

Effects of Yeast Culture in Broiler Diets on Performance and Immunomodulatory Functions

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ABSTRACT A study was conducted to evaluate the effect of supplemental yeast culture (Diamond V XP Yeast Culture; YC) in broiler diets on performance, digestibility, mucosal development, and immunomodulatory functions. One-day-old Arbor Acres chicks (n = 960) were randomly assigned to 1 of 4 dietary treatments based on corn and soybean meal and containing 0, 2.5, 5.0, and 7.5 g/kg of YC in the diet for 42 d. Each treatment consisted of 12 replicates of 20 broilers each. Nutrient digestibility was determined on d 15 and 35 by total fecal collection. On d 21 and 42, 12 birds per treatment were sacrificed to evaluate gut morphology and secretory IgA. Broilers were vaccinated with Newcastle disease vaccine by eye drop on d 7 and 28 and antibody titer was determined on d 14, 21, 35, and 42. Dietary supplemental YC at 2.5 g/kg improved average daily gain and feed conversion during grower and overall periods ($P \leq 0.05$). Yeast cul-

ture supplementation increased digestibility of Ca (linear and quadratic, $P = 0.01$) and P (linear, $P = 0.01$) on d 35, but did not affect ($P > 0.05$) protein retention and energy digestibility. Villus height to crypt depth ratios in the duodenum and jejunum (d 42) and ileum (d 21) were increased ($P \leq 0.05$) in broilers fed 2.5 g/kg of YC. Yeast culture increased antibody titers to Newcastle disease virus (linear, $P \leq 0.05$), serum lysozyme activity (linear and cubic, $P \leq 0.05$), and IgM (linear, $P \leq 0.05$) and secretory IgA concentrations in the duodenum (linear, $P = 0.01$). Results of this study indicate that dietary supplemental YC at 2.5 g/kg improved growth performance. Dietary YC affected immune functions, digestibility of Ca and P, and intestinal mucosal morphology of broilers. Growth performance was optimized at 2.5 g/kg of YC in the present study. Immune function could be modified with dietary YC supplementation.

Key words: yeast culture, broiler, immune function, mucosal morphology, digestibility

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INTRODUCTION

It is well documented that antibiotics benefit animal growth, performance, and health. However, increasing concerns regarding overuse of antibiotics has prompted extensive investigation into alternatives to use of subtherapeutic antibiotics in production diets. Yeast products are important natural growth promoters. Eckles and Williams (1925) first reported the use of *Saccharomyces cerevisiae* as a growth promoter for ruminants. Commercial yeast products specifically for animal feeding are used worldwide in animal production particularly in ruminant diets. Beneficial effects of yeast products in ruminants are due to increased concentration of total and cellulolytic ruminal bacteria (Wallace, 1994; Newbold et al., 1995), which may

increase availability of ME from diets, thereby increasing production.

Effects of yeast products on production and their mode of action in monogastrics have been reported in poultry (Hayat et al., 1993; Bradley and Savage, 1995; Stanley et al., 2004a; Zhang et al., 2005) and pigs (Mathew et al., 1998; van Heugten et al., 2003; Shin et al., 2005a). However, mode of action of yeast products in these animals is less clear. Some studies have confirmed the effects of yeast culture (YC) in increasing concentrations of commensal microbes or suppressing pathogenic bacteria (Stanley et al., 2004a). However, these effects were not reported by others (Mathew et al., 1998; White et al., 2002; van Heugten et al., 2003). We hypothesize that there may be other mechanisms responsible for effects of YC in monogastrics other than modulation of microbial ecology. Mannan-oligosaccharide and 1,3/1,6 β -glucan are components of the yeast cell wall that modulate immunity (Shashidhara and Devegowda, 2003), promote growth of intestinal microflora (Spring et al., 2000; Stanley et al., 2000), and increase growth (Parks et al., 2001). Yeast culture

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Table 1. Composition and nutrient content of starter (d 1 to 21) and grower (d 22 to 42) basal diets for broiler chicks (g/kg as fed-basis)

Ingredients (g/kg)	Starter	Grower
Yellow corn	554.2	580.4
Soybean meal	354.5	321.5
Soybean oil	41.0	53.2
Dicalcium phosphate	19.2	17.2
Limestone (38% Ca)	12.2	11.0
Salt (NaCl)	3.7	3.5
Choline chloride	2.6	2.0
DL-Methionine	2.2	1.1
Mineral premix ¹	2.0	2.0
Vitamin premix ²	0.2	0.2
L-Lysine HCl	0.3	0
Yeast culture or carrier ³	7.5	7.5
Antioxidants	0.4	0.4
Total	1,000.0	1,000.0
Nutrient content		
AME ⁴ (kcal/kg)	3,000	3,100
CP ⁵ (g/kg)	217.2	191.6
Ca ⁵ (g/kg)	10.5	8.6
Total P ⁵ (g/kg)	7.0	6.1
Available P ⁴ (g/kg)	4.5	4.1

¹Mineral premix contained the following per kilogram of diet: Fe, 80 g; Cu, 8 mg; Mn, 60 mg; Zn, 40 mg; I, 0.4 mg; Se, 0.2 mg.

²Vitamin premix contained the following per kilogram of diet: vitamin A, 1,100 IU; vitamin D₃, 240 IU; vitamin E, 6 IU; menadione sodium bisulfite, 0.6 mg; vitamin B₁₂, 0.004 µg; biotin, 0.15 mg; folic acid, 0.2 mg; nicotinic acid, 50 mg; D-pantothenic acid, 5 mg; pyridoxine hydrochloride, 1.2 mg; riboflavin, 2.2 mg; thiamine mononitrate, 1.6 mg.

³The diets of treatments contained Diamond V XP (Diamond V Mills, Cedar Rapids, IA) yeast culture 0, 2.5, 5.0, or 7.5 g/kg, and carrier (zeolite powder) 7.5, 5.0, 2.5, or 0 g/kg, respectively.

⁴Calculated according to Chinese Feed Database News Web Center (2005).

⁵Determined value.

contains viable cells, cell wall components, metabolites, and the media on which the yeast cells were grown (Miles and Bootwalla, 1991). In a recent *in vitro* study (Jensen et al., 2007), the addition of a soluble fraction of YC showed an antiinflammatory effect in conjunction with activation of natural killer cells and B lymphocytes. In addition, others have reported that yeast products affect nutrient digestibility (Thayer and Jackson, 1975; Thayer et al., 1978; Bradley and Savage, 1995; Shin et al., 2005b) and intestinal mucosal development (Santin et al., 2001; Zhang et al., 2005). Therefore, the objective of this study was to evaluate effects of YC in broiler diets on performance, nutrient digestibility, intestinal morphology, and immune function in poultry.

MATERIALS AND METHODS

Experimental Design and Dietary Treatments

Arbor Acres broiler chicks (n = 960; 1 d old; 480 males) were randomly assigned to 1 of 4 dietary treatments containing 0, 2.5, 5.0, and 7.5 g/kg diet of a commercial YC product (Diamond V XP YC, Diamond V Mills Inc., Cedar Rapids, IA). Two corn-soybean based basal diets were formulated to be fed during starter (d 1 to 21) and grower (d 22 to 42; Table 1) periods. Each treatment consisted of

12 replicates with 20 chicks (10 males and 10 females) per replicate. Diets were fed in mash form. All bird management was consistent with recommendations of Arbor Acres Broiler Commercial Management Guide.

Growth Performance

Body weight and feed consumption were measured on d 21 and 42. Mortality was recorded during the experiment. Average weight gain, average daily gain (ADG), average daily feed consumption, and feed conversion (feed/gain) were calculated for starter, grower, and overall periods.

Apparent Digestibility of Nutrients

Nutrient digestibility was determined on d 15 and 35 by total fecal collection. Total excreta collection was made from half of the birds (5 males and 5 females) in each pen by inserting a wire net to split the group in 2 equal sizes. One subgroup from each pen was randomly selected for total excreta collection. Wire nets were placed only during the excreta collection periods and removed afterward. This modification was made to facilitate the handling of the excreta samples. All droppings were collected once daily for 5 consecutive days from d 15 and again from d 35. Excreta from each pen were collected, mixed, and weighed, and a 10% aliquot was sampled and frozen (−20°C). Daily aliquots of excreta were combined per pen within each period and a 10% aliquot was taken and dried. Feed samples were collected daily and pooled to produce a single composite of each diet. Diet and excreta samples were analyzed for Ca, P, CP, and gross energy. Gross energy was determined in an oxygen bomb calorimeter (Parr 1281 Automatic Bomb Calorimeter, Parr Instrument Company, Moline, IL). Crude protein was analyzed by a Kjeltac 2300 Analyzer Unit (Foss Tecator AB, Höganäs, Sweden). Samples were digested in concentrated nitric acid to solubilize Ca and P in the diet and excreta. Concentration of Ca in the supernatant was determined using flame atomic absorption spectrometry (Z-8200, Hitachi, Japan). The concentration of P in the supernatant was measured by ultraviolet and visible range spectrophotometer.

Intestinal Morphology Development

On d 21 and 42, 6 female and 6 male chicks from each treatment were killed by cervical dislocation for measurement of intestinal villus height and crypt depth by the method of Sun et al. (2005). Five-centimeter sections of duodenum (medial portion), jejunum (medial portion posterior to the bile ducts and anterior to Meckel's diverticulum), and ileum (medial portion posterior to Meckel's diverticulum and anterior to the ileocecal junction) were removed, rinsed in Tris-buffered saline, cut into 5 equal pieces, and fixed in 10% neutral buffered formalin. Each intestinal piece was subsequently cut into 5-mm sections and placed into tissue cassettes. Cassettes were embedded

in paraffin, cut into thicknesses of 5 μm , and mounted onto slides. Tissue slides were stained using hematoxylin and eosin for light microscope measurement of villus height and crypt depth. Villus height was measured from the tip of a villus to the top of the crypt, whereas crypt depth was defined as the depth of the invagination between adjacent villi. All reported villi and crypt values were an average of 5 measurements per tissue.

Blood Collection and Analysis

Blood was collected on d 21 and 42 from the wing vein of 12 birds per treatment (1 bird/replicate) and serum was collected by centrifugation. Serum was harvested and stored (-20°C) before analysis. Serum lysozyme activity was measured using the method of Kreukniet et al. (1994) using *Micrococcus lysodeikticus* cells as substrate. Serum IgG, IgA and IgM were measured by double-antibody sandwich ELISA using commercial kits (Bethyl Laboratories, Montgomery, TX).

Secretory IgA Content

Six chicks per treatment were randomly selected and used for determination of secretory IgA (sIgA) in the duodenum on d 21 and 42 using the immunohistochemical method. Tissue samples were fixed in 2.5% glutaraldehyde-polyoxymethylene fixative. Paraffin sections were prepared by routine method and blocked with 3% peroxide-methanol at room temperature for endogenous peroxidase ablation after deparaffinage. Sections blocked with normal goat serum were incubated for 2 h at 37°C after adding mouse anti-chicken IgA (Southern Biotechnology Associates Inc., Birmingham, AL). A biotin-conjugated secondary antibody (Goat anti-mouse IgG/Bio, Beijing Zhongshan Goldenbridge Company, Beijing, China) was added and the sections were incubated for 30 min at 37°C . Following incubating in avidin-biotin-peroxidase complex (Beijing Zhongshan Goldenbridge Company), sections were stained with 3, 3'-diaminobenzidine and dyed with hematoxylin. Negative control was conducted with the same steps as described above with the exception of substituting PBS-Tween for the mouse anti-chicken IgA. Sections were mounted with neutral gums and were observed by using a Motic Digital Biological Microscope (DMB5, Motic China Group Co. Ltd., Xiamen, China) equipped with a digital analysis system of medical photo software (Motic Med 6.0 CMIAS). Results were expressed as the ratio of the positive areas covered with sIgA to the whole visual field. Five visual fields were randomly selected per slide.

Antibody Titer Against Newcastle Disease Virus

Broilers were vaccinated with Newcastle disease "LaSota" vaccine (Intervet International B.V., Boxmeer, Holland) by eye drop on d 7 and 28, and antibody titer in serum was measured by hemagglutination-inhibition test

as described by Alexander et al. (1983) on d 14, 21, 35, and 42. One bird from each replicate was randomly selected and blood was collected by wing venipuncture. Antibody titer against Newcastle disease virus (NDV) was detected by hemagglutination-inhibition test using 4 hemagglutinin units of the NDV antigen (China Institute of Veterinary Drug Control, Beijing, China).

Statistical Analysis

Data were analyzed using one-way ANOVA of SAS 8.02 for Windows (SAS Institute, 2001) and means were separated by the Fisher's multiple range tests. The effect of supplemental levels of YC was determined using orthogonal polynomials for linear, quadratic, and cubic effects. Data were assumed to be statistically significant when $P \leq 0.05$.

RESULTS

Growth Performance

Growth performance of broilers was affected by dietary YC. Yeast culture effect was not apparent during the starter period (d 1 to 21; Table 2). However, during the grower (d 22 to 42) and overall (d 1 to 42) periods, supplemental YC at 2.5 g/kg improved ADG and feed conversion compared with 0 g/kg. Dietary treatments did not affect feed consumption. No significant differences in growth performance were found between the YC-treated birds at 5.0 or 7.5 g/kg and the control birds in the entire experimental period.

With the increase of dietary YC, BW on d 42 and ADG exhibited quadratic responses during the growing and overall periods, with the 2.5 g/kg feeding level being the most effective. Feed conversion tended to be improved (quadratic, $P = 0.08$) during the same periods as the dietary YC increased. No difference in mortality among treatments was observed. In general, compared with the control, supplementation with YC at 2.5 g/kg increased growth performance, but its effect at greater inclusion levels (5.0 or 7.5 g/kg) was not significant.

Nutrient Digestibility

Dietary YC affected the apparent digestibility of Ca and P on d 35 (Table 3). Digestibility of Ca on d 35 increased (linear, $P = 0.01$; quadratic, $P = 0.01$) as dietary YC increased. Digestibility of P also increased linearly ($P = 0.01$) when YC fed to birds increased. Retention of CP and digestibility of gross energy were unaffected by supplemental YC.

Intestinal Morphology

Villus height was affected variably by YC, sample site, and age of birds (Table 4). Generally, YC increased villus height in the duodenum, particularly on d 42, when villus height increased linearly ($P = 0.01$) with increasing level

Table 2. Effect of supplemental yeast culture in broiler diets on growth performance¹

Item	Yeast culture supplementation (g/kg of diet)				SEM	P-value ²		
	0	2.5	5.0	7.5		L	Q	C
BW (g/bird)								
d 1	43.2	43.2	43.2	43.2				
d 21	767.7	757.9	755.9	758.3	3.9	0.39	0.44	0.92
d 42	2,378 ^b	2,459 ^a	2,404 ^{ab}	2,357 ^b	14.1	0.34	0.02	0.24
ADG ³ (g/bird per d)								
d 1 to 21	34.5	34.0	33.9	34.1	0.2	0.39	0.44	0.92
d 22 to 42	76.7 ^b	81.0 ^a	78.5 ^{ab}	76.1 ^b	0.6	0.44	0.01	0.20
d 1 to 42	55.6 ^b	57.5 ^a	56.2 ^{ab}	55.1 ^b	0.3	0.34	0.02	0.24
Feed consumption (g/bird per d)								
d 1 to 21	52.1 ^{ab}	50.9 ^b	51.2 ^b	52.9 ^a	0.3	0.17	0.01	0.90
d 22 to 42	177.0	176.1	175.7	172.4	2.1	0.45	0.78	0.88
d 1 to 42	112.8	112.2	112.1	110.8	1.0	0.48	0.87	0.83
Feed conversion (feed/gain)								
d 1 to 21	1.51	1.50	1.51	1.55	0.01	0.14	0.18	0.85
d 22 to 42	2.31 ^a	2.17 ^b	2.24 ^{ab}	2.27 ^{ab}	0.02	0.70	0.08	0.23
d 1 to 42	2.03 ^a	1.95 ^b	2.00 ^{ab}	2.01 ^{ab}	0.01	0.89	0.08	0.20

^{ab}Means within a row lacking a common superscripts differ significantly ($P \leq 0.05$).

¹n = 12 replicates of 20 birds (10 males, 10 females) each.

²Orthogonal contrasts: L = linear, Q = quadratic, and C = cubic effect of supplemental yeast culture.

³ADG = average daily gain.

of YC. Changes in jejunal villus height were variable with YC supplementation. Ileal villus height declined on both d 21 (linear, $P = 0.01$; quadratic, $P = 0.02$; cubic, $P = 0.01$) and d 42 (quadratic and cubic, $P = 0.01$).

Changes in intestinal crypt depth also varied with YC supplementation, sample site, and age of birds. Supplementation with YC increased crypt depth in the duodenum on d 42 (linear and quadratic, $P = 0.01$), jejunum on d 21 (linear and cubic, $P = 0.01$) and d 42 (linear and quadratic, $P = 0.01$), and ileum on d 42 (quadratic, $P = 0.01$).

Inclusion of YC increased the villus height to crypt depth ratio (VCR) in duodenum on d 21 (cubic, $P = 0.01$) and d 42 (quadratic, $P = 0.01$), decreased in jejunum on d 21 (linear, $P = 0.01$) and d 42 (linear and quadratic, $P = 0.01$) and in ileum on d 21 (linear, quadratic and cubic, $P = 0.01$) and d 42 (linear, $P = 0.04$). In general, compared with controls, YC at 2.5 g/kg increased VCR but greater

inclusion of YC (5.0 or 7.5 g/kg) decreased or had no effect on VCR.

Lysozyme Contents and Antibody Titers to NDV

Yeast culture inclusion in broiler diets increased (linear and cubic) serum lysozyme content (Table 5). Compared with the control group, supplemental YC increased serum lysozyme concentration up to 49%.

The antibody titers to NDV were also increased by YC supplementation at all sampling times except d 28 when the increase was only numerical (Table 5). Antibody titers increased as dietary YC concentration increased on d 14 (linear, $P = 0.02$; cubic, $P = 0.01$), d 21 ($P = 0.01$), d 35 (linear and quadratic, $P = 0.01$), and d 42 (linear and cubic, $P = 0.01$).

Table 3. Effect of yeast culture supplementation in broiler diet on nutrient digestibilities¹

Digestibility, %	Yeast culture supplementation (g/kg of diet)				SEM	P-value ²		
	0	2.5	5.0	7.5		L	Q	C
Ca (d 15)	38.93	45.28	42.88	44.70	1.14	0.13	0.32	0.21
Ca (d 35)	24.89 ^c	38.47 ^b	46.12 ^a	44.28 ^{ab}	1.73	0.01	0.01	0.75
P (d 15)	50.48	52.78	51.61	50.72	0.80	0.95	0.34	0.62
P (d 35)	34.71 ^b	38.67 ^{ab}	40.20 ^a	40.54 ^a	0.84	0.01	0.26	0.86
CP (d 15)	62.17	64.13	62.46	61.16	0.63	0.41	0.21	0.49
CP (d 35)	56.62	56.47	57.88	58.28	0.75	0.35	0.86	0.71
Gross energy (d 15)	73.39	74.03	74.21	73.26	0.37	0.95	0.29	0.84
Gross energy (d 35)	77.37	76.65	77.35	77.59	0.33	0.65	0.48	0.55

^{a-c}Means within a row with no common superscripts differ significantly ($P \leq 0.05$).

¹n = 12 replicates of 10 birds (5 males, 5 females) each.

²Orthogonal contrasts: L = linear, Q = quadratic, and C = cubic effect of supplemental yeast culture.

Table 4. Effect of dietary yeast culture supplementation on villus height, crypt depth, and villus height to crypt depth ratio (VCR) in duodenum, jejunum, and ileum in broiler chicks¹

Item	Yeast culture supplementation (g/kg of diet)				SEM	P-value ²		
	0	2.5	5.0	7.5		L	Q	C
Villus height (µm)								
Duodenum (d 21)	1,092 ^b	1,313 ^a	987 ^b	1,030 ^b	28.3	0.01	0.06	0.01
Duodenum (d 42)	685 ^c	1,009 ^b	1,518 ^a	1,579 ^a	66.2	0.01	0.10	0.08
Jejunum (d 21)	997 ^{ab}	1,065 ^a	768 ^c	856 ^{bc}	29.9	0.01	0.84	0.01
Jejunum (d 42)	1,158	992	1,009	1,099	37.2	0.63	0.09	0.74
Ileum (d 21)	687 ^b	819 ^a	604 ^{bc}	568 ^c	21.6	0.01	0.02	0.01
Ileum (d 42)	987 ^a	631 ^c	850 ^{ab}	801 ^b	33.2	0.18	0.01	0.01
Crypt depth (µm)								
Duodenum (d 21)	305.7	280.9	336.2	305.1	9.9	0.54	0.87	0.07
Duodenum (d 42)	132.7 ^c	111.4 ^c	166.2 ^b	247.7 ^a	9.1	0.01	0.01	0.29
Jejunum (d 21)	230.4 ^c	299.9 ^{ab}	265.5 ^{bc}	330.0 ^a	9.5	0.01	0.87	0.01
Jejunum (d 42)	178.9 ^b	106.5 ^d	144.4 ^c	215.2 ^a	8.0	0.01	0.01	0.12
Ileum (d 21)	215.1	179.8	225.8	228.8	7.4	0.17	0.18	0.06
Ileum (d 42)	155.6 ^a	112.9 ^b	118.9 ^b	166.3 ^a	6.2	0.44	0.01	0.88
VCR								
Duodenum (d 21)	3.58 ^{bc}	4.76 ^a	3.00 ^c	3.70 ^b	0.14	0.15	0.28	0.01
Duodenum (d 42)	5.51 ^b	9.28 ^a	9.52 ^a	6.66 ^b	0.45	0.29	0.01	0.90
Jejunum (d 21)	4.34 ^a	3.66 ^b	2.92 ^c	2.65 ^c	0.13	0.01	0.29	0.54
Jejunum (d 42)	6.75 ^b	9.34 ^a	7.42 ^b	5.15 ^c	0.34	0.01	0.01	0.09
Ileum (d 21)	3.25 ^b	4.70 ^a	2.89 ^{bc}	2.52 ^c	0.16	0.01	0.01	0.01
Ileum (d 42)	6.89 ^a	6.64 ^a	7.21 ^a	4.98 ^b	0.30	0.04	0.08	0.16

^{a-c}Means within a row with no common superscripts differ significantly ($P \leq 0.05$).

¹n = 12.

²Orthogonal contrasts: L = linear, Q = quadratic, and C = cubic effect of supplemental yeast culture.

IgG, IgA, IgM, and sIgA Contents

Effect of dietary YC on serum immunoglobulins and sIgA is shown in Table 6. As the dietary YC increased, IgM increased linearly on d 21 ($P = 0.01$) and on d 42 ($P = 0.03$). Serum IgA and IgG content were not affected by dietary inclusion of YC.

Dietary YC supplementation in broiler diets significantly increased sIgA content in the duodenum on d 21 (linear and cubic) and d 42 (linear). Compared with the control, YC supplementation increased sIgA content in the duodenum up to 160% on d 21 and up to 79% on d 42.

DISCUSSION

At appropriate levels, dietary supplemental YC improved ADG and feed conversion of broilers in the present study. There was a quadratic effect of concentration of YC on performance, with the lower concentration (2.5 g/kg) being the most effective. The improved growth performance with YC supplementation, however, was not attributed to increased feed consumption. Similar results with regard to the effect of YC supplementation on feed consumption were reported in pigs (Shin et al., 2005a). Yeast culture contains yeast cells as well as metabolites

Table 5. Effect of dietary yeast culture supplementation on lysozyme content and on antibody titers to Newcastle disease virus (NDV) in broiler chicks^{1,2}

Item	Yeast culture supplementation (g/kg of diet)				SEM	P-value ³		
	0	2.5	5.0	7.5		L	Q	C
Lysozyme content (µg/mL)								
d 21	3.37 ^c	5.01 ^a	4.01 ^{bc}	4.91 ^{ab}	0.20	0.02	0.28	0.01
d 42	3.85 ^b	3.75 ^b	5.62 ^a	5.07 ^a	0.24	0.01	0.61	0.02
Antibody titers to NDV (log ₂)								
d 14	2.92 ^b	4.42 ^a	3.58 ^{ab}	4.25 ^a	0.17	0.02	0.17	0.01
d 21	4.33 ^{bc}	3.91 ^c	5.00 ^{ab}	5.92 ^a	0.20	0.01	0.06	0.28
d 35	4.00 ^b	5.50 ^a	6.33 ^a	5.42 ^a	0.22	0.01	0.01	0.53
d 42	5.67 ^b	7.33 ^a	6.25 ^b	7.83 ^a	0.20	0.01	0.90	0.01

^{a-c}Means within a row with no common superscripts differ significantly ($P \leq 0.05$).

¹Broiler chicks were vaccinated with NDV vaccine at 7 and 28 d of age.

²n = 12.

³Orthogonal contrasts: L = linear, Q = quadratic, and C = cubic effect of supplemental yeast culture.

Table 6. Effect of dietary yeast culture supplementation on serum immunoglobulin G, A, and M content and on secretory IgA (sIgA) content in duodenum in broiler chicks

Immunoglobulin	Yeast culture supplementation (g/kg of diet)				SEM	P-value ¹		
	0	2.5	5.0	7.5		L	Q	C
IgA ² (µg/mL)								
d 21	54.03	51.29	47.90	29.60	4.81	0.08	0.42	0.74
d 42	49.68 ^{ab}	97.05 ^a	39.36 ^b	40.59 ^b	10.00	0.33	0.24	0.07
IgM ² (ng/mL)								
d 21	261.9 ^b	416.0 ^{ab}	391.1 ^{ab}	598.2 ^a	46.23	0.01	0.76	0.32
d 42	242.3 ^b	395.1 ^{ab}	465.4 ^a	450.9 ^a	35.57	0.03	0.23	0.99
IgG ² (ng/mL)								
d 21	87.6	72.3	141.6	135.7	15.84	0.13	0.88	0.27
d 42	106.7	140.7	142.6	100.4	15.52	0.91	0.24	0.93
sIgA ³								
d 21	8.62 ^c	13.63 ^b	21.19 ^a	22.46 ^a	1.26	0.01	0.06	0.05
d 42	8.44 ^b	13.15 ^a	13.93 ^a	15.11 ^a	0.74	0.01	0.13	0.39

^{a-c}Means within a row with no common superscripts differ significantly ($P \leq 0.05$).

¹Orthogonal contrasts: L = linear, Q = quadratic, and C = cubic effect of supplemental yeast culture.

²n = 12.

³n = 6. The value was expressed by the area ratio of positive areas covered with sIgA to whole visual field.

such as peptides, organic acids, oligosaccharides, amino acids, flavor and aroma substances, and possibly some unidentified growth factors, which have been proposed to produce beneficial performance responses in animal production. In agreement with this study, beneficial effects of YC on performance were also observed in broiler chicks (Zhang et al., 2005) and nursery pigs (Mathew et al., 1998). Other studies, however, reported that yeast products had no effect on performance in turkey poults (Bradley and Savage, 1995) and early weaned pigs (White et al., 2002). Differences in animal response may be related to differences in product formulations: yeast products are interchangeably classified as active dried yeast, live YC, or fermented YC, making comparisons difficult among studies.

No antibiotics were included in the experimental diets of the current study. Whether there is a synergistic effect between YC and antibiotics is not clear because of the limited studies available. van Heugten et al. (2003) observed that live yeast supplementation had a positive effect on nursery pig performance when diets contained growth-promoting Cu, Zn, and antibiotics, whereas no positive effect was found in yeast-treated pigs fed antibacterial-free diets. Under stress conditions, YC was reported to have a beneficial effect on broiler performance when birds were challenged with *Eimeria* spp. (Stanley et al., 2004a) or aflatoxin (Stanley et al., 2004b). The response to antimicrobial agents was greater in a "dirty" environment (Cromwell, 2000). Likewise, experimental conditions under which birds are reared could influence the effect of supplemental YC. Broiler chicks in this study were reared in cages with good ventilation and little environmental stress. Further studies are warranted to investigate the effect of dietary YC under stressed conditions such as health, environmental, or nutritional challenges.

Supplemental YC increased P and Ca digestibility. Earlier studies (Thayer et al., 1978; Bradley and Savage, 1995)

also reported improved utilization of phosphorus or calcium in phosphorus-insufficient or sufficient diets of poultry when YC was supplemented. Kornegay et al. (1995) reported that YC contains 1,400 units/kg of phytase. The improvement of P or Ca utilization could partly be attributed to phytase activity of YC. Ohta et al. (1995) reported that dietary fructooligosaccharide increased digestibility of Ca, Mg, Fe, Zn, and Cu. The role of oligosaccharide components in YC on digestibility of Ca and P or other minerals warrants further research. The utilization of gross energy and CP was unaffected by YC in the present study although others reported that supplemental YC improved efficiency of energy utilization in birds (Tonkinson et al., 1965; Savage et al., 1985; Bradley and Savage, 1995) and nitrogen utilization in weaned pigs (Shin et al., 2005b).

Development of intestinal morphology could reflect the health status of the GI tract of an animal. New epithelial cells are produced in the intestinal mucosal crypts and migrate along with the villi to the top (Schat and Myers, 1991). The crypt, therefore, can be regarded as the villus factory. Effects of YC supplementation on intestinal morphology were dose-dependent. Broilers fed YC at 2.5 g/kg had greater VCR. A deeper crypt may indicate faster tissue turnover to permit renewal of the villus, which suggests that the host's intestinal response mechanism is trying to compensate for normal sloughing or atrophy of villus due to inflammation from pathogens and their toxins. More energy would be required to support faster tissue turnover. Taller villi indicate more mature epithelia and enhanced absorptive function due to increased absorptive area of the villus. Greater villus height increases the activities of enzymes secreted from the tips of the villi (Hampson, 1986), resulting in improved digestibility. The greater performance of birds fed the lowest level of YC (2.5 g/kg) compared with greater levels (5.0 and 7.5 g/kg) may partly be attributed to reduced energy partitioning

toward tissue turnover. In agreement with this study, Bradley et al. (1994) reported that goblet cell number and crypt depth in the ileal mucosa were reduced when the broiler diet was supplemented with *S. cerevisiae*. Also, Santin et al. (2001) and Zhang et al. (2005) reported greater villus height and improved performance in birds with supplementation of whole yeast or yeast cell wall. Cell wall components of YC (β -glucans and α -mannans) may provide a protective function to mucosa by preventing pathogens from binding to villi and allowing fewer antigens to be in contact with the villi. Zhang et al. (2005) reported the positive role of yeast cell wall in ileal mucosal development of broiler chicks. Different yeast products contain varying amounts of cell wall fraction; therefore, prediction of response to various yeast products may require additional data regarding levels of β -glucans and α -mannans.

The immune system guards the body against foreign substances and protects from invasion by pathogenic organisms. It can be divided into the innate (nonspecific) immune system and the acquired (specific) immune system. In this study, dietary YC linearly increased the content of serum lysozyme, which is mainly secreted by phagocytes and is a nonspecific immune effector. The increased lysozyme concentration in birds supplemented with YC can break down the polysaccharide walls of many types of bacteria and thus provides protection against infection. The increased lysozyme in YC-treated birds suggests that more phagocytes were activated with the inclusion of YC. Therefore, YC may intensify the non-specific immunity of birds. A recent study by Jensen et al. (2008) supports the role of YC in innate immune function. The authors reported that the addition of cell-wall-free soluble extract of YC showed an antiinflammatory effect in conjunction with activation of natural killer cells and B lymphocytes.

Antibody titer responses have been used as measures of humoral immune status of birds (Sklan et al., 1994). Antibody titers to NDV increased linearly when the level of dietary YC increased, which suggests that YC may also influence systemic or humoral immunity of birds. This result was in agreement with increased IgM in YC-fed birds. It was proposed that oligosaccharides in the yeast cell wall could bind to viruses and work as adjuvants of vaccines to increase the titers of antibody in YC-treated birds (Newman, 1994). Mucosal immunity is an important part of humoral immunity and secretory IgA is the effector of mucosal immunity. It is the most prominent antibody present at mucosal surfaces, and provides passive immunoprotection against invading pathogens in the gastrointestinal tract. Reports pertaining to the effect of YC inclusion in broiler diets on mucosal immunity are sparse. In the present study, birds fed YC-supplemented diets had greater sIgA content in the duodenum. With increasing concentration of dietary YC, sIgA content increased linearly. This implies that YC may stimulate the humoral immune system to produce more antibodies. Increased antibodies cover the surface of intestinal mucosa and can protect villi from damage. This could be

partly responsible for the changes in intestinal morphology in this study. The intestine is one of the organs subject to contact with exotic pathogens and toxins. Secretory IgA can function in eliminating antigens from tissues via immune complex formation (Robinson et al., 2001) and intraepithelial neutralization of virus replication (Fujioka et al., 1998). It is difficult for serum immunoglobulins to reach the gut; therefore, the direct protective effects of intestinal sIgA are efficacious. Because IgA is a non-inflammatory antibody that binds complement only weakly, it protects the tissues from excessive immune-mediated damage.

The effect of YC supplementation on broiler performance was more apparent during the grower period. In addition, significantly improved digestibility was also observed during the grower period. It seems that a period of adaptation is needed before the effects of YC supplementation can be significant because the changes in intestinal morphology and immune responses take time.

Responses to YC supplementation on growth performance (ADG and feed conversion) were quadratic, with 2.5 g/kg being the most effective feeding level. However, the digestibility of Ca and P, and immune indices such as IgM, sIgA, lysozyme, and antibody titers were mainly linear, with greater levels (5.0 and 7.5 g/kg) being more effective. These results suggest that under low challenge or stress conditions, a lower level of YC would be more effective in improving performance because the demand for immune response is minimal. Greater levels of YC could direct energy to prime the immune system and compromise potential growth performance. However, under challenged conditions (disease or heat stress), broilers fed greater levels of YC may perform better because they are more immune competent and less susceptible to diseases.

In summary, the results of the current study indicate that YC improves growth performance and affects immune functions, Ca and P digestibilities, and intestinal mucosal morphology of broilers. Growth performance varied with the levels of YC supplemented and was best when 2.5 g/kg of YC was supplemented under the experimental conditions of this study. Immune function could be modified with dietary YC supplementation.

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