wk feeding period, all pigs were housed in a temperature-controlled nursery room (25 to 27°C). Feed and water were available ad libitum. All diets were formulated according to the nutrient requirements of NRC (1998; Table 1). Feed intake and BW were measured at the beginning and end of the experiment to determine ADG, ADFI, and G:F ratio.

Exp. 2. Potential modes of action of YC that could explain the improved growth performance observed in Exp. 1 were investigated in Exp. 2. Twenty-four nursery pigs (Large White × Landrace; barrows; 5.8 ± 0.1 kg of BW; 21 d of age) were randomly allotted to 3 treatments: 1) control (without AGP or YC); 2) AGP (chlortetracycline, 80 mg/kg, Jinhe Biotechnology Co. Ltd.); 3) 5 g/kg of YC (Diamond V XP Yeast Culture, Diamond V Mills). Pigs were housed individually. Basal diet and feeding management were identical to Exp. 1. Feed intake and BW of pigs were measured weekly for calculation of ADG, ADFI, and G:F.

All pigs were killed by a lethal injection of sodium pentobarbital to obtain intestinal tissues and digesta samples. Intestinal tissue from middle sections of duodenum, jejunum, and ileum were aseptically isolated, flushed with 0.9% salt solution, fixed in 10% formaldehyde-phosphate buffer, and kept at 4°C for microscopic assessment of mucosal morphology. Sections of jejunum (4 cm) were collected and processed to determine cytokine content [IL-10 and interferon (**IFN**)-γ] according to van Halteren et al. (1997). The large intestine was removed immediately and divided into 3 segments (cecum, colon, and rectum) for microbial counting (Mathew et al., 1998). In addition, the representative digesta from cecum and colon were gently squeezed into a preweighed tube and then stored in a freezer at −80°C until analysis for VFA concentrations (Mathew et al., 1998).

Feces were collected during the last 3 d of the study. On each day, approximately 50 g of fecal sample was collected from each pig into sterile plastic bottles and immediately stored at −20°C until further analysis for nutrient content. The 3-d composite sample was oven-dried for 72 h at 55°C and ground through a 1.0-mm screen. Digestibility estimates were measured using the indicator (Cr_2O_3) method according to the procedure of Fan and Sauer (2002). On d 0, 7, 14, and 21 of the experiment, 10-mL blood samples were collected from a jugular vein from all pigs by a 10-mL vacutainer K_2 EDTA tube (Greiner Bio-One GmbH, Kremsmunster, Austria), which contained 1.8 mg/mL of spray-dried K_2 EDTA. Two milliliters of the blood was used for the determination of $CD4^+$ and $CD8^+$ concentrations. The remainder of the blood was used to obtain plasma by centrifugation at 3,500 × *g*, 4°C for 5 min. The plasma was stored at −20°C until needed for the determination of cytokines (Lai et al., 2005).

Chemical Analysis of Feed and Feces

Feed samples were collected at the start of Exp. 2. Feed and fecal samples were dried in an oven (65°C) and ground to pass through a 1-mm sieve. Each sample was analyzed for DM, GE, CP (N × 6.25), Ca, total P, and Cr. Analysis of proximate nutrients was conducted according to the methods of AOAC (1997). Gross energy was determined by an automatic adiabatic oxygen bomb calorimeter (Parr 1281 Automatic Energy Analyzer, Moline, IL). Chromium content was analyzed using an atomic absorption spectrophotometer (Hitachi

Table 1. Ingredient and chemical composition of the experimental diet, as-fed basis

Item	Control	AGP[1]	Dietary yeast culture, g/kg			
			2.5	5	10	20
Ingredient, g/kg						
Corn, yellow	508.4	508.4	505.9	503.4	498.4	488.4
Soybean meal, 45% CP	242.5	242.5	242.5	242.5	242.5	242.5
Spray-dried porcine plasma	30.0	30.0	30.0	30.0	30.0	30.0
Fish meal, 61% CP	50.0	50.0	50.0	50.0	50.0	50.0
Dried whey	100.0	100.0	100.0	100.0	100.0	100.0
Soybean oil	30.8	30.8	30.8	30.8	30.8	30.8
Dicalcium phosphate	18.2	18.2	18.2	18.2	18.2	18.2
Limestone	3.4	3.4	3.4	3.4	3.4	3.4
Salt	2.5	2.5	2.5	2.5	2.5	2.5
Yeast culture[2]	0	0	2.5	5.0	10.0	20.0
DL-Met	0.4	0.4	0.4	0.4	0.4	0.4
L-Lys·HCl	1.3	1.3	1.3	1.3	1.3	1.3
Vitamin and mineral premix[3]	10.0	10.0	10.0	10.0	10.0	10.0
Chromic oxide	2.5	2.5	2.5	2.5	2.5	2.5
Calculated composition, g/kg						
ME, kcal/kg	3,400	3,400	3,400	3,400	3,400	3,400
CP	200.0	200.0	200.1	200.2	200.4	200.8
Ca	9.0	9.0	9.0	9.0	9.0	9.0
P	8.0	8.0	8.0	8.0	8.0	8.0
Lys	13.5	13.5	13.5	13.5	13.5	13.5

[1]Pigs in the antibiotic growth promoters (AGP) group fed a basal diet supplemented with 80 mg/kg of chlortetracycline (Jinhe Biotechnology Co. Ltd., Tuoketuo, China).

[2]Yeast culture (Diamond V Mills, Cedar Rapids, IA) contained 3,395 kcal/kg of ME, 120 g/kg of CP, 0.5 g/kg of Ca, 6 g/kg of P, 5.5 g/kg of Lys, and 2.5 g/kg of Met+Cys.

[3]Vitamin and mineral premix provided the following per kilogram of feed: vitamin A, 12,000 IU as vitamin A acetate; vitamin D, 2,500 IU as vitamin D_3; 30 IU of vitamin E; 12 μg of vitamin B_{12}; vitamin K, 3 mg as menadione sodium bisulfate; D-pantothenic acid, 15 mg as calcium pantothenate; 40 mg of nicotinic acid; choline chloride, 400 mg as choline chloride; Mn, 40 mg as manganous oxide; Zn, 100 mg as zinc oxide; Fe, 90 mg as iron sulfate; Cu, 8.8 mg as copper oxide; I, 0.35 mg as ethylenediamine dihydroiodide; and Se, 0.3 mg as sodium selenite.

Z-5000 Automatic Absorption Spectrophotometer, Tokyo, Japan) according to Williams et al. (1962). Phosphorus content was analyzed using the UV-visible spectrophotometer (Hitachi, U-1000).

Bacteriological Analysis

For microbial analysis, about 1 g of digesta sample was placed in a sterile bottle and serially diluted 10-fold with a sterile-physiological salt solution (NaCl, 9 g/L). The sample was homogenized using an ultra-turrax, and diluted in sterile saline ranging from 10^{-1} to 10^{-8} for viable counts of total aerobic and anaerobic bacteria, *Lactobacillus*, and *Escherichia coli*. *Escherichia coli* were cultured using MacConkey agar (Beijing Haidian Microbiological Culture Factory, Beijing, China, CM908). *Lactobacillus* was determined using MRS agar (De Man, Rogosa, Sharpe, Oxoid Ltd., CM0361). For anaerobic culture, 100 μL of each serial dilution was incubated in an anaerobic chamber. For aerobic culture, 100 μL of each serial dilution was planted in a nutrient agar plate. All plates and tubes were incubated in 35°C, 5% CO_2 for 2 d. All dilutions were duplicated and counts from duplicated plates were averaged. Digesta microbial enumerations were expressed as $\log_{10}$ cfu per gram.

VFA Analysis

Volatile fatty acid concentrations in digesta from the cecum, colon, and rectum were determined by a gas chromatographic method following the procedures of Franklin et al. (2002). In the determination, approximately 1.5 g of thawed digesta was suspended in 1.5 mL of distilled water in a screw-capped tube. The entire sample was centrifuged (15,000 × *g*) at 4°C for 10 min. After centrifugation, 1 mL of supernatant was transferred into an ampoule and mixed with 200 μL of metaphosphoric acid (HPO_3). The ampoules were placed in an ice bath for 30 min then centrifuged (15,000 × *g*) at 4°C for 10 min. One microliter of sample was injected into a model 5890 Hewlett Packard gas chromatograph (Hewlett Packard, Avondale, PA). The gas chromatograph was equipped with a flame ionization detector and a polyethylene glycol column. The column was operated at 100 to 150°C with highly purified N_2, at 1.8 mL/min, as the carrier gas. The least detectable limit for all VFA was 0.1 mmol/L.

Small Intestinal Morphology

Fixed intestinal segments were embedded in paraffin wax. Cross sections at 6 μm thickness of each intesti-

Table 2. Effect of dietary yeast culture (YC) and antibiotic growth promoters (AGP) on growth performance of weaning pigs (Exp. 1)[1]

			Dietary yeast culture (g/kg)					
Item	Control	AGP	2.5	5	10	20	SEM	*P*-value
ADG, g	362[c]	412[ab]	375[bc]	438[a]	384[bc]	379[bc]	7	0.02
ADFI, g	655[c]	749[ab]	673[bc]	820[a]	741[ab]	708[bc]	14	<0.01
G:F, g/kg	558	538	594	538	523	543	9	0.31

[a–c]Means in the same row with different superscripts differ ($P < 0.05$).

[1]Values represent means of 32 pigs (4 replicates per pen). Initial average BW of pigs was 7.51 kg, and pigs were tested for 21 d. AGP (positive control supplemented with 80 mg/kg of chlortetracycline, Jinhe Biotechnology Co. Ltd., Tuoketuo, China); control (negative control fed a basal diet); YC (supplemented with Diamond V XP Yeast Culture, Cedar Rapids, IA).

nal segment were stained with hematoxylin and eosin. Intestinal morphological measurements including villus height and crypt depth were determined at 40× magnification using an Olympus CK 40 microscope (Olympus Optical Company, Shenzhen, China). Lengths of 10 well-oriented intact villi and their associated crypt were measured in triplicate for each segment of each pig according to the procedure of Touchette et al. (2002).

Cytokine and CD4$^+$/CD8$^+$

Plasma IL-10 and IFN-γ were measured using a commercially available swine ELISA kit (Moraes et al., 2007; BioSource International Inc., Camarillo, CA). The minimum detectable dose was 3.0 pg/mL for IL-10 and 2.0 pg/mL for IFN-γ. Assays were performed colorimetrically using a plate reader.

To measure the cytokine content in the small intestine, 4-cm sections of the jejunum were collected and processed according to the method of van Halteren et al. (1997). Protein lysates of the jejunum were prepared with a cocktail of proteinase inhibitors and centrifuged at 10,000 × g at 4°C for 10 min. Concentrations of IL-10 and IFN-γ were determined by ELISA according to the manufacturer's instructions (Biosource, Camarillo, CA). Cytokine contents were standardized to the weight of jejunum tissue in each sample (Nakajima-Adachi et al., 2006).

Blood CD4$^+$ and CD8$^+$ lymphocyte subsets, as well as CD4$^+$:CD8$^+$ ratio, were analyzed by flow cytometry according to the method of Lai et al. (2005). Whole blood (100 μL) samples were stained with 25 μL of diluted primary antibody (IgG2b mouse anti-swine-CD4 FITC-labeled and IgG2a mouse anti-swine-CD8α PE-labeled; Southern Biotechnology Associates, Birmingham, AL), as well as the isotype control antibodies mouse IgG2b-FITC (Sigma-Aldrich, St. Louis, MO) for anti-CD4 and mouse IgG2a-Quantum Red (Sigma-Aldrich) for anti-CD8. After incubation for 30 min at room temperature, the tubes were washed twice with PBS and centrifuged for 30 min at 3,000 × *g*. Hemolysin solution (300 μL) diluted in PBS (1:25) was added to the tubes. Two-color flow cytometric analyses were conducted with a Coulter XL (Beckman Coulter Corp, Fullerton, CA).

Statistical Analysis

All data in Exp. 1 were analyzed using GLM procedure (SAS Inst. Inc., Cary, NC). In Exp.2, data of growth performance, apparent total track digestibility, small intestinal morphology, intestinal microflora, IL-10, IFN-γ, VFA level, blood CD4$^+$, and CD8$^+$ lymphocyte subsets, as well as CD4$^+$:CD8$^+$ ratio were subjected to ANOVA for a randomized complete block design by using the GLM procedure of SAS. Bacterial concentration was transformed ($\log_{10}$) before statistical analysis. Statistical differences among treatments were separated by Duncan's multiple range tests. Results were expressed as least squares means and SEM. Probability values less than 0.05 were used as the criterion for statistical significance.

Table 3. Effect of dietary yeast culture (YC) and antibiotic growth promoters (AGP) on growth performance of weaning pigs (Exp. 2)[1]

Item	Control	AGP	YC	SEM	*P*-value
ADG, g	275[a]	338[b]	346[b]	13	0.034
ADFI, g	467	500	526	12	0.113
G:F, g/kg	586	675	660	18	0.081

[a,b]Means in the same row with different superscripts differ ($P < 0.05$).

[1]Values represent means of 8 pigs. AGP (positive control supplemented with 80 mg/kg of chlortetracycline, Jinhe Biotechnology Co. Ltd., Tuoketuo, China); control (negative control fed a basal diet); YC (supplemented with 5 g/kg of Diamond V XP Yeast Culture, Cedar Rapids, IA).

Table 4. Effects of dietary yeast culture (YC) and antibiotic growth promoter (AGP) supplementation on apparent digestibility (%) of GE, DM, CP, Ca, and P in weaning pigs (Exp. 2)[1]

Item	Control	AGP	YC	SEM	*P*-value
GE	74.8[a]	82.3[b]	82.0[b]	1.46	0.04
DM	72.6[a]	81.3[b]	80.1[b]	1.48	0.01
CP	71.4[a]	79.0[b]	78.5[b]	1.37	0.02
Ca	46.7	56.6	50.8	1.81	0.07
P	42.8	54.2	50.1	2.42	0.15

[a,b]Means in the same row with different superscripts differ ($P < 0.05$).

[1]Values represent means of 8 pigs. AGP (positive control supplemented with 80 mg/kg of chlortetracycline, Jinhe Biotechnology Co. Ltd., Tuoketuo, China); control (negative control fed a basal diet); YC (supplemented with 5 g/kg of Diamond V XP Yeast Culture, Cedar Rapids, IA).

RESULTS

Performance

In Exp. 1, dietary supplementation of 5 g/kg of YC and AGP improved ADG ($P < 0.05$) compared with pigs fed the control diet (Table 2). There was no difference in ADG between pigs supplemented with 5 g/kg of YC and AGP. Greater ($P < 0.01$) ADFI was observed for pigs supplemented with AGP, 5 g/kg of YC, and 10 g/kg of YC than for pigs fed the control diet. However, G:F was not affected by YC or AGP supplementation. Pigs supplemented with 5 g/kg of YC and AGP also had greater ADG ($P < 0.05$) than those fed the control diet in Exp. 2 (Table 3) as observed in Exp. 1. Overall, 5 g/kg of YC and 80 mg/kg of chlortetracycline improved growth performance of weaning pigs at a similar magnitude.

Apparent Digestibility

Total tract apparent digestibility of DM ($P < 0.05$), GE ($P < 0.05$), and CP ($P < 0.05$) was increased by dietary supplementation of YC and AGP (Table 4). There were no significant differences of the apparent digestibility of Ca and P among treatments.

Intestinal Microflora

The number of *E. coli* in the cecum was decreased ($P < 0.05$) by dietary supplementation of YC and AGP (Table 5). However, this action of YC and AGP was not observed in the colon or in samples obtained from the rectum. In terms of the lactobacilli counts, no effect of dietary YC or AGP was observed in the hindgut. Total aerobes and total anaerobes in different segments of the hindgut were also not affected by dietary treatment.

Table 5. Effects of dietary yeast culture (YC) and antibiotic growth promoter (AGP) supplementation on microbial concentrations ($\log_{10}$ cfu/g of digesta) at various locations along the intestinal tract of weaning pigs (Exp. 2)[1]

Item	Control	AGP	YC	SEM	*P*-value
Cecum					
Escherichia coli	5.33[a]	4.71[b]	4.52[b]	0.13	0.03
Lactobacilli	7.39	7.62	7.52	0.18	0.87
Total anaerobes	7.89	8.28	7.62	0.20	0.42
Total aerobes	5.89	5.85	6.04	0.16	0.89
Colon					
E. coli	5.54	4.95	4.90	0.17	0.27
Lactobacilli	7.34	7.77	7.62	0.20	0.69
Total anaerobes	7.66	7.95	8.33	0.19	0.38
Total aerobes	6.22	5.94	5.55	0.15	0.17
Rectum					
E. coli	4.40	4.20	4.09	0.11	0.53
Lactobacilli	6.36	6.38	6.50	0.17	0.94
Total anaerobes	6.76	6.82	6.94	0.16	0.90
Total aerobes	4.94	4.96	5.15	0.14	0.82

[a,b]Means in the same row with different superscripts differ ($P < 0.05$).

[1]Values represent means of 8 pigs. AGP (positive control supplemented with 80 mg/kg of chlortetracycline, Jinhe Biotechnology Co. Ltd., Tuoketuo, China); control (negative control fed a basal diet); YC (supplemented with 5 g/kg of Diamond V XP Yeast Culture, Cedar Rapids, IA).

Table 6. Effect of yeast culture (YC) and antibiotic growth promoters (AGP) on VFA concentrations on various sites along the gastrointestinal tract[1]

Item	Control	AGP	YC	SEM[2]	*P*-value
Acetic acid[3]					
Cecum	48.9	49.8	59.6		0.22
Colon	41.2	36.7	37.3	2.73	0.61
Rectum	32.2	34.8	37.2		0.60
Propionic acid[3]					
Cecum	25.8	28.1	30.1		0.56
Colon	22.6	22.7	21.7	1.61	0.96
Rectum	18.0	23.1	22.3		0.34
Iso-butyric acid[3]					
Cecum	0.49	0.36	0.52		0.09
Colon	0.97	0.81	0.92	0.09	0.60
Rectum	0.84	1.14	1.28		0.13
n-Butyric acid					
Cecum	5.6	5.6	6.7		0.57
Colon	6.8	6.2	4.7	0.59	0.32
Rectum	4.8	6.4	7.8		0.12
Iso-valeric acids[3]					
Cecum	1.1	0.9	1.2		0.14
Colon	2.6	2.1	2.5	0.29	0.58
Rectum	2.4	3.4	3.7		0.13
n-Pentanoic acids[3]					
Cecum	1.2	1.1	1.3		0.42
Colon	1.8	1.6	1.6	0.10	0.92
Rectum	1.6	2.2	2.5		0.19

[1]Data are in millimoles per liter and represent least squares means from 3 replicate measurements (n = 8/treatment). AGP (positive control supplemented with 80 mg/kg of chlortetracycline, Jinhe Biotechnology Co. Ltd., Tuoketuo, China); control (negative control fed a basal diet); YC (supplemented with 5 g/kg of Diamond V XP Yeast Culture, Cedar Rapids, IA).

[2]SEM = maximum SEM observed among all test segments.

[3]Location effect, $P < 0.001$.

VFA Analysis

No treatment effects were detected on individual VFA concentrations measured from samples obtained from the cecum, colon, and rectum (Table 6). However, iso-butyric acid tended to be greater ($P < 0.09$) in cecal samples in pigs receiving YC compared with those fed the AGP diet.

The VFA concentrations at various sites of gastrointestinal tract were different. The location effect of individual VFA was significant ($P < 0.001$) except for *n*-butyric acid. Generally, concentrations of major VFA (acetic and propionic acids) were in the order of cecum > colon > rectum, whereas the concentrations of iso-butyric, *n*-butyric, iso-valeric, and *n*-pentanoic acid followed the opposite order of rectum > colon > cecum.

Table 7. Effects of yeast culture (YC) and antibiotic growth promoter (AGP) supplementation on small intestinal morphology (Exp. 2)[1]

Item	Control	AGP	YC	SEM	*P*-value
Villus height, μm					
Duodenum	348	392	400	21	0.57
Jejunum	302[a]	362[ab]	400[b]	15	0.02
Ileum	309	309	306	10	0.99
Crypt depth, μm					
Duodenum	125	110	121	5.3	0.53
Jejunum	122	99	112	4.5	0.09
Ileum	113	96	97	5.6	0.41
Villus height to crypt depth ratio					
Duodenum	2.97	3.68	3.35	0.21	0.41
Jejunum	2.49[a]	3.78[b]	3.71[b]	0.21	0.01
Ileum	2.91	3.38	3.23	0.16	0.51

[a,b]Means in the same row with different superscripts differ ($P < 0.05$).

[1]Values represent means of 8 pigs. AGP (positive control supplemented with 80 mg/kg of chlortetracycline, Jinhe Biotechnology Co. Ltd., Tuoketuo, China); control (negative control fed a basal diet); YC (supplemented with 5 g/kg of Diamond V XP Yeast Culture, Cedar Rapids, IA).

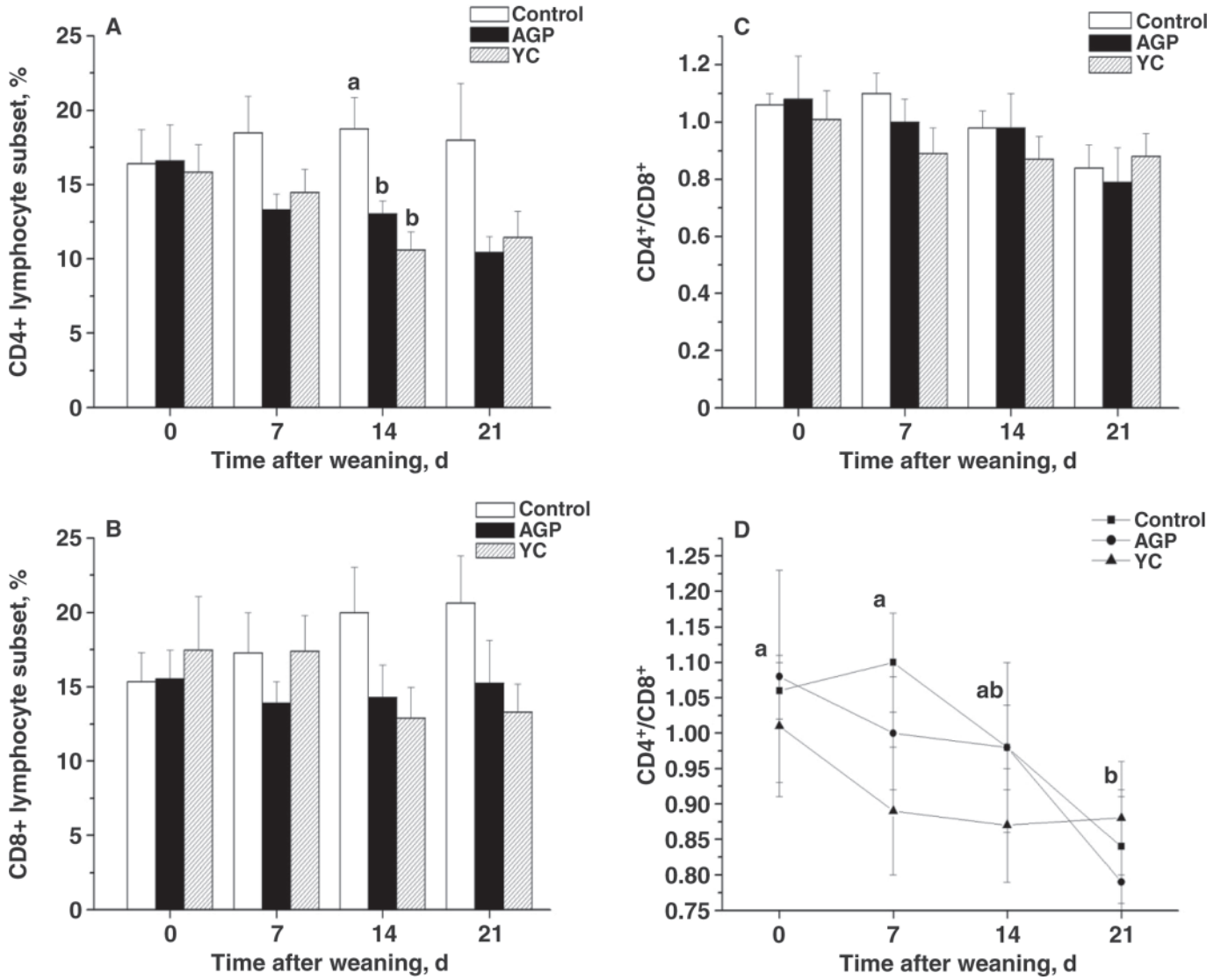

Figure 1. Effects of yeast culture (YC) and antibiotic growth promoter (AGP) supplementation on $CD4^+$ (A), $CD8^+$ (B) lymphocyte subset, and $CD4^+/CD8^+$ ratio (C) in plasma and age effect on $CD4^+/CD8^+$ ratio (D). Each bar represents the mean ± SE of 8 pigs. [a,b]Values with different letters are different at $P < 0.05$. The percentage of $CD4^+$ and $CD8^+$ is a percentage of total lymphocytes. The $CD4^+$ to $CD8^+$ ratio was greater ($P < 0.05$) on d 0 and 7 compared with d 21 only in control group. AGP (positive control supplemented with 80 mg/kg of chlortetracycline, Jinhe Biotechnology Co. Ltd., Tuoketuo, China); control (negative control fed a basal diet); YC (supplemented with 5 g/kg of Diamond V XP Yeast Culture, Cedar Rapids, IA).

Intestinal Morphology

Histological evaluation of duodenum and ileum indicated no effect of dietary supplementation of YC and AGP on villus height, crypt depth, and ratio of villus height and crypt depth (Table 7). However, jejunal villus height and villus height to crypt depth ratio of pigs fed YC were greater ($P < 0.05$) than those pigs fed the basal diet. Jejunal villus height to crypt depth ratio was also increased ($P < 0.05$) by AGP.

Cytokine and $CD4^+/CD8^+$

Percentage of CD4+ was decreased ($P < 0.01$) by YC and AGP on d 14 in blood (Figure 1). However, no differences were observed among treatments on d 0, 7, and 21. There was no time (age of pig) effect on percentage of CD4+ lymphocytes in blood in any of the 3 treatments. The percentage of CD8+lymphocytes and the CD4+ to CD8+ ratio did not differ among treatments. However, the CD4+ to CD8+ ratio was decreased with age of pigs in control group. The CD4+ to CD8+ ratio was greater ($P < 0.05$) on d 0 and 7 compared with d 21 in the control group.

Plasma IFN-γ concentration was decreased ($P < 0.05$) for the YC and AGP groups compared with control group on d 7 (Figure 2). However, this response was not observed on d 0, 14 and 21. Interferon-γ concentration in plasma reached its peak ($P < 0.05$) on d 7 for control groups and decreased to the initial level on d 14 and 21. No age effect was observed for YC and AGP on plasma IFN-γ concentrations. Plasma IL-10 concentration was similar among treatments and was not affected by age of the pig. Gut IFN-γ level was increased ($P < 0.01$) by dietary YC. No treatment effect on level of gut IL-10 was observed.

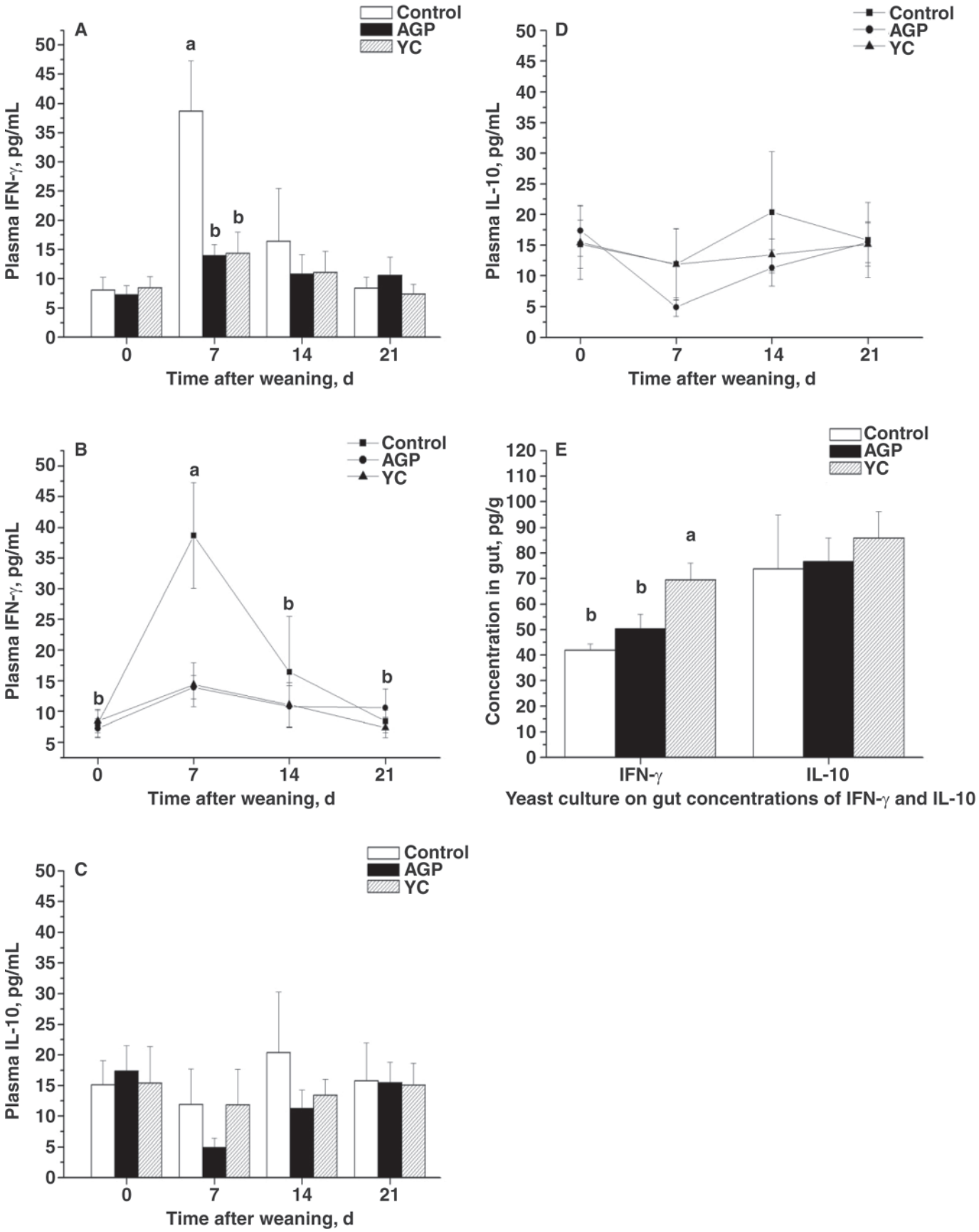

Figure 2. Effects of yeast culture (YC) and antibiotic growth promoter (AGP) supplementation on plasma interferon (IFN)-γ (A) and effect of age on plasma IFN-γ (B). Effects of YC and AGP supplementation on plasma IL-10 (C), effect of age on plasma IL-10 (D), and effects of YC and AGP supplementation on gut IFN-γ and IL-10 concentrations (E). Each bar represents the mean ± SE of 8 pigs (5.8 ± 0.1 kg of BW). AGP (positive control supplemented with 80 mg/kg of chlortetracycline, Jinhe Biotechnology Co. Ltd., Tuoketuo, China); control (negative control fed a basal diet); YC (supplemented with 5 g/kg of Diamond V XP Yeast Culture, Cedar Rapids, IA). [a,b]Values with different letters are different at $P < 0.05$.

DISCUSSION

Performance

Dietary supplementation of 5 g/kg of YC improved growth performance of nursery pigs in both Exp. 1 and 2. Positive effects of YC in weaning or nursery diets have been reported by Kornegay et al. (1995). Mathew et al. (1998) reported that direct-fed YC could improve ADG of weanling pigs. Gao et al. (2008) also reported diet supplementation of YC could improve ADG and G:F during grower and overall periods in broiler chicken. However, growth performance response to YC has been shown to be variable by other investigators. In studies of Veum and Bowman (1973) and Jurgens (1995), YC addition did not affect the performance of neonatal pigs. In this study (Exp. 1), addition of YC to diet improved feed intake, and this may be a possible explanation for increased ADG. This result is consistent with Mathew et al. (1998), who also observed an improved feed intake with YC. However, Kornegay et al. (1995) and van der Peet-Schwering et al. (2007) did not observe improved feed intake with YC supplementation.

Some studies indicated possible benefits of other yeast products such as live yeast (van Heugten et al., 2003). van Heugten et al. (2003) reported that live yeast supplementation had a positive effect on nursery pig performance when diets contained growth promoting antimicrobials. However, the action of YC (prebiotics) may be different from live yeast (probiotics). Modulation of the immune system (van der Peet-Schwering et al., 2007), accommodation of a beneficial intestinal environment (van Heugten et al., 2003), and improvement in intestinal morphology (van der Peet-Schwering et al., 2007) have all been proposed as potential mechanism of YC.

In our study, we showed that only 5 g/kg of YC supplementation can improve performance. Greater YC supplementation showed only a numerical improvement in ADG and ADFI. A possible explanation would be that YC interacted with immune systems by triggering immune responses in the intestinal tract (van Heugten et al., 2003; Davis et al., 2004) and energy was partitioned toward immune function rather than to support the growth. Under low disease challenge or stress conditions, a decreased level of YC would be more effective in improving performance because the demand for immune response is minimal. Similar responses were observed in broilers (Gao et al., 2008).

Intestinal Microflora and VFA Analysis

Microflora in the digestive system of pigs plays a very important role in the defense mechanisms of the body. One of the important abilities of stable microflora in gastrointestinal tract is colonization resistance. Microbial composition in the gastrointestinal tract can be affected by dietary differences (Anderson et al., 1999). Several studies have demonstrated that YC or yeast cell wall components can affect the composition of intestinal microflora (Firon et al., 1987; Naughton et al., 2001; White et al., 2002). However, in the present study, dietary supplementation of YC and AGP did not affect microbial populations tested. Only the number of *E. coli* in the cecum of YC and AGP group was decreased compared with the control group. Our result is consistent with the result of Mathew et al. (1998), who reported YC did not change microflora in the stomach, duodenum, ileum, cecum, or colon. van der Peet-Schwering et al. (2007) also reported that YC did not have a clear effect on microbial composition in the ileum. It is interesting to note that AGP showed little effect on intestinal microbial population and is similar to YC effects. This result is consistent with the result of van der Peet-Schwering et al. (2007).

Volatile fatty acids are the major end products that are produced by bacterial metabolism in the large intestine of swine (Bergman, 1990). Increased fermentation in the large intestine, leading to an increased production of VFA, can reduce the incidence of digestive disorders, which contribute substantially to the overall energy supply (Bellier and Gidenne, 1996; Mourão et al., 2005). In our study, VFA concentrations were used as an index of the changes in microbial fermentation. Consistent with the minimal changes in microbial population with YC or AGP supplementation, no difference was observed in VFA concentrations (Table 6). This result was consistent with the results reported by Mathew et al. (1998).

Intestinal Morphology and Nutrient Digestibility

Significant postweaning reduction in villus height has been observed by several researchers (Cera et al., 1988; McCracken et al., 1999; Tang et al., 1999; Berkeveld et al., 2007). Villus atrophy is due to an increased rate of cell apoptosis or programmed death and decreased rate of renewal, which can be affected by cellular factors or endogenous stressors (van der Peet-Schwering et al., 2007). An increase in villus height and villus:crypt ratio in the jejunum was observed in this study in pigs fed AGP and YC after weaning when compared with pigs fed the control diet. These results agree with those from a study conducted by Gao et al. (2008), in which dietary supplementation of YC improved the villus:crypt ratio in the duodenum and jejunum in broiler chicken. However, van der Peet-Schwering et al. (2007) reported that dietary supplementation of YC did not change villus height and crypt depth as well as villus:crypt ratio. Decreased number of pathogenic bacteria in the gut may improve proliferation of epithelial cells to build villus in the gut and thus enhance intestinal morphology (Mourão et al., 2005). However, in this study the change of microflora composition was observed only in the cecum, but not in jejunum where intestinal morphology was measured, suggesting that the enhanced

intestinal morphology as shown in this study may be only partly due to decreased *E. coli* colonization.

Nutrient digestibility and absorption capacity of the small intestine was affected by villus:crypt ratio (Montagne et al., 2003). This study demonstrated that dietary supplementation of YC improved the digestibility of DM, GE, and CP, which may be due to increased villus height and villus:crypt ratio in the jejunum. However, these results are not consistent with other studies. Kornegay et al. (1995) observed that the digestibility of protein and energy was not affected by YC supplementation.

Cytokine and CD4$^+$/CD8$^+$

Recent studies indicated that yeast cell wall components may interact with immunity systems, triggering immune responses (Seljelid et al., 1987; Muchmore et al., 1990; Podzorski et al., 1990; Davis et al., 2004). Seljelid et al. (1987) reported that β-glucan could stimulate macrophage activities in mice, whereas Djeraba and Quere (2000) reported that mannans (another component of yeast cell wall) increased the macrophage activation in chickens. However, little information is available describing the exact mechanism of YC on immune responses of the pig. To obtain more insight into the exact mechanism of YC, gut and plasma cytokine excretion and blood CD4$^+$/CD8$^+$ were measured.

In this study, T-helper1 (**Th-1**) cytokine IFN-γ level in gut was increased by YC (Figure 2E). This indicated that YC might change immune function of the gut by triggering a Th-1 response in the gut. This result is supported by a study using broiler chickens conducted by Gao et al. (2008), which reported that YC increased the lysozyme in gut indicating increased nonspecific immune function in gut. Our result was also supported by the results of Baran et al. (2007), who reported that β-glucans stimulated a Th-1 response which increased production of the Th-1 cytokine IFN-γ (possibly by intestinal mucosal macrophages). Therefore, a possible explanation of our result may be that YC triggers gut epithelium or gut-associated lymphoid tissue Th-1 response by activating macrophages.

The CD4$^+$ T cells normally induce IFN-γ secretion in blood (Oyaizu et al., 1994). A systemic abnormal increase of proinflammatory cytokines IFN-γ decreases feed intake, resulting in starvation (Johnson, 1995). In this study, plasma IFN-γ increased dramatically after weaning in the control group. The IFN-γ produced in plasma peaked on d 7 in the control group, indicating that immune reaction was increased during the first week of the nursery period. The CD4$^+$:CD8$^+$ ratio also declined with time postweaning. This indicated that weaning stress was alleviated 2 or 3 wk after weaning. This result was consistent with the report by Verdonk (2005).

In this study, we showed that IFN-γ, which can activate phagocytosis by macrophages, was increased in gut mucosa by YC supplementation. It was also shown that CD4$^+$ lymphocyte numbers increased after weaning in the case of control group, whereas the number of CD4$^+$ lymphocytes in YC group did not increase by 14 d postweaning. Kaiserlian et al.(2005) reported that rapid and efficient phagocytosis of bacteria by macrophages prevents their migration outside the mucosal immune system and preserves systemic ignorance. Thus, weaning-induced systemic immune response alleviated by YC may be due to improvements of Th-1 response in gut mucosa.

Collectively, the results of the current study indicated that supplementation of 5 g/kg of YC improves growth performance of pigs probably by improving villus height, gut immune response, and nutrient digestibility. The results of the study also suggest that YC could be an alternative to AGP in diets for the weaning pigs.

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