Effect of yeast cell wall on the growth performance and gut health of broilers challenged with aflatoxin B_1 and necrotic enteritis

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ABSTRACT This study aimed to investigate the effect of yeast cell walls (YCW) on the growth performance, visceral lesions, intestinal integrity, enterotoxicity, and bacteria of broilers challenged with aflatoxin B_1 (AF) and necrotic enteritis (NE) from 1 to 21 d of age. A total of 576 one-day-old broilers were assigned to a 2 × 2 × 2 design for diets containing AFB_1 (0 or 40 μg/kg), NE (challenged or unchallenged), or YCW (0 or 500 mg/kg). The main effect analysis showed that AF depressed ($P < 0.01$) average daily feed intake (ADFI), average daily body weight gain (ADG), the mRNA profiles of polymeric Ig receptor (pIgR), claudin-1, and occludin, but increased ($P < 0.001$) liver lesion scores, serum endotoxin, and diamine oxidase (DAO). The NE challenge depressed ($P < 0.01$) ADFI, ADG, secretory IgA (sIgA), pIgR, claudin-1, occludin, and the populations of *Lactobacilli and Bifidobacteria*, but increased ($P < 0.001$) visceral lesions, endotoxins, and DAO. The main effect of YCW on growth performance, visceral lesions, and intestinal integrity was not significant, but decreased ($P < 0.01$) mortality, endotoxin, DAO, and *C. perfringens*, and increased ($P < 0.05$) the populations of *Lactobacilli* and *Bifidobacteria*. There were 3-way interactions ($P < 0.05$) on growth performance, intestinal lesions, integrity, and gut bacteria. Compared with the treatment with the dual challenges, there were pronounced effects ($P < 0.05$) of YCW on ADFI, ADG, lesions, DAO, pIgR, and *Bifidobacteria*. The results suggest that with the concurrent challenges of AF and NE, the YCW can partially protect the growth performance and intestinal health of broilers.

Key words: aflatoxin B_1, broiler, gut health, necrotic enteritis, yeast cell wall

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INTRODUCTION

Due to the global climate change, aflatoxins are becoming more prevalent and persistent in vegetable seeds and derived products (Paterson and Lima, 2010). Of the aflatoxins congeners, aflatoxin B_1 (**AFB_1**) is the most prevalent and toxic. Chronic exposure to AFB_1 caused weight loss, immunosuppression, mutagenesis, reproductive alterations, and carcinogenesis in many species including broilers (Nesbitt et al., 1962; Rawal et al., 2010; Chen et al., 2013). With the restriction on feed antibiotics and cage rearing, necrotic enteritis (**NE**) is increasingly more threating to gut health and livability for broilers. The NE could be caused by *Clostridium perfringens* (***C. perfringens***) and further exacerbated with some undesirable or negative dietary factors (Riddell and Kong, 1992).

Recently, increasing evidence have shown that yeast cell walls (**YCW**) are effective in adsorbing mycotoxins as well as pathogenic microbes in animal gastrointestinal tract (Yannikouris et al., 2004; Tian et al., 2016). The functional ingredients in YCW are beta-glucan and mannan oligosaccharides, and the bioadsorbing feature is dependent upon their purity quotients or supplemental doses in the feed since the affinity is reversible and saturable (Moran, 2004). Khadem et al. (2012) found that supplementing 0.5% yeasts improved the body weight gain and feed intake of broiler chicks fed on diets containing 200 μg/kg of AFB_1. Gao et al. (2008) reported that yeast culture added at 0.25% increased antibody titers to Newcastle disease virus, serum lysozyme, duodenal IgM, and secretary IgA (**sIgA**). Also, mannan oligosaccharides activated innate immune response by up-regulating toll-like receptor 2b, toll-like receptor 4, interleukin-12p35, and interferon-gamma in broilers fed on organic diets and challenged with *C. perfringens* (Yitbarek et al., 2012). Glucans increased antibody levels against *C. perfringens*, and reduced intestinal *C. perfringens* populations and gut lesions (Tian et al., 2016).

Clearly, there is a significant effect of YCW against either AF or *C. perfringens* in broilers. However, when AF and NE concurrently occurred the CYW effect still remains unclear. This study aimed to evaluate the effect

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of an YCW product on growth performance, visceral lesions, enterotoxicity, intestinal integrity, and bacteria under the dual challenges of AF and NE through a 3-way design in broilers.

MATERIALS AND METHODS

Animal Ethic Statement

The trial protocol was approved by the Institutional Committee for Animal Use and Ethics of the College of Animal Science of Henan University of Science and Technology (Luoyang, Henan, China).

Aflatoxin B_1, Yeast Cell Walls and Diets

A 2 × 2 × 2 factorial arrangement of treatments included AFB_1 (0 or 40 μg/kg), NE (challenged or not), and YCW (0 or 500 mg/kg). The AFB_1 was produced using *Aspergillus flavus* from the China General Microbiological Culture Collection Center to yield moldy corn according to the method by Liu et al. (2017a). The AFB_1 concentration in the moldy corn meal was 3876 μg/kg and modulated to 40 μg/kg of feed.

The YCW (D-glucose 48.3% and D-mannose 32.3%) were provided by Luoyang Hongxiang Biological Feed Laboratory of Henan University of Science and Technology (Henan, China), and added at 500 mg/kg of feed at the expense of corn in the formulation. The nutrition levels of basal diet were recommended by China Agricultural Standard (NY/T 33–2004), and the water contents of all ingredients and diets were controlled under 12% and stored in a cool, dry, dark, and well-ventilated place. All diets were fed as mash on air-dried basis. No antibiotics were offered to broilers via either feed or water throughout the trial. The formulation of basal diet was listed in Table 1.

Animal Management and NE Challenge

A total of 576 one-day-old male Cobb broilers were randomly distributed into 8 groups with 6 pens of 12 chicks each. All chicks were reared on floor pens (1.2 m × 1.2 m) in an environmentally controlled facility at the Research Center of the Henan University of Science and Technology and given ad libitum access to diets and water throughout the study. The room temperature was maintained at 34°C for 1 to 5 d and then gradually decreased to 24°C by 21 d old. Birds received 24 h light at 1 d, and then 23L:1D for the remainder days. Birds and feed in each pen were weighed weekly and feed efficiency was adjusted for mortality on a pen basis. All birds were monitored for general health twice a day.

From 1 d, birds were placed on the recently used litter from chickens challenged via the feed with *C. perfringens* cultures (China Veterinary Culture Collection Center, China Institute of Veterinary Drug Control, Beijing, China), whereas unchallenged birds were placed on the clean litter.

Table 1. Ingredients and nutrient levels of basal diet[1] (air-dry basis).

Ingredients	Contents (%)	Nutrients[2]	Contents (%)
Corn	54.10	Crude protein	21.48
Soybean meal	25.80	Crude fat	3.31
Corn gluten meal	6.00	Ca	0.99
Corn DDGS	6.00	Total P	0.73
Soybean oil	2.90	ME (MJ/kg)	12.41
Lysine	0.25	Crude fiber	2.84
Methionine	0.15	Non-phytate P	0.51
Salt	0.40	Methionine	0.51
Dicalcium phosphate	2.10	Methionine+cysteine	0.86
Limestone	1.15	Lysine	1.18
Titanium dioxide	0.50		
Choline chloride	0.15		
Premix[2]	0.50		

[1]Aflatoxin B_1 was not detectable (<2 μg/kg).
[2]Provided per kg diet: vitamin A (retinyl acetate), 8,000 IU; cholecalciferol, 1,000 IU; vitamin E (DL-tocopheryl acetate), 20 IU; vitamin K, 0.5 mg; thiamin, 2.0 mg; riboflavin, 8.0 mg; d-pantothenic acid, 10 mg; niacin, 35 mg; pyridoxine, 3.5 mg; biotin, 0.18 mg; folic acid, 0.55 mg; vitamin B_{12}, 0.010 mg; Mn, 120 mg; I, 0.70 mg; Fe, 100 mg; Cu, 8 mg; Zn, 100 mg; and Se, 0.30 mg.
[2]Calculated by Chinese Feed Database, version 21, 2010.

Sample Collection and Intestinal Lesion Scoring

On 21 d, 5 birds per pen were randomly selected, weighed, euthanized by carbon dioxide, and then dissected. Blood was immediately drawn from the heart with a syringe and aliquoted into sterile vials for serum preparation as described by Liu et al. (2008). Liver, duodenum, jejunum, and ileum were collected and scored for lesions on a scale of 0 to 3 using lesion visibility. Briefly, 0—lesion is not seen; 1—lesion is poorly seen; 2—lesion is visible; 3—the lesion is easily visible. Approximately a 1 cm segment from the middle part of jejunum was dissected, flushed with phosphate buffer solution, and stored in RNA later solution at –20°C for mRNA expression analysis. Approximately 2 g ileal digesta was collected and stored at −40°C for gut bacteria analysis.

Chemical and Biological Analysis

The contents of D-glucose and D-mannose in YCW were determined according to China National Standard GB/T 18,104–2000. The concentrations of AFB_1 in moldy corn or diets were detected using an enzyme-linked immunosorbent assay kit (detection limit 2 μg/kg, Longke Fangzhou Biotech, Beijing, China).

The concentrations of serum endotoxin were measured using a limulus amoebocyte lysate-based kit (Lonza, Walkersville, MD). Briefly, samples and standards were incubated for 10 min at 37°C with limulus amoebocyte lysate and then for another 6 min with

Table 2. Information of primers for quantitative real-time PCR.

Name	GenBank	Primers (5′→3′)		Length (bp)
		Forward	Reverse	
Claudin-1	AY750897.1	cggctggatgggtatcatca	agtcgtacaccttgcactgg	158
Occludin	D21837.1	aaccccgagttggatgagtc	ttcaggtcggtgtcgaactc	158
sIgA	S40610	acctccaaagtgaccctcct	ggggtcatctcctcgttgtc	137
pIgR	XM_01,529,8928	caggtggaaatgcagggcta	tcttgcattccacgtcaggtt	202
Beta-actin	NM_205,518	gccgagagagaaattgtgcg	cacaggactccatacccaaga	208

sIgA, secretory IgA; pIgR, polymeric Ig receptor.

colorimetric substrate. Internal control for recovery calculation was included in the assessment. The reaction was stopped with 25% acetic acid and then the absorbance was read at 405 nm. The activity of diamine oxidase (**DAO**) in serum (1 mL) was examined by a spectrophotometric assay. The DAO standard (D7876–250) was purchased from a Sigma-Aldrich supplier in Beijing of China.

Total mRNA isolation and cDNA synthesis for jejunal samples were carried out as described by Liu et al. (2008), and the transcript levels were expressed as the relative expression to beta-actin gene. Primer information for qPCR was listed in Table 2. The qPCR reactions were set at 10 μL with 5 μL of SYBR Green Master Mix, 1 μL of primer, 4 μL of 10 × diluted cDNA or DNA. Plates were run on the ABI Prism 7900HT Fast Real-Time PCR System. All qPCRs were run in triplicates on the same thermal cycles (50°C 2 min, 95°C 10 min, 40 cycles of 95°C 15 s and 60°C 1 min). No amplification signal was detected in water or no-RT RNA samples. Primers synthesis and qPCR reagents were provided by Dalian TaKaRa Co., Ltd. (Liaoning, China).

Numeration of Bacteria

Approximately 1 g of each ileal digesta was diluted with 9 mL of ice-cold sterile buffer peptone water and homogenized. The suspension of each sample was serially diluted between 10^{-1} to 10^{-7} dilutions, and 100 μL of each diluted sample was spread onto duplicate selective agar plates for bacterial counting. The count of colony forming units was expressed as a logarithmic transformation per gram of intestinal digesta. Commercial media (Qingdao Hopebio Co., Ltd., Shandong, China) were used for the cultivation and isolation of *Lactobacilli* (HB0392), *Bifidobacteria* (HB0394), *Escherichia coli* (***E. coli***; HB7001), and *C. perfringens* (HB0256).

Statisical Analysis

Data were analyzed using a 3-way Model of General Linear procedure (IBM SPSS, version 23.0, Armonk, NY). Differences among treatments were analyzed by ANOVA Model. Pen was the experimental unit for growth performance, and the average of liver, jejunal, ileal, and serum samples of 5 birds was the statistical unit for the analysis of lesion scores, gene expression ($2^{-\Delta\Delta Ct}$), bacteria population ($\log_{10}$ cfu) and serum toxic markers. Differences of variables of ANOVA analysis were separated using Duncan's multiple-range test at $P < 0.05$ level of significance, and the Tamhane T2 test was used in case of equal variances not assumed.

RESULTS

Growth Performance

The main effect analysis showed that average daily feed intake (**ADFI**) and average daily body weight gain (**ADG**) were decreased ($P < 0.01$) by the AF or NE challenge, but unaffected by supplemented YCW (Table 3). The feed conversion ratio (**FCR**) was worsen ($P < 0.01$) by dietary AF, but not by NE challenge. The mortality rate of broilers was increased ($P < 0.05$) by NE and decreased ($P < 0.05$) by YCW. The interactions of 3-way were significant ($P < 0.01$) on ADFI, ADG, and FCR. Among treatments, increased effects of YCW on ADFI and ADG were found ($P < 0.05$) when with NE challenge with or without AF challenge.

Intestinal Lesion Scores and Serum Toxic Markers

In Table 4, the AF increased ($P < 0.001$) liver lesion scores, serum endotoxin, and DAO, whereas NE challenge increased ($P < 0.001$) lesion scores in liver and intestine, endotoxin, and DAO. The YCW had no effects on lesion scores in liver and intestine, but decreased ($P < 0.01$) endotoxin and DAO. A 3-way interaction ($P < 0.001$) on intestinal lesions was found. With the concurrent challenges by AF and NE, the YCW effects on liver lesions and serum DAO were more pronounced ($P < 0.05$).

Intestinal Integrity

In Table 5, the AF down-regulated ($P < 0.001$) the mRNA profiles of polymeric Ig receptor (**pIgR**), claudin-1, and occludin, but did not affect secretory IgA (**sIgA**). The NE challenge down-regulated ($P < 0.01$) sIgA, pIgR, claudin-1, and occludin. The YCW did not affect these genes. 3-way interactions ($P < 0.05$) on sIgA, claudin-1, and occludin were

Table 3. Effect of experimental factors on the growth performance and mortality of broilers at 1 to 21 d of age.

Items			Growth performance			
			ADFI (g/bird)	ADG (g/bird)	FCR	Mortality (%)
Treatments						
AF[1]	NE[2]	YCW[3]				
–	–	–	43.06[a]	28.95[a]	1.49[b,c]	2.50[a,b]
–	–	+	43.50[a,b]	29.25[a]	1.49[b,c]	1.67[b]
–	+	–	41.47[a,b]	27.45[c]	1.51[b]	5.83[a,b]
–	+	+	42.20[a,b]	28.78[a,b]	1.47[c]	3.33[a,b]
+	–	–	42.79[b,c]	28.50[b]	1.50[b,c]	4.17[a,b]
+	–	+	42.70[c]	28.58[a,b]	1.49[b,c]	2.50[a,b]
+	+	–	39.13[d]	25.80[d]	1.52[a,b]	6.67[a]
+	+	+	40.31[e]	25.97[d]	1.55[a]	3.33[a,b]
SEM			0.651	0.563	0.015	1.428
P values						
AF			**	***	**	NS
NE			***	***	NS	*
YCW			NS	NS	NS	*
AF × NE			***	***	**	NS
AF × YCW			*	**	*	NS
NE × YCW			***	***	NS	*
AF × NE × YCW			***	***	**	NS

[a–e]Means within a column with no common superscripts differ significantly ($P < 0.05$).
[1]AFB_1 at 40 μg/kg of feed.
[2]Induced using a litter contaminated with *Clostridium perfringens* from 1 d old.
[3]Containing D-glucose 48.3% and D-mannose 32.3%, and added at 500 mg/kg of feed.
–/+ Without/with an experimental factor.
ADFI, average daily feed intake; ADG, average daily body weight gain; AF, aflatoxin; FCR, ratio of feed to gain; FI, feed intake; NE, necrotic enteritis; SEM, standard error of mean; YCW, yeast cell walls.
NS, $P \geq 0.05$; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Table 4. Effect of experimental factors on the visceral lesion scores and serum toxic markers of broilers.

Items			Lesion scores		Serum toxic markers (U/mL)	
			Liver	Intestine	Endotoxin	DAO
Treatments						
AF[1]	NE[2]	YCW[3]				
–	–	–	0.17[e]	0.27[c]	0.25[c,d]	0.72[d]
–	–	+	0.13[e]	0.23[c]	0.23[d]	0.72[d]
–	+	–	1.00[b,c]	3.50[a]	0.33[a,b]	1.33[b]
–	+	+	0.80[c]	2.80[a]	0.29[b,c]	1.24[b]
+	–	–	0.47[d]	1.13[b]	0.29[b,c]	0.97[c]
+	–	+	0.57[d]	1.00[b,c]	0.26[c,d]	0.73[d]
+	+	–	1.43[a]	4.60[a]	0.37[a]	1.82[a]
+	+	+	1.13[b]	3.27[a]	0.33[a,b]	1.35[b]
SEM			0.078	0.662	0.015	0.063
P-values						
AF			***	NS	***	***
NE			***	***	***	***
YCW			NS	NS	**	***
AF × NE			NS	***	NS	**
AF × YCW			NS	NS	NS	NS
NE × YCW			*	***	NS	NS
AF × NE × YCW			NS	***	NS	NS

[a–e]Means within a column with no common superscripts differ significantly ($P < 0.05$).
[1]AFB_1 at 40 μg/kg of feed.
[2]Induced using a litter contaminated with *Clostridium perfringens* from 1 d old.
[3]Containing D-glucose 48.3% and D-mannose 32.3%, and added at 500 mg/kg of feed.
–/+ Without/with experimental factor.
AF, aflatoxin; NE, necrotic enteritis; SEM, standard error of mean; YCW, yeast cell walls.
NS, $P \geq 0.05$; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

found. Compared with the treatment with the dual challenges, the YCW up-regulated ($P < 0.05$) pIgR. Compared with the treatment with AF alone, the YCW decreased ($P < 0.05$) claudin-1. Also, the YCW increased ($P < 0.05$) occludin when no challenge occurred.

Ileal Bacterial Population

The main effect analysis showed that AF had no effect on ileal bacterial population. The NE challenge decreased ($P < 0.01$) the populations of *Lactobacilli*, *Bifidobacteria*, and *E. coli*, but increased ($P < 0.05$)

Table 5. Effect of experimental factors on the profiles of genes of intestinal integrity of broilers.

Items			mRNA expression ($2^{-\Delta\Delta Ct}$)			
			sIgA	pIgR	Claudin-1	Occludin
Treatments						
AF[1]	NE[2]	YCW[3]				
–	–	–	5.38[a]	1.13[a,b]	5.98[a]	4.46[b]
–	–	+	5.07[a,b]	1.25[a]	5.74[a]	5.05[a]
–	+	–	3.10[a–c]	1.06[b,c]	3.38[d]	3.81[c,d]
–	+	+	1.68[c]	1.07[b,c]	3.50[d]	3.56[d]
+	–	–	4.07[a–c]	0.99[c,d]	4.53[c]	4.12[b,c]
+	–	+	2.57[b,c]	0.92[d]	4.89[b]	4.19[b]
+	+	–	2.27[c]	0.45[f]	3.21[d]	2.97[e]
+	+	+	1.58[c]	0.59[e]	3.47[d]	2.96[e]
SEM			0.079	0.114	0.103	0.123
P-values						
AF			NS	***	***	***
NE			**	***	***	***
YCW			NS	NS	NS	NS
AF × NE			**	***	***	NS
AF × YCW			NS	***	*	NS
NE × YCW			**	***	NS	*
AF × NE × YCW			***	***	NS	*

[a–f]Means within a column with no common superscripts differ significantly ($P < 0.05$).
[1]AFB_1 at 40 μg/kg of feed.
[2]Induced using a litter contaminated with *Clostridium perfringens* from 1 d old.
[3]Containing D-glucose 48.3% and D-mannose 32.3%, and added at 500 mg/kg of feed.
–/+ Without/with experimental factor.
AF, aflatoxin; NE, necrotic enteritis; pIgR, polymeric Ig receptor; SEM, standard error of mean; sIgA, secretory IgA; YCW, yeast cell walls.
NS, $P \geq 0.05$; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Table 6. Effect of experimental factors on the populations of gut bacteria of broilers.

Item			Ileal populations ($\log_{10}$ cfu/g of ileal digesta)			
			Lactobacilli	*Bifidobacteria*	*C. perfringens*	*E. coli*
Treatment						
AF[1]	NE[2]	YCW[3]				
–	–	–	7.50[a,b]	8.37[a,b]	2.70[d,e]	5.07[a,b]
–	–	+	7.80[a]	8.60[a]	2.65[e]	5.20[a]
–	+	–	6.97[c]	7.87[c,d]	3.85[a]	4.68[b]
–	+	+	7.52[a,b]	8.38[a,b]	3.17[b,c]	4.99[a,b]
+	–	–	7.27[b,c]	8.20[a–c]	3.10[b–d]	4.83[a,b]
+	–	+	7.57[a,b]	8.43[a,b]	2.90[c–e]	5.05[a,b]
+	+	–	6.95[c]	7.58[d]	4.25[a]	4.93[a,b]
+	+	+	7.12[b,c]	8.12[b,c]	3.36[a]	4.77[b]
SEM			0.195	0.184	0.252	0.139
P-values						
AF			NS	NS	NS	NS
NE			**	**	***	*
YCW			*	**	**	NS
AF × NE			**	**	NS	NS
AF × YCW			*	**	NS	NS
NE × YCW			**	***	**	NS
AF × NE × YCW			**	***	***	NS

[a–e]Means within a column with no common superscripts differ significantly ($P < 0.05$).
[1]AFB_1 at 40 μg/kg of feed.
[2]Induced using a litter contaminated with *Clostridium perfringens* from 1 d old.
[3]Containing D-glucose 48.3% and D-mannose 32.3%, and added at 500 mg/kg.
–/+ Without/with experimental factor.
AF, aflatoxin; NE, necrotic enteritis; SEM, standard error of mean; YCW, yeast cell walls.
NS, $P \geq 0.05$; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

C. perfringens population (Table 6). The YCW increased ($P < 0.05$) the populations of *Lactobacilli* and *Bifidobacteria*, and decreased ($P < 0.01$) *C. perfringens* population. There were 3-way interactions ($P < 0.01$) on *Lactobacilli*, *Bifidobacteria*, and *C. perfringens*. With the challenge of NE alone, the YCW increased ($P < 0.05$) *Lactobacilli*, and decreased ($P < 0.05$) *C. perfringens*. Regardless of AF, with NE challenge, the YCW increased ($P < 0.05$) *Bifidobacteria* population.

DISCUSSION

In the present study, both AF and NE challenge decreased ADFI and ADG, and the NE challenge also increased FCR and mortality. These results were consistent with previous studies. Cravens et al. (2013) reported that the AF decreased ADG and ADFI, and further resulted in poorer FCR, and increased mortality, while the NE challenge also decreased ADFI and ADG. Tian et al. (2016) found that the ADG of broilers at 0 to 42 d old decreased by NE challenge, while higher ADG and feed efficiency were observed in the glucan-supplemented birds during 21 to 42 d and the whole experimental period, but no interaction between the two factors. However, literature about 3-way interaction among AF, NE, and YCW is not available. In the present study, the more pronounced effects of YCW with challenges AF, NE, or both caused significant 3-way interactions on ADFI, ADG, and FCR, indicating that the growth was partially protected by YCW, but the main effects of YCW on ADFI and ADG were not significant.

The different effects of YCW on the growth performance may be caused by its varied quality or functionality. The radical development in the fuel alcohol has created vast quantities of yeast byproducts, including a commercial-scale source of yeast extract, yeast glucose and mannose polysaccharides. The YCW contains three major constituents: glucans (glucose polysaccharides), mannans (mannose polysaccharides), and a protein fraction. The separation of these natural polymers is simple and inexpensive. However, it is difficult and expensive to obtain more than 65% pure fractions and, therefore, these components are produced and sold at these low levels of purity. The glucans and mannans are the functional components, which vary with the microbial strains and processing. Their largest commercial application are as nutritional supplements for animal feed (Moran, 2004). The differences in the product purity and supplemental doses caused the different efficacy in animal application.

The specific structure is the center of the bioactivity of YCW as well as both fractions of polysaccharides in practical application as animal feed nutritional supplements. Glucans are a part of the cell wall's triple helix tridimensional structure, with spring-like mechanical properties, responsible for yeast cell walls strength and ability to absorb toxins (Yannikouris et al., 2004). The major role of glucans in animal nutrition is to adsorb mycotoxins and detoxify feed. Mannans have a brush-like structure, which creates a specific combination of various functionalities that also involve protein conjugates (Vinogradov et al., 1998). It can fit with various receptors present on the walls of animal digestive tracts (Mansour and Levitz, 2003) and with the receptors on the membranes of pathogenic bacteria (Wellens et al., 2008).

Mannan/protein-conjugates are involved in interactions with animal immune systems, and as a result enhance immune system activity (Wismar et al., 2010), and contribute to animal antioxidant and antimutagenic defenses (Krizkova et al., 2006). The functions of YCW on immune system strength, blockage of bacterial adhesion to the gut, and modification of the gut structure improve survival and growth of young animals. The YCW product in the present study was prepared with glucans 48.3% and mannans 32.3%, considering the dual functions as adsorbents against feed toxins and gut pathogens. Indeed, the beneficial effects of YCW on enterotoxin clearance, mucosal integrity, and microbiota equilibrium were demonstrated in the present study.

With the inhibition of antibiotics as animal growth promoters, coupled with the susceptibility to pathogens for floor free range rearing poultry, NE disease is rising as a significant threat to gut health and livability. The main pathogen causing NE is the *C. perfringens* which exists not only in the exogenous environment but also in the gut bacteria. Furthermore, the dietary compositions can also influence the incidence of this disease (Riddell and Kong, 1992). Cravens et al. (2013) reported that the effect of NE was dependent on AF concentration, and AF increased lesion scores in NE challenged birds.

Based on the mechanism of YCW adhering bacteria in the broiler gut, the YCW and its two fractions are potential alternatives to antibiotics against *C. perfringens*. Indeed, studies showed the significance of yeast glucan or mannan oligosaccharides decreasing intestinal lesion scores as well as the amount of *C. perfringens* in the broiler gut (Hofacre et al., 2003; Tian et al., 2016). In the present study, the main effect of YCW was significant on serum endotoxins and DAO, and ileal *C. perfringens* count. Further, compared with the treatment with the dual challenges, YCW deceased liver lesions, indicating the positive effect of YCW as an adsorbent of pathogens. This also implied that there are still opportunities for further enhancements in the product quality or supplemental amount.

The intestinal integrity is critical to defend pathogenic microbiota in farm animals, especially broilers that are susceptible to disease due to genetic selective rapid growth. The main defenders of intestinal mucosal barrier include sIgA and the flowing mucus (Deplancke and Gaskins, 2001). The sIgA is secreted by plasma cells in lamina propria and transported by pIgR across epithelial cells into mucosa layers, and as a result, pIgR bridges innate and adaptive immune responses at mucosal surfaces (Kaetzel, 2005). Claudin-1 and occludin are integral membrane proteins localizing at tight junctions (Furuse et al., 1998). Tight junctions represent one mode of cell-to-cell adhesion in epithelial or endothelial cell sheets, forming continuous seals around cells and serving as a physical barrier to prevent solutes and water from passing freely through the paracellular space (Hahn-Strömberg et al., 2017). Therefore, the mRNA profiles of sIgA, pIgR, claudin-1, and occludin can reflect the intestinal mucosal barrier function.

The immune modulatory effect of YCW is in that its components can interact with not only the receptors present on the walls of animal digestive tracts (Mansour and Levitz, 2003) but also the receptors on the membranes of pathogenic bacteria (Wellens et al., 2008). In the present study, the main effects of YCW on the four genes were not significant, but in some levels of the two challenged factors, YCW up-regulated pIgR, claudin-1, and occludin, and interactions were found on sIgA, claudin-1, and occludin, indicating the partial modulation of YCW on intestinal integrity. Tian et al. (2016) reported that yeast beta-glucan supplementation increased intestinal anti- *C. perfringens* IgA antibody levels at 7 and 14 d post infection of broilers, but no significant interaction. Rajput et al. (2013) found that living yeast cells increased IgA-positive cells in the lumen of the intestinal villus and modulated intestinal ultrastructure through increasing occludin, claudin-2, and claudin-3 levels in broiler intestine.

In addition to the adsorption to toxins and pathogens as well as the immune promotion, the components of YCW are also the most popular prebiotics used nowadays in foods and feeds (Fowler et al., 2015; Markazi et al., 2017; Liu et al., 2017b). Undoubtedly, in the present study, the addition of YCW improved the populations of *Lactobacilli* and *Bifidobacteria*, decreased *C. perfringens*, but did not affect the *E. coli.* Meanwhile, the AF had no effect on ileal microbial populations, and the NE challenge decreased the counts of *Lactobacilli*, *Bifidobacteria* and *E. coli*, but increased *C. perfringens* count. Importantly, facing the dual challenges by AF and NE, the main effect analysis showed that the YCW was unable to completely compensate the growth performance, intestinal lesion scores, and mocusal barrier function, but the main effect was significant on the improvement in gut bacterial equilibrium.

These findings indicated that the YCW are more predominant as the food of gut probiotics than as the adsorbent and the immunoadjuvant in this complicated scenario of 3-way interaction. Similarly, Tian et al. (2016) found that broilers fed diets containing yeast beta-glucans had higher levels of cecal lactic acid bacteria and *Bifidobacteria* at 14 d post infection, and lower concentrations of cecal *C. perfringens* at 7 d post infection and coliforms and lactose-negative bacteria at 21 d post infection compared with un-treated birds. Kim et al. (2011) reported that dietary mannans decreased the populations of *C. perfringens* and *E. coli*, and increased *Lactobacilli* and total bacteria in the ileum of broilers.

CONCLUSIONS

In the 3-way design, both AF and NE challenge decreased growth performance and gut health of broilers. The YCW reduced mortality, endotoxin, and DAO, and increased the counts of *Lactobacilli* and *Bifidobacteria*. There were 3-way interactions on growth performance, intestinal lesions, integrity, and bacteria. Compared with the treatment with the dual challenges, there were pronounced effects of YCW on ADFI, ADG, lesions, DAO, *Bifidobacteria*, *C. perfringens*. The results suggest that with the concurrent challenges of AF and NE, the YCW can partially protect the growth performance and intestinal health of broilers.

SUPPLEMENTARY DATA

Supplementary data are available at *Poultry Science* online.

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