Effects of a yeast-derived product on growth performance, antioxidant capacity, and immune function of broilers

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ABSTRACT Yeast culture plus enzymatically hydrolyzed yeast cell wall (**YC-EHY**) contains crude protein, mannan-oligosaccharide, β-glucan and yeast culture. This study was carried out to explore the effects of dietary YC-EHY at different levels on growth performance, antioxidant capacity, and immune function of broiler chickens. A total of 320 one-day-age male broiler chicks were allocated into 4 groups and were fed with a basal diet supplemented with 0 mg/kg (the control group), 50 mg/kg, 100 mg/kg, 150 mg/kg YC-EHY for 42 d. Dietary YC-EHY improved average daily gain and feed efficiency during the starter, grower, and overall periods as well as average body weight of broiler chickens on 42 d (linear and quadratic, $P < 0.05$). Broiler chickens fed with YC-EHY quadratically increased jejunal sucrase activity on 21 d (quadratic, $P < 0.05$), and linearly and quadratically enhanced maltase activity on 21 and 42 d (linear and quadratic, $P < 0.05$). Supplementing YC-EHY linearly and quadratically enhanced jejunal superoxide dismutase (**SOD**) activity on 21 and 42 d and glutathione peroxidase (**GPX**) activity on 42 d whereas decreased malonaldehyde (**MDA**) concentration on 42 d (linear and quadratic, $P < 0.05$). Consistently, the jejunal genes expression of nuclear factor erythroid 2-related factor 2 (**Nrf2**) and SOD1 on 21 and 42 d, heme oxygenase-1 (**HO-1**) and GPX1 on 42 d were enhanced by YC-EHY supplementation (linear and quadratic, $P < 0.05$). The concentrations of jejunal immunoglobulin G (**IgG**) on 21 and 42 d and secreted immunoglobulin A (**SIgA**) on 42 d were linearly and quadratically elevated by supplementing YC-EHY (linear and quadratic, $P < 0.05$). Dietary YC-EHY quadratically increased jejunal IgG and IgM genes expression on 21 d (quadratic, $P < 0.05$), and linearly and quadratically enhanced the genes expression of IgG and IgM on 42 d (linear and quadratic, $P < 0.05$). Overall, this study indicated that supplementing YC-EHY could exert beneficial effects on growth performance, intestinal antioxidant capacity and immune function in broiler chickens.

Key words: yeast-derived product, broiler chicken, growth performance, antioxidant capacity, immune function

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INTRODUCTION

Nowadays, the intensive breeding models make broiler chickens directly exposed to various stress stimulus such as immunologic and oxidative stress, thus causing damage to cells and tissues in the body (Zhang et al., 2020). The oxidative damage is induced by the imbalance between the free radical generation system and antioxidant defense system (Huang and Ahn, 2019). As the firstline to defense against the external environment, the intestine is first damaged when oxidative stress occurs, which would negatively impact the digestion and absorption of nutrients, thereby leading to diseases and even death in broiler chickens (Bai et al., 2018). Therefore, it is necessary to improve the intestinal antioxidant capacity and immune function through nutritional efforts, thus consequently improving the growth performance and health status of broiler chickens.

Yeast-derived products, such as yeast cell call (Wang et al., 2017; Ma et al., 2020), yeast hydrolysate (Fu et al., 2019; Rahimnejad et al., 2020), dried yeast extracts (Kim et al., 2019b), and yeast culture (Samarasinghe et al., 2004; Sun et al., 2020), are natural feed additives that have been used in animals for decades. Supplementing animals with yeast cell wall improved the growth performance, digestive function, and antioxidant capacity of animals (Li et al., 2016; Wang et al., 2017; Salinas-Chavira et al., 2018). Yeast culture contains cell wall and various metabolites, which

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have been proposed to stimulate bacterial growth in the digestive tract and optimize animal feed intake to improve growth performance as well as active the natural killer cells and B lymphocytes to promote immune function (Gao et al., 2008; Sun et al., 2020). The addition of yeast culture could improve intestinal nutrients digestibility (Samarasinghe et al., 2004), enhance disease resistance (Bu et al., 2019), and alleviate inflammatory responses (Bu et al., 2020) in animals. The extracts and fractions of yeast cell wall have been shown beneficial effects on the growth performance, antioxidant capacity and immune function in animal production (Lei et al., 2015; Cheng et al., 2018). Additionally, dietary mannan-oligosaccharide and β-glucan supplementation could improve the growth performance as well as nonspecific and specific immunity, and mitigate the oxidative stress and inflammation caused by *Escherichia coli* in broilers (Fadl et al., 2020).

The yeast culture plus enzymatically hydrolyzed yeast cell wall (**YC-EHY**) is a yeast-derived product developed by *Saccharomyces cerevisiae*. Several previous studies have been shown that YC-EHY effectively improved the performance and health responses in animals (Nocek et al., 2011; Silva et al., 2018). Nocek et al. (2011) and Yuan et al. (2015) showed that supplementing dairy cows with YC-EHY improved the milk production performance and milk protein percentage, regulated the uterine inflammatory signals, elevated the humoral and mucosal immunity and promoted the mammary gland health. However, to the best of our knowledge, there is no available information regarding the effects of YC-EHY in broiler chickens, especially in terms of intestinal antioxidant capacity and immune function. According to the favorable characteristics on the performance and health responses of YC-EHY, we hypothesized that dietary YC-EHY could show positive effects on growth performance, antioxidant capacity, and immune function in broiler chickens. The present study was thus conducted to investigate the effects of YC-EHY on the growth performance, intestinal disaccharidase activities, antioxidant capacity, and immune function in broiler chickens.

MATERIALS AND METHODS

Yeast-Derived Product

The yeast-derived product used in this study (YC-EHY; Celmanax; Church & Dwight Co., Inc.; Princeton, NJ) consists of the liquid medium used to grow strains of *Saccharomyces cerevisiae*; hence composed of dead cell walls, the medium, and an undetermined number of live yeast cells. Enzymatically hydrolyzed *Saccharomyces cerevisiae* cell wall and its metabolites, including mannan-oligosaccharide (**MOS**) and β-glucan components are added to the liquid medium, which is then dried on a grain-based carrier (by proprietary processes; Church & Dwight Co., Inc.). The main components of YC-EHY are crude protein (> 30%), β-glucan (> 8%), and MOS (> 6%).

Animals, Diets, and Experimental Design

The experiment was approved and conducted under the supervision of the Animal Care and Use Committee, Nanjing Agricultural University, Nanjing, P. R. China (GB14925-2010, NJAU-CAST-2011-093).

A total of 320 one-day-old male Arbor Acres broiler chicks with similar hatching weight (39.50 ± 0.30 g) were obtained from a commercial hatchery (Land Animal Husbandry Co., Ltd, Yantai, Shandong, China) and randomly assigned into 4 treatments for a 42-d feeding trial after separate weighing. Each group had 8replicate cages and each replicate cage had 10 birds. Birds in the control group (**CON**) were provided with a basal diet, and the other 3 groups were respectively fed the basal diet supplemented with 50 mg/kg, 100 mg/kg, and 150 mg/kg YC-EHY. The inclusion rate of YC-EHY was according to manufacturer's recommendation (Church & Dwight Co., Inc.). The basal diet was formulated based on the NRC (1994) guidelines to meet the nutrient requirements of the broilers (Table 1). All broiler chickens were raised in 3-level cages (120 cm × 70 cm × 60 cm; 0.08 m^2 per chicken), and water and feed were given ad libitum with a light schedule of 23-h light and 1-h darkness per day during the entire experimental procedure. The environmental temperature in the house was controlled ranged from 34 to 36°C during 1 to 7 d and subsequently declined to a final temperature of 24°C until the end of the experiment.

Table 1. Composition and nutrient content of experimental diets (g/kg, as-fed basis unless otherwise stated).

Items	1 to 21 d	22 to 42 d
Ingredient		
Corn	556.00	552.00
Soybean meal	290.00	240.00
Cottonseed meal	25.00	30.00
Wheat flour	40.00	40.00
Hydrolyzed feather meal	15.00	15.00
Dicalcium phosphate	9.00	8.00
Limestone	15.00	15.00
Amargosite	1.00	10.00
Soybean oil	20.00	70.00
Premix[1]	20.00	20.00
Calculated nutrient levels[2]		
Crude protein	215.00	195.10
Calcium	9.60	8.40
Total phosphorus	6.60	5.50
Lysine	14.50	14.00
Methionine	5.40	5.00
Threonine	9.10	8.00
Metabolisable energy (MJ/kg)	12.46	13.38
Analyzed composition[3]		
Crude protein	202.20	194.90
Calcium	12.80	10.20
Total phosphorus	6.40	5.80

[1]Premix provided per kilogram of diet: VA 10,000 IU, VD_3 3,000 IU, VE 30 IU, VK_3 1.3 mg, thiamine 2.2 mg, riboflavin 8 mg, niacin 40 mg, choline chloride 600 mg, calcium pantothenate 10 mg, pyridoxine 4 mg, biotin 0.04 mg, folic acid 1 mg, VB_{12} 0.013 mg, zinc 65 mg, iron 80 mg, copper 8 mg, manganese 110 mg, iodine 1.1 mg, selenium 0.3 mg.

[2]The nutrient levels were as fed basis.

[3]Values based on analysis of triplicate samples of diets.

Growth Performance Measurement

All broilers in each replicate cage were weighed respectively after a 12-h fasting on 21 and 42 d of age to calculate the average body weight (**ABW**) of each treatment. The feed consumption of each replicate cage was measured weekly from 1 d of age to calculate the average daily feed intake (**ADFI**) of broiler chickens. The weight of each group of broiler chickens was weighed on 1, 21, and 42 d to calculate the average daily gain (**ADG**) during the starter (1−21 d), grower (22 −42 d), and overall (1−42 d) periods. The ratio between ADFI and ADG was also calculated (F/G).

Sample Collection

On 21 and 42 d of age, 8 birds (1 bird per pen) from each replicate cage were randomly selected from each treatment and weighted after a 12-h feed deprivation. Birds were euthanized by cervical dislocation and necropsied. After that, the small intestine was dissected free of the mesentery and placed on a chilled stainless steel tray. The jejunum (from the end of the duodenum to the Meckel' diverticulum) was opened longitudinally and flushed the residual digesta with ice-cold phosphate buffer solution for collecting mucosa. The jejunal mucosa was collected by directly scraping using a sterile glass microscope slide at 4°C, which were then immediately frozen in liquid nitrogen and stored at −80°C until analysis.

Preparation of Jejunal Homogenate

About 1 g jejunal mucosal samples were cut off and added with ice-cold sodium chloride solution (154 mmol/L), then homogenized (1:4, wt/vol) using an ultraturrax homogenizer (Tekmar Co., Cincinnati, OH). Afterward, the above homogenate was centrifuged at 4,000 × *g* for 15 min at 4°C. The top supernatant was promptly collected and stored at −80°C for subsequent analysis.

Disaccharidase Activities Determination

The disaccharidase (sucrase and maltase) activities in the jejunum were measured by assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China) according to the methods of Zhu et al. (2014) and Li et al. (2015). The obtained results were normalized against the total protein level of each sample for intersample comparisons. The total protein level of each sample was measured by the Coomassie brilliant blue protein assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China).

Analysis of Antioxidant Status

The total antioxidant capacity (**T-AOC**), superoxide dismutase (**SOD**) and glutathione peroxidase (**GPX**) activities, reduced glutathione (**GSH**), and malondialdehyde (**MDA**) contents were determined using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China) according to the manufacturer's instructions. The results were normalized against total protein concentration in each sample for intersample comparison.

Determination of Immunoglobulin Levels

The chicken-specific ELISA quantification kits (Angle Gene, Nanjing, China) were used to measure the concentrations of secreted immunoglobulin A (**SIgA**) and immunoglobulin G (**IgG**) in the jejunal mucosal samples according to the instructions of the manufacturer. The obtained results were normalized against total protein concentration in each sample for intersample comparison.

Quantitative Real-Time PCR Analysis

Total RNA was extracted from the jejunal mucosa in line with the instructions of manufacturer using the Trizol Reagent (Vazyme Biotech Co., Ltd, Nanjing, China). The RNA concentration and purity were determined from OD260/OD280 readings (ratio > 1.8) using a NanoDrop ND-1000 UV spectrophotometer (NanoDrop Technologies, Wilmington, DE), and simultaneously the ratio for OD260/OD230 (> 2) was also measured in order to identify the presence of organic contaminants. After that, 1 μg of total RNA reverse-transcribed into complementary DNA using the PrimeScript RT reagent kit (Vazyme Biotech Co., Ltd) following the manufacturer's protocols. The complementary DNA samples were amplified with the ChamQ SYBR qPCR Master Mix Kit (Vazyme Biotech Co., Ltd) according to the manufacturer's requirement. Real-time PCR was carried out on an ABI StepOnePlusTM Real-Time PCR system (Applied Biosystems, Grand Island, NY) according to the manufacturer's instructions. All primers were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China) and the sequences were showed in Table 2. The reaction mixture was prepared using 10 μL of 2 × ChamQ SYBR qPCR Master Mix (Vazyme Biotech Co., Ltd), 0.4 μL of forward primer, 0.4 μL of reverse primer, 0.4 μL of 50 × ROX Reference Dye (Vazyme Biotech Co., Ltd), 2 μL of complementary DNA template and 6.8 μL of double-distilled water. The process of PCR consisted of a pre-run at 95°C for 30 s and 40 cycles of denaturation at 95°C for 5 s, followed by a 60°C annealing step for 30 s. The conditions of the melting curve analysis were as follows: one cycle of denaturation at 95°C for 10 s, followed by an increase in temperature from 65 to 95°C at a rate of 0.5°C/s. The melting curve analysis was to check and verify the specificity and purity of all PCR products. The standard curve of each gene was run in duplicate and three times for obtaining reliable amplification efficiency. The relative levels of mRNA expression were calculated using

Table 2. Primer sequence of target and reference genes.

Gene name[1]		Primer sequence (5′-3′)	Accession number
β-actin	Forward	TTGGTTTGTCAAGCAAGCGG	NM_205518.1
	Reverse	CCCCCACATACTGGCACTTT	
Nrf2	Forward	GATGTCACCCTGCCCTTAG	NM_205117.1
	Reverse	CTGCCACCATGTTATTCC	
HO-1	Forward	GGTCCCGAATGAATGCCCTTG	HN237181.1
	Reverse	ACCGTTCTCCTGGCTCTTGG	
SOD1	Forward	CCGGCTTGTCTGATGGAGAT	NM_205064.1
	Reverse	TGCATCTTTTGGTCCACCGT	
GPX1	Forward	GACCAACCCGCAGTACATCA	NM_001277853.1
	Reverse	GAGGTGCGGGCTTTCCTTTA	
CAT	Forward	GGTTCGGTGGGGTTGTCTTT	NM_001031215.1
	Reverse	CACCAGTGGTCAAGGCATCT	
IgM	Forward	GCATCAGCGTCACCGAAAGC	X01613.1
	Reverse	TCCGCACTCCATCCTCTTGC	
IgG	Forward	ATCACGTCAAGGGATGCCCG	X07174.1
	Reverse	ACCAGGCACCTCAGTTTGG	

[1]Abbreviations: CAT, catalase; GPX1, glutathione peroxidase 1; HO-1, heme oxygenase-1; IgM, immunoglobulin M; IgG, immunoglobulin G; Nrf2, nuclear factor erythroid 2-related factor 2; SOD1, superoxide dismutase 1.

$2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001) after normalization against the reference gene, β-actin. The values of CON group were used as a calibrator.

Statistical Analysis

All obtained data were processed by Excel 2010 first, and then analyzed by one-way ANOVA procedure using Statistical Analysis System (SAS Institute, 2000). A replicate cage was the experimental unit for the growth performance data, whereas an individual bird from each replicate cage was the experimental unit for other measured parameters (disaccharidase activities, antioxidant-related parameters, and immune indicators). Duncan's multiple range test was used to determine differences among treatments. The orthogonal polynomial contrasts were employed to test the linear and quadratic effects of the increasing levels of YC-EHY. Data were presented as means and their pooled standard errors. The differences were regarded as statistically significant when $P < 0.05$.

RESULTS

Growth Performance

In Table 3, birds fed the basal diet with 150 mg/kg YC-EHY showed improved ABW compared with the CON group (linear and quadratic, $P < 0.05$). Dietary YC-EHY addition, regardless of its level, increased ADG during the starter and growth periods (linear and quadratic, $P < 0.05$). In addition, supplementing YC-EHY, irrespective of its level, linearly and quadratically decreased the F/G during the starter, growth and overall periods (linear and quadratic, $P < 0.05$).

Table 3. Effects of graded levels of dietary YC-EHY supplementation on growth performance of broiler chickens from 1 to 42 d of age.

	Treatment[4]					*P*-value	
Items[1]	CON	YC-EHY1	YC-EHY2	YC-EHY3	SEM[2]	Linear[3]	Quadratic[3]
1 d ABW (g)	39.41	39.47	39.80	39.61	0.092	0.588	0.661
21 d ABW (g)	805.75	811.18	824.56	823.13	4.936	0.087	0.219
42 d ABW (g)	2474.72[b]	2539.67[ab]	2581.52[ab]	2604.99[a]	15.983	0.006	0.021
1 to 21 d							
ADFI (g/d/bird)	47.40	48.56	47.29	47.19	0.234	0.338	0.218
ADG (g/d/bird)	34.63[b]	37.54[a]	37.98[a]	37.77[a]	0.323	0.014	0.004
F/G (g/g)	1.37[a]	1.29[b]	1.25[b]	1.25[b]	0.012	0.004	0.005
22 to 42 d							
ADFI (g/d/bird)	162.88	165.54	165.13	166.28	1.275	0.477	0.759
ADG (g/d/bird)	75.53[b]	82.86[a]	82.08[a]	83.10[a]	0.739	0.023	0.025
F/G (g/g)	2.16[a]	2.00[b]	2.01[b]	2.01[b]	0.015	0.017	0.007
1 to 42 d							
ADFI (g/d/bird)	106.30	106.55	106.21	106.49	0.669	0.556	0.770
ADG (g/d/bird)	55.25[b]	59.65[a]	59.34[ab]	60.19[a]	0.460	0.007	0.003
F/G (g/g)	1.78[a]	1.62[b]	1.66[b]	1.63[b]	0.009	0.004	0.001

[a-b]Means within the same row with no common superscript differ significantly ($P < 0.05$).
[1]Abbreviations: ABW, average body weight; ADFI, average daily feed intake; ADG, average daily gain; F/G, feed to gain ratio.
[2]Standard error of the means (n = 8).
[3]Orthogonal polynomials were used to evaluate linear and quadratic responses to the levels of YC-EHY treatment.
[4]CON, birds fed the basal diet; YC-EHY1, YC-EHY2, and YC-EHY3, birds fed the basal diet adding 50, 100, and 150 mg/kg yeast culture plus enzymatically hydrolyzed yeast cell wall, respectively.

Table 4. Effects of graded levels of dietary YC-EHY supplementation on the jejunal disaccharidase activities of broiler chickens.

	Treatment[3]					*P*-value	
Items	CON	YC-EHY1	YC-EHY2	YC-EHY3	SEM[1]	Linear[2]	Quadratic[2]
21 d							
Sucrase (U/mg protein)	14.01[b]	17.86[ab]	18.94[a]	16.23[ab]	0.661	0.266	0.043
Maltase (U/mg protein)	169.99[b]	294.77[a]	299.69[a]	255.89[a]	10.245	0.048	0.002
42 d							
Sucrase (U/mg protein)	15.17	15.85	15.10	15.44	1.247	0.406	0.482
Maltase (U/mg protein)	258.16[a]	281.43[ab]	307.08[b]	292.63[b]	12.149	0.028	0.022

[a-b]Means within the same row with no common superscript differ significantly ($P < 0.05$).
[1]Standard error of the means (n = 8).
[2]Orthogonal polynomials were used to evaluate linear and quadratic responses to the levels of YC-EHY treatment.
[3]CON, birds fed the basal diet; YC-EHY1, YC-EHY2 and YC-EHY3, birds fed the basal diet adding 50, 100 and 150 mg/kg yeast culture plus enzymatically hydrolyzed yeast cell wall, respectively.

Jejunal Disaccharidase Activities

Table 4 indicated that YC-EHY addition linearly and quadratically enhanced jejunal maltase activity on 21 and 42 d of age compared with the CON group (linear and quadratic, $P < 0.05$). In addition, the activity of sucrase in the jejunum on 21 d of age was quadratically improved by supplementing YC-EHY ($P < 0.05$).

Jejunal Antioxidant Status

As revealed in Table 5, in comparison with the CON group, YC-EHY addition quadratically enhanced jejunal GSH content except in the YC-EHY3 group on 21 d of age ($P < 0.001$). Both linear and quadratic increases of jejunal SOD activity on 21 and 42 d of age as well as GPX activity on 42 d of age by YC-EHY were observed (linear and quadratic, $P < 0.05$). In contrast, supplementing YC-EHY, irrespective of its level, decreased jejunal MDA concentration on 42 d of age (linear and quadratic, $P < 0.05$).

Jejunal Immunoglobulin Levels

Table 6 indicated that dietary YC-EHY addition linearly and quadratically enhanced IgG content on 21 and 42 d of age as well as SIgA level on 42 d of age in the jejunum compared with the CON group (linear and quadratic, $P < 0.05$). The highest jejunal IgG and SIgA levels both on 21 d and 42 d were presented in the YC-EHY2 group.

Jejunal Antioxidant Genes Expression

As summarized in Table 7, compared with the CON group, dietary YC-EHY increased jejunal Nrf2 gene expression on 21 and 42 d of age except in the YC-EHY1 group (linear and quadratic, $P < 0.05$). Dietary YC-EHY supplementation, regardless of its level, enhanced SOD1 gene expression on 21 and 42 d of age as well as HO-1 and GPX1 genes expression on 42 d of age in the jejunum (linear and quadratic, $P < 0.05$).

Jejunal Immunoglobulin Genes Expression

Table 8 displayed that supplementing YC-EHY linearly and quadratically enhanced jejunal IgM and IgG genes expression on 42 d of age (linear and quadratic, $P < 0.05$). Besides, the genes expression of IgG and IgM in the jejunum on 21 d of age showed quadratic increases by YC-EHY supplementation ($P < 0.05$). The highest

Table 5. Effects of graded levels of dietary YC-EHY supplementation on the jejunal antioxidant capacity of broiler chickens.

	Treatment[4]					*P*-value	
Items[1]	CON	YC-EHY1	YC-EHY2	YC-EHY3	SEM[2]	Linear[3]	Quadratic[3]
21 d							
T-AOC (U/mg protein)	0.86	0.82	0.89	0.88	0.027	0.667	0.871
GSH (mg/g protein)	14.32[b]	19.86[a]	20.61[a]	17.01[ab]	0.563	0.114	<0.001
SOD (U/mg protein)	405.35[b]	480.86[a]	477.63[a]	465.77[ab]	8.263	0.030	0.006
GPX (U/mg protein)	27.30	31.21	34.32	26.92	1.582	0.950	0.309
MDA (nmol/mg protein)	0.48	0.48	0.48	0.47	0.013	0.355	0.238
42 d							
T-AOC (U/mg protein)	0.97[b]	1.21[a]	1.13[ab]	1.01[ab]	0.033	0.973	0.090
GSH (mg/g protein)	9.20[a]	15.68[b]	9.85[b]	10.37[b]	0.474	0.635	0.088
SOD (U/mg protein)	464.78[b]	521.98[ab]	558.47[a]	543.39[ab]	10.521	0.031	0.041
GPX (U/mg protein)	21.08[c]	49.73[ab]	46.33[b]	60.50[a]	1.815	<0.001	<0.001
MDA (nmol/mg protein)	0.63[a]	0.52[b]	0.51[b]	0.47[c]	0.020	0.006	0.027

[a-c]Means within the same row with no common superscript differ significantly ($P < 0.05$).
[1]Abbreviations: GSH, glutathione; GPX, glutathione peroxidase; MDA, malonaldehyde; SOD, superoxide dismutase; T-AOC, total antioxidant capacity.
[2]Standard error of the means (n = 8).
[3]Orthogonal polynomials were used to evaluate linear and quadratic responses to the levels of YC-EHY treatment.
[4]CON, birds fed the basal diet; YC-EHY1, YC-EHY2, and YC-EHY3, birds fed the basal diet adding 50, 100, and 150 mg/kg yeast culture plus enzymatically hydrolyzed yeast cell wall, respectively.

Table 6. Effects of graded levels of dietary YC-EHY supplementation on the jejunal IgG and SIgA concentrations of broiler chickens.

	Treatment[4]					*P*-value	
Items[1]	CON	YC-EHY1	YC-EHY2	YC-EHY3	SEM[2]	Linear[3]	Quadratic[3]
21 d							
IgG (ng/mg protein)	139.59[b]	178.54[a]	202.51[a]	194.13[a]	5.062	0.005	0.004
SIgA (ng/mg protein)	286.45	289.59	313.21	312.72	12.718	0.456	0.762
42 d							
IgG (ng/mg protein)	119.34[b]	152.37[a]	153.85[a]	149.79[a]	3.517	0.044	0.015
SIgA (ng/mg protein)	221.07[b]	284.26[a]	286.84[a]	245.92[ab]	7.692	0.004	0.038

[a-b]Means within the same row with no common superscript differ significantly ($P < 0.05$).
[1]Abbreviations: IgG, immunoglobulin G; SIgA, secreted immunoglobulin A.
[2]Standard error of the means (n = 8).
[3]Orthogonal polynomials were used to evaluate linear and quadratic responses to the levels of YC-EHY treatment.
[4]CON, birds fed the basal diet; YC-EHY1, YC-EHY2, and YC-EHY3, birds fed the basal diet adding 50, 100, and 150 mg/kg yeast culture plus enzymatically hydrolyzed yeast cell wall, respectively.

Table 7. Effects of graded levels of dietary YC-EHY supplementation on the expression levels of jejunal antioxidant-related genes of broiler chickens.

	Treatment[4]					*P*-value	
Items[1]	CON	YC-EHY1	YC-EHY2	YC-EHY3	SEM[2]	Linear[3]	Quadratic[3]
21 d							
Nrf2	1.00[b]	1.35[ab]	1.58[a]	1.62[a]	0.059	<0.001	0.001
HO-1	1.00	1.08	1.14	1.12	0.060	0.497	0.748
GPX1	1.00	1.19	1.43	1.26	0.071	0.127	0.149
CAT	1.00	1.05	0.96	1.10	0.064	0.664	0.819
SOD1	1.00[b]	1.58[a]	1.87[a]	1.58[a]	0.053	0.009	0.001
42 d							
Nrf2	1.00[b]	1.49[ab]	1.62[a]	1.63[a]	0.060	0.016	0.022
HO-1	1.00[a]	1.39[b]	1.40[b]	1.35[b]	0.057	0.032	0.016
GPX1	1.00[b]	1.64[a]	1.81[a]	1.56[a]	0.029	0.032	0.004
CAT	1.00	0.96	1.10	1.12	0.074	0.393	0.684
SOD1	1.00[b]	1.51[a]	1.71[a]	1.64[a]	0.055	0.004	0.002

[a-b]Means within the same row with no common superscript differ significantly ($P < 0.05$).
[1]Abbreviations: CAT, catalase; GPX1, glutathione peroxidase 1; HO-1, hemeoxygenase-1; Nrf2, nuclear factor erythroid 2-related factor 2; SOD1, superoxide dismutase 1.
[2]Standard error of the means (n = 8).
[3]Orthogonal polynomials were used to evaluate linear and quadratic responses to the levels of YC-EHY treatment.
[4]CON, birds fed the basal diet; YC-EHY1, YC-EHY2, and YC-EHY3, birds fed the basal diet adding 50, 100, and 150 mg/kg yeast culture plus enzymatically hydrolyzed yeast cell wall, respectively.

IgG and IgM genes expression were observed in the YC-EHY2 group.

DISSCUSION

So far, the effects of dietary YC-EHY supplementation on the growth performance have not been investigated in broiler chickens. But the rationale with the yeast products on growth performance have been researched in broilers previously (Zhang et al., 2005; Sun et al., 2020). In the present study, we firstly found that dietary supplementation with YC-EHY from 50 to 150 mg/kg improved the growth performance in broiler chickens. It is worth mentioning that during the starter, growth, and overall periods, the YC-EHY1 group

Table 8. Effects of graded levels of dietary YC-EHY supplementation on the expression levels of jejunal immunoglobulin genes of broiler chickens.

	Treatment[4]					*P*-value	
Items[1]	CON	YC-EHY1	YC-EHY2	YC-EHY3	SEM[2]	Linear[3]	Quadratic[3]
21 d							
IgM	1.00[b]	2.05[a]	2.19[a]	1.70[a]	0.090	0.057	<0.001
IgG	1.00[b]	1.48[a]	1.52[a]	1.27[a]	0.046	0.125	0.001
42 d							
IgM	1.00[b]	1.77[a]	1.87[a]	1.76[a]	0.119	0.049	0.035
IgG	1.00[b]	1.64[a]	1.67[a]	1.60[a]	0.093	0.008	0.001

[a-b]Means within the same row with no common superscript differ significantly ($P < 0.05$).
[1]Abbreviations: IgG, immunoglobulin G; IgM, immunoglobulin M.
[2]Standard error of the means (n = 8).
[3]Orthogonal polynomials were used to evaluate linear and quadratic responses to the levels of YC-EHY treatment.
[4]CON, birds fed the basal diet; YC-EHY1, YC-EHY2, and YC-EHY3, birds fed the basal diet adding 50, 100, and 150 mg/kg yeast culture plus enzymatically hydrolyzed yeast cell wall, respectively.

enhanced ADG by 8.4, 9.7, and 8.0%, and decreased F/G by 5.8, 7.4, and 9.0%, respectively. These results suggested that a lower level of YC-EHY would be enough to improve growth performance of broiler chickens effectively. In this study, the enhanced feed efficiency probably related to the improvement of ADG, which was similar with the previous results of Chen et al. (2020). Similarly, supplemental YC-EHY increased the ADG and feed efficiency in ruminants (Salinas-Chavira et al., 2018; Silva et al., 2018). Besides, Huff et al. (2013) showed that supplementing a commercial yeast culture feed intermittently during times of stress at the level of 200 mg/kg prevented the decrease of body weight in turkeys with transport stress/*Escherichia coli* challenge. Moreover, supplementing above yeast feed continuously at 100 mg/kg could enhance the overall feed efficiency by 21 point in challenged turkeys. In addition, plentiful researches displayed that yeast culture and yeast cell wall components showed positive effects on growth performance in different animals (Gao et al., 2008; Ma et al., 2020; Sun et al., 2020). The beneficial effects of YC-EHY on growth performance of broiler chickens might be due to that the yeast-derived additive or its components could improve health responses, immune, and anti-inflammatory effects in animals, as previously reported (Salinas-Chavira et al., 2018; Silva et al., 2018; Bu et al., 2020).

The intestinal epithelial cells cannot directly absorb disaccharides, the hydrolysates of starch, which must be hydrolyzed into monosaccharides by mucosal disaccharidases before absorption and utilization. Thus, disaccharidases play crucial roles in digestion and absorption of carbohydrates. Sucrase, maltase, and isomaltase are 3 disaccharidases existed in the intestine of broiler chickens, which are the signs of intestinal functional maturation and also the key of final digestion (Hung et al., 2020). In current study, dietary YC-EHY enhanced jejunal sucrase activity on 21 d of age as well as maltase activity on 21 and 42 d of age in broiler chickens, and the dosage of 100 mg/kg YC-EHY addition exhibited a better effect. Notably, the improved disaccharidases activities suggested that the utilization of intestinal nutrients in broiler chickens was strengthened, which probably promoted the improvement of growth performance in this study, that was in agreement with the outcomes of Wan et al. (2016) and Hung et al. (2020). Consistent with our results, dietary yeast and yeast cell wall components supplementation enhanced intestinal maltase activity (Yang et al., 2008) as well as amylase, protease and lipase activities (Castro et al., 2013) in animals. Although there were no studies on the improvement of disaccharidase activities by YC-EHY, abundant researches showed that other yeast-derived products could present prodigestive effects in animals. For example, live yeast and yeast culture addition enhanced intestinal ash retention, the digestibility of organic matter as well as calcium and phosphor in animals (Gao et al., 2008; Attia et al., 2020).

Reactive oxygen species (**ROS**) produced inside body during normal metabolism play essential roles in multiple biochemical pathways and physiological processes (Cheng et al., 2020). However, excessive ROS produced in individuals would pose a threat to cellular biological macromolecules such as proteins and nucleic acids, thereby resulting in damages in tissues and causing negative effects on growth performance and health status further (Bai et al., 2018; Cheng et al., 2020). Both enzymatic (SOD, GPX, CAT) and non-enzymatic antioxidants (like GSH) in living cells collectively defense oxidative stress. SOD could transform the superoxide anion into oxygen and hydrogen peroxide, which are then disintegrated to water by antioxidant enzymes including GPX and CAT. GPX is an important peroxidase, which protects the structure and function of cell membrane from the interference and damage of oxides (Luo et al., 2003). However, when antioxidative enzymes activities decrease, the polyunsaturated fatty acids would be attacked by oxygen free radicals, resulting in lipid peroxidation (Cheng et al., 2020). MDA is an end product of lipid peroxidation. In this work, YC-EHY supplementation enhanced the SOD activity of 21 d and 42 d as well as the GPX activity of 42 d in the jejunum, and the level ranged from 50 to 100 mg/kg showed better outcomes. Also, supplementing YC-EHY linearly and quadratically lowered jejunal MDA content on 42 d of age, the dosage of 150 mg/kg exhibited the best lipid oxidation inhibition effect. These results suggested that the antioxidant capacity of broiler chickens was improved by YC-EHY supplementation. Consistently, supplementing yeast-derived products including yeast culture improved serum and hepatic SOD, GPX, and CAT activities but decreased MDA concentration in animals (Bu et al., 2019; Timothée Andriamialinirina et al., 2020; He et al., 2021). Up to date, the effect of YC-EHY on antioxidant capacity has not been found in any animals. But the rationale with the yeast-derived products and their mode of action on antioxidant capacity have been expounded in poultry previously (Li et al., 2016; Zhang et al., 2021). Many researches demonstrated that natural carbohydrates are crucial macromolecules which deeply affect the antioxidant system (Krizkova et al., 2001; Lei et al., 2015; Liu et al., 2018; Guo et al., 2019). Plentiful studies indicated that yeast cell wall components which contain carbohydrates could enhance antioxidant-related enzymes activities (Li et al., 2016; Bu et al., 2019) whereas decreased the production of ROS and the lipid peroxidation level in animals (Zhang et al., 2005; Kim et al., 2019a). Thus, we inferred that carbohydrates in YC-EHY might play a part role in improving antioxidant capacity. From the molecular perspective, the improved antioxidative enzymes activities were associated with their gene transcription. YC-EHY supplementation elicited favorable changes in relative genes expression of Nrf2-related mRNAs. Nrf2, a transcription factor, can regulate antioxidant-related genes expression such as HO-1, GPX1, and SOD1. When cells are damaged, Nrf2 will upregulate and activate the phase II enzymes to enhance the tolerance of cells to oxidative stress so as to maintain the cellular redox homeostasis (Song et al., 2018). In the

present study, HO-1 gene expression was enhanced with increasing YC-EHY addition. HO-1 catalyzes the decomposition of hemoglobin into Fe^{2+}, carbon monoxide and biliverdin, which could be converted into bilirubin by biliverdin reductase further. Both biliverdin and bilirubin are crucial antioxidants against the oxidation of protein (Bai et al., 2018). Consistent with the corresponding activities of antioxidative enzymes, the genes expression of SOD1 and GPX1 were also upregulated. Similarly, supplementing other yeast-derived products could improve the antioxidative enzymes activities, meanwhile, enhancing corresponding genes expression (El-Murr et al., 2019; Rahimnejad et al., 2020). We deduced that YC-EHY, as an antioxidant, might improve the antioxidant capacity of broiler chickens by regulating the related genes expression in Nrf2 signaling pathway and thereby enhancing the activities of antioxidant enzymes and reducing the damage of lipid peroxidation. Whereas, how YC-EHY activates the transcription factor, Nrf2, thus resulting in initiating the transcription of relative antioxidative target genes is not understood, additional exploration is clearly needed in this aspect.

IgG, IgM, and SIgA are 3 main immunoglobulins in broiler chickens to fight against various sources of viruses and toxins, thereby protecting the immune system and maintaining the health status of the body (Bai et al., 2018; Song et al., 2018). IgG, secreted by plasma cells, has antiviral, antibacterial, and toxins-neutralizing effects. IgM presents powerful bactericidal, complement activation and immunomodulatory functions. SIgA, as the first-line to defense against pathogen invasion, could slow down the reproduction of viruses and improve the intestinal mucosal barrier function (Song et al., 2018). Also, SIgA has antibody activity against some viruses, bacteria and common antigens. The rationale with the yeast products and their mode of action on immune function has been reported in detail before (Ganner and Schatzmayr, 2012). In this study, dietary YC-EHY increased jejunal IgG concentration on 21 and 42 d of age and SIgA content on 42 d of age, which suggested that YC-EHY might stimulate the humoral immunity to produce more antibodies. Similar with our results, Yuan et al. (2015) indicated that dietary YC-EHY enhanced the humoral and mucosal immunity as well as the blood's bactericidal capacity through increasing serum IgG content, fecal IgA concentration and uterine neutrophil numbers in transition dairy cows. Huff et al. (2013) demonstrated that dietary yeast feed supplementation protected the turkeys against the harmful effects of transport stress/*Escherichia coli* challenge and declined the colonization of harmful pathogen. Additionally, yeast cultures contain different immunostimulants (β-glucans, mannoproteins, chitin and nucleotides), which could produce a more general immune response to regulate the nuclear factor kappa-B signaling pathway and improve immune status in animals (Bu et al., 2019). Gao et al. (2008) showed that yeast culture supplementation could enhance antibody titers to Newcastle disease virus, serum lysozyme activity and duodenal IgM and SIgA contents in broiler chickens. At mRNAs level, intestinal IgM and IgG genes expression were simultaneously enhanced by YC-EHY in broiler chickens. The results indicated that YC-EHY supplementation increased the expression of immunoglobulins by enhancing the transcription of immunoglobulin genes. Macpherson et al. (2008) showed that the balance between anti-inflammatory and proinflammatory cytokines is important in SIgA and IgG productions. Thus, researchers showed that supplementing yeast-derived products could decrease the mRNA expression levels of intestinal proinflammatory genes, thereby improving intestinal barrier function and immune status in animals (Alizadeh et al., 2016; Wang et al., 2016b; Li et al., 2017; Ma et al., 2020), which probably indirectly promoted the production of immunoglobulin. Interestingly, Lessard et al. (2009) and Wang et al. (2016a) found that live yeast addition decreased the challenge-induced excessive augment of intestinal SIgA level to a normal level in animals. This indirectly reflected the intestinal structure and immune function were improved because the alleviated intestinal SIgA concentration might be the outcome of relieved irritation and enhanced intestinal barrier function in animals (Lessard et al., 2009). Besides, yeast cell wall components containing immune carbohydrates (MOS and β-glucan) have been shown to stimulate humoral immunity and the production of immunoglobulin (Davis et al., 2004). Both MOS and β-glucan are effective immunopotentiators. The mannose sugars could reproduce probiotic bacteria and restrain the adherence and colonization of pathogen in gut (Walker et al., 2017), and β-glucan could elicit immune responses after recognition by metazoan carbohydrate receptor (Brown and Gordon, 2005). However, because of the intricacy nutrient composition in YC-EHY and the unclear mechanism by which YC-EHY improves the immune function of broiler chickens, further investigation of YC-EHY is still warranted.

CONCLUSIONS

In summary, the results of the present study indicated that YC-EHY supplementation could improve the growth performance, intestinal disaccharidase activities, antioxidant capacity and immune function in broiler chickens. The level ranged from 50 to 150 mg/kg improved growth performance and the level ranged from 50 to 100 mg/kg enhanced antioxidant capacity and immune function in broiler chickens. Herein, the recommended level of YC-EHY is 50 to 100 mg/kg in broiler chicken diet.

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DISCLOSURES

All authors approve the submission of this manuscript and declare no conflict of interest. The manuscript has not been published previously, and not under consideration for publication elsewhere.

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