

Effects of combination of mannan-oligosaccharides and β -glucan on growth performance, intestinal morphology, and immune gene expression in broiler chickens

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ABSTRACT The study was conducted to evaluate the effects of the combination of mannan-oligosaccharides (MOS) and β -glucan on growth performance, intestinal morphology, and immune gene expression in broiler chickens. A total of 640, one-day-old male Cobb 500 broilers were randomly allocated into 32 pens with 8 replicates and 20 birds per pen. Thirty-two pens were divided into 4 treatments, including control, 0.04% MOS, 0.002% β -glucan, and a combination of MOS and β -glucan. Growth performance was measured on d14, 28, and 35. The ileum and cecal tonsils were collected from one bird per pen at 21 and 35 d of age for further analyses of immune gene

expression. Duodenum, jejunum, and ileum were collected for intestinal morphology on d 35. Results indicated that both MOS and β -glucan improved growth performance during starter phase ($P < 0.05$). In addition, β -glucan further increased body weight gain of birds from d 0 to 28 ($P < 0.05$). Furthermore, the combination of MOS and β -glucan presented higher villi height in the jejunum on d 35 ($P < 0.05$). There were no significant differences for gene expressions of immune responses on d 21 and 35. In conclusion, the application of prebiotic combination of MOS and β -glucan might perform multiple pathways, improving growth performance in broiler chickens.

Key words: Mannan-oligosaccharide, β -glucan, prebiotic, broiler chicken

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INTRODUCTION

The “Antibiotic Free”, “Raise Without Antibiotic”, and “No Antibiotic Ever” are new trends of production strategies in the poultry industry because the emergence of antibiotic resistance in pathogens has led to high risk of human health and raised the public concern (Marshall and Levy, 2011). Exploring new feed additives has been one of the approaches to replace antibiotics, especially the antibiotic growth promoters which were banned by the EU on January 1, 2006 (Official Journal of the European Union, 2003). Prebiotic is one of the potential feed additives which regulate host immunity, intestinal microbiota, and the intestinal ecosystem. The prebiotic is defined as a selectively fermented ingredient that allows specific changes in the composition and/or activity in the intestinal microbiota that confers benefits upon the host’s well-being and health (Gibson et al., 2004). The intestinal health-promoting bacteria can ferment prebiotics, further improving intestinal microbial

structures, integrity of intestine epithelial cells, and eventually overall health of the host (Teng and Kim, 2018). Mannan-oligosaccharides (MOS), yeast β -glucan, and fructans are the 3 major prebiotics which have been studied broadly and applied successfully in animal production (Adhikari et al., 2018; Teng and Kim, 2018).

MOS and β -glucan are derived from yeast cell walls of *Saccharomyces cerevisiae*. Either *Saccharomyces cerevisiae* itself or extracted MOS and β -glucan have been confirmed their potential on improving growth performance, regulating intestinal microbiota, and stimulating immune responses of broiler chickens (Teng et al., 2017a; Teng and Kim, 2018; Ricke et al., 2020). MOS are capable of binding pathogenic bacteria, such as *Salmonella* and *E. coli*, to reduce pathogen infection in the intestine of animals (Spring et al., 2000). It has been reported that MOS could increase several *Lactobacillus* species, enhanced villus height in the intestine, and regulated gene expression of toll-like receptors and cytokines in the ileum and cecal tonsils (Corrigan et al., 2011; Yitbarek et al., 2012; Corrigan et al., 2015; Teng and Kim, 2018). MOS also acts as a proinflammatory factor inducing immune responses (Tada et al., 2002). Moreover, MOS can enhance antibody production against infectious bursal diseases virus, Newcastle disease virus, and avian

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influenza virus (Shashidhara and Devegowda, 2003; Tohid et al., 2010; Salehimanesh et al., 2016).

β -glucans, long-chain polysaccharides linked with D-glucose monomers by 1-3, and 1-6 β -glycosidic bonds, could act as immunomodulators which trigger cytokine production and enhance proliferation of lymphocytes (ŚWiĄTkiewicz et al., 2014; Teng and Kim, 2018). Furthermore, intestinal macrophages could recognize β -glucans, consequently inducing their phagocytic ability and proinflammatory cytokine production, such as interleukin-1 and tumor necrosis factor- α (Guo et al., 2003; Cox et al., 2010b). Additionally, previous reports indicated that β -glucan enhanced gene expression of antimicrobial peptides in chickens infected with *Salmonella* (Shao et al., 2013; Shao et al., 2016). The authors also suggested that β -glucan protected chickens from pathogen infections by inducing specific IgA in the intestine (Shao et al., 2013; Shao et al., 2016).

Several review papers have emphasized the importance of prebiotics on controlling pathogens infection and strengthening intestinal health (Teng and Kim, 2018; Ricke et al., 2020). However, limited research has investigated if the combination of 2 prebiotics performs more substantial outcomes than single prebiotic applications. We hypothesized that MOS and β -glucan might positively interact to improve intestinal health and growth performance in chickens; thus, the current study was conducted to evaluate the effects of dietary supplementation of MOS or/and β -glucan on growth performance, intestinal morphology, and immune gene expression in broiler chickens.

MATERIALS AND METHODS

Experimental Design and Growth Performance

The study was approved by the Institutional Animal Care and Use Committee and conducted at the Poultry Research Center, University of Georgia, Athens, GA. A total of 640 one-day-old male broiler chickens were randomly allocated to 4 treatments with 8 replicates, and 20 birds per pen. A 2 \times 2 factorial arrangement was used in the study. The main factors were MOS and β -glucan. The treatments included a control group, a MOS treatment, a β -glucan treatment, and a combination group with both MOS and β -glucan. Chickens in the MOS treatment were fed corn-soybean meal-based diet added with 0.04% MOS (with a minimum 40% MOS concentration) produced by Kerry Inc (Beloit, WI), whereas birds in the β -glucan treatment were fed additional 0.002% β -glucan produced by Kerry Inc (Everwell). Moreover, 0.04% MOS and 0.002% β -glucan were both added in the basal diets for the combination treatment. The diet formulation is shown in Table 1. Feed and water were provided ad libitum during whole experiment and the environmental temperature program was followed to the recommendation of Cobb Broiler Management Guide (Cobb 2018). Feed and body weight of birds were weighted on d 1, 14, 28, and 35 to calculate feed intake (**FI**), body weight gain (**BWG**), and feed conversion rate (**FCR**).

Table 1. Ingredient and nutrient composition for broiler starter, grower and finisher diets (%).

Item	Starter (0–14 d)	Grower (14–28 d)	Finisher (29–35 d)
Ingredient (% of diet)			
Corn	60.00	63.55	66.86
Meat and bone meal	1.97	1.23	1.16
Distiller's dried grains with solubles	2.50	2.50	2.50
Soybean meal (48%)	31.00	27.00	23.80
Poultry fat	1.37	2.53	2.80
Limestone	0.61	0.62	0.59
Defluorinated phosphate	1.14	1.22	1.04
Salt	0.30	0.30	0.30
Vitamin mix ¹	0.25	0.25	0.25
Mineral mix ²	0.08	0.08	0.08
DL-Methionine	0.30	0.25	0.22
L-Lysine	0.24	0.24	0.16
Threonine	0.05	0.05	0.03
Sand	0.20	0.20	0.20
ME (kcal/kg)	3,000	3,100	3,150
CP (%)	22.43	20.40	19.00
Ca (%)	0.90	0.84	0.76
Available P (%)	0.45	0.42	0.38
TSAA (%)	0.98	0.89	0.82
Met (%)	0.63	0.57	0.52
Lys (%)	1.32	1.19	1.05
Thr (%)	0.86	0.78	0.71

¹Provided per kilogram of DSM Vitamin premix: vitamin A, 2,204,586 IU; vitamin D3, 200,000 ICU; vitamin E, 2,000 IU; vitamin B12, 2 mg; biotin, 20 mg; menadione, 200 mg; thiamine, 400 mg; riboflavin, 800 mg; d-pantothenic acid, 2,000 mg; vitamin B6, 400 mg; niacin, 8,000 mg; folic acid, 100 mg; and choline, 34,720 mg.

²Provided per kilogram of mineral premix: Ca, 0.72 g; Mn, 3.04 g; Zn, 2.43 g; Mg, 0.61 g; Fe, 0.59 g; Cu, 22.68 g; I, 22.68 g; and Se, 9.07 g.

Intestinal Morphology

Intestinal morphometric analyses were followed by the method described by (Teng et al., 2017b). One bird per pen was randomly selected and killed by cervical dislocation for collecting intestinal tissue on d 35. Three cm long tissue was cut from the center of the duodenum, jejunum, and ileum, rinsed with phosphate buffer saline, and immediately fixed in 10% formalin solution. The fixed tissues were embedded in paraffin and cut into 4 μm , followed by staining process of the hematoxylin and eosin method (Ferldman and Wolfe, 2014). The villi height and crypt depth were observed and captured by a light microscope with 1.6X (duodenum and jejunum) or 5X (ileum) magnification (Leica DC500 camera, Leica Microsystems Inc., Buffalo Grove, IL). The villi height and crypt depth were measured in 5 randomly-selected villi or crypt per slide, using the LAS v4.8 software (Leica Microsystems Inc.). Besides villi height and crypt depth, the ratio of villi height to crypt depth was calculated from each sample.

Real-Time PCR Analysis

On d 21 and 35, ileum and cecal tonsils were collected from one bird per pen. Intestinal samples were frozen with liquid nitrogen immediately and stored in -80°C for further analyses of immunity gene expression. Approximately 80 to 150 mg samples were cut from frozen samples and homogenized in QiAzol lysis reagents (Qiagen, Valencia, CA) to collect total RNA. The quantity and purity of extracted RNA were measured by a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA). The cDNA was reverse-transcribed from total RNA by High Capacity cDNA synthesis kits (Applied BioSystems, Life Technologies, CA). Real-time PCR reaction was conducted by using SYBR Green Master mix with a Step One thermo-cycler (Applied Biosystem, Foster City, CA). The cDNA samples were run in duplicate in the real-time PCR analysis. The target genes expression normalized by a housekeeping gene was calculated and analyzed by the $2^{-\Delta\Delta\text{Ct}}$ method (Livak and Schmittgen, 2001). The outliers were removed from the data set if the data point was exceeded ± 3 standard deviations from the mean. The glyceraldehyde-3-phosphate dehydrogenase (GAPDH, forward primer: CCTCTCTGGCAAAGTCCAAG; reverse primers: GGTCACGCTCCTGGAAGATA) was used as a housekeeping gene, whereas interleukin-6 (IL-6, forward primer: CAGGACGAGATGTGCAAGAAG; reverse primers: TAGCACAGAGACTCGACGTT), interleukin-10 (IL-10, forward primer: AGCAGATCAAGGAGACGTT; reverse primers: ATCAGCAGGTACTCCTCGAT), and interferon- γ (IFN- γ , forward primer: CTGAAGAACTGGACAGAGAG; reverse primers: CACCAGCTTCTGTAAGATGC) were used as target genes in the experiment.

Statistical Analyses

All data were analyzed in the PROC GLM program of SAS software (SAS Institute Inc., Cary, NC). The comparison of treatments was subjected to the two-way ANOVA where MOS and β -glucan were considered as the main factors. Statistical significance of all analyses was set at $P < 0.05$, and trends were considered at $P < 0.10$.

RESULTS AND DISCUSSION

Supplementation of MOS or β -glucan increased BW and BWG of the birds during the first 2 wk in the current study ($P < 0.05$, Table 2); MOS treatment significantly increased BW and BWG ($P = 0.038$ and 0.045 , respectively), and β -glucan treatment improved BW, BWG, and FCR ($P = 0.001$, 0.001 , and 0.042 ,

Table 2. Effects of combination of mannan-oligosaccharides (MOS) and β -glucan on growth performance of broiler chickens.

Items ¹		FI	BW	BWG	FCR
D 1–14	Control	591	377	339	1.74
	MOS	606	388	350	1.73
	β -glucan	592	395	357	1.66
	MOS + β -glucan	604	403	364	1.66
	SEM	7.16	2.67	2.68	0.02
	MOS -	592	386 ^b	348 ^b	1.70
	+	605	396 ^a	357 ^a	1.70
	β -glucan -	599	383 ^b	345 ^b	1.74 ^a
	+	598	399 ^a	361 ^a	1.66 ^b
	<i>P</i> value				
MOS	0.375	0.038	0.045	0.895	
β -glucan	0.959	0.001	0.001	0.042	
MOS \times β -glucan	0.891	0.65	0.642	0.895	
D 1–28	Control	2,467	1,388	1,349	1.83
	MOS	2,504	1,428	1,389	1.80
	β -glucan	2,537	1,460	1,421	1.79
	MOS + β -glucan	2,486	1,440	1,401	1.77
	SEM	14.30	9.77	9.77	0.01
	MOS -	2,502	1,424	1,385	1.81
	+	2,495	1,434	1,395	1.79
	β -glucan -	2,486	1,408 ^b	1,369 ^b	1.82
	+	2,512	1,450 ^a	1,411 ^a	1.78
	<i>P</i> value				
MOS	0.803	0.557	0.569	0.459	
β -glucan	0.36	0.026	0.027	0.156	
MOS \times β -glucan	0.132	0.102	0.099	0.824	
D 1–35	Control	3,896	2,169	2,130	1.83
	MOS	3,895	2,205	2,167	1.80
	β -glucan	3,960	2,212	2,173	1.82
	MOS + β -glucan	3,895	2,224	2,185	1.78
	SEM	22.06	13.64	13.68	0.01
	MOS -	3,928	2,191	2,152	1.83
	+	3,895	2,215	2,176	1.79
	β -glucan -	3,896	2,187	2,149	1.82
	+	3,928	2,218	2,179	1.82
	<i>P</i> value				
MOS	0.472	0.389	0.391	0.177	
β -glucan	0.486	0.282	0.286	0.606	
MOS \times β -glucan	0.482	0.661	0.66	0.892	

¹MOS, supplementation of 0.04% of mannan-oligosaccharides in diets; β -glucan, supplementation of 0.002% β -glucan in diet; MOS + β -glucan, supplementation of 0.04% of mannan-oligosaccharides and 0.002% β -glucan in diet.

^aN = 8; Data is present as the average of 8 replicates per treatment.

^bBirds were fed with different treatment diets from d 1 to 35 to investigate effects of combination of MOS and β -glucan on growth performance, intestinal morphology and immune responses of broiler chickens.

respectively). Moreover, β -glucan further improved BW and BWG till d 28 ($P = 0.026$ and 0.027 , respectively). However, there was no significant main effect of MOS on growth performance during the same phase (d 1–28). Additionally, there was no significant interaction between MOS and β -glucan, suggesting that the combination of MOS and β -glucan did not provide further improvement on growth performance. Though MOS and β -glucan did not significantly increase overall growth performance from d 1 to 35, applying these prebiotics in the diets numerically improved BWG and FCR. Similarly, a previous study reported that combination of MOS and β -glucan did not significantly, but numerically improved growth performance (Awaad et al., 2011).

Administration of MOS and β -glucan presents positive outcomes on ecosystems of the intestine in the broiler chickens by regulating the three major elements in the intestine of the host, 1) intestinal epithelial cell linings, 2) immunity, and 3) microbial community (Teng and Kim, 2018). First, combination of MOS and β -glucan significantly increased villus height in the jejunum in the current study ($P = 0.035$, Table 3). Furthermore, duodenal and ileal villus height was numerically increased by the combined prebiotic group (Tables 4 and 5). These results are consistent to the previous studies which indicated that MOS increased villi height, enhanced goblet cell numbers, as well as decreased crypt depth in the intestine of broilers (Baurhoo et al., 2009; Chee et al., 2010). Additionally, MOS can be used by beneficial bacteria in the intestine of birds. Previous studies have demonstrated significant increases of *Lactobacillus* and *Bifidobacteria* species in the ceca of birds fed MOS (Baurhoo et al., 2007; Baurhoo et al., 2009; Chee et al., 2010). These microorganisms can ferment MOS to produce organic acids, protecting the host from pathogen invasion (Teng and Kim, 2018). It has been reported that MOS could efficiently reduce *E. coli*, *C.*

Table 3. Effects of combination of mannan-oligosaccharides (MOS) and β -glucan on intestinal morphology of broiler chickens on d 35 (Duodenum).

Items ¹	VH	CD	VH: CD	
Control	2,471	244	10.70	
MOS	2,360	252	9.51	
β -glucan	2,538	253	10.03	
MOS + β -glucan	2,543	246	10.55	
SEM	49.48	6.61	0.23	
MOS	-	2,505	249	10.37
+	2,452	249	10.03	
β -glucan	-	2,416	248	10.11
+	2,541	250	10.29	
<i>P</i> value				
MOS	0.603	0.563	0.467	
β -glucan	0.225	0.476	0.689	
MOS \times β -glucan	0.570	0.271	0.069	

N = 8; Data is present as the average of 8 replicates per treatment.

¹VH, villi height; CD, crypt depth; VH:CD, ratio of villi height to crypt depth; MOS, supplementation of 0.04% of mannan-oligosaccharides in diets; β -glucan, supplementation of 0.002% β -glucan in diet; MOS + β -glucan, supplementation of 0.04% of mannan-oligosaccharides and 0.002% β -glucan in diet.

Table 4. Effects of combination of mannan-oligosaccharides (MOS) and β -glucan on intestinal morphology of broiler chickens on d 35 (Jejunum).

Items ¹	VH	CD	VH: CD	
Control	1,419 ^{ab}	246	5.55	
MOS	1,324 ^{ab}	221	6.37	
β -glucan	1,280 ^b	211	6.08	
MOS + β -glucan	1,487 ^a	212	7.23	
SEM	35.21	6.73	0.30	
MOS	-	1,350	229	5.82
+	1,406	217	6.80	
β -glucan	-	1,372	234	5.96
+	1,384	212	6.66	
<i>P</i> value				
MOS	0.418	0.36	0.106	
β -glucan	0.858	0.102	0.248	
MOS \times β -glucan	0.035	0.346	0.778	

¹VH, villi height; CD, crypt depth; VH:CD, ratio of villi height to crypt depth; MOS, supplementation of 0.04% of mannan-oligosaccharides in diets; β -glucan, supplementation of 0.002% β -glucan in diet; MOS + β -glucan, supplementation of 0.04% of mannan-oligosaccharides and 0.002% β -glucan in diet.

^{ab}N = 8; Data is present as the average of 8 replicates per treatment.

Table 5. Effects of combination of mannan-oligosaccharides (MOS) and β -glucan on intestinal morphology of broiler chickens on d 35 (Ileum).

Items ¹	VH	CD	VH: CD	
Control	766	220	3.57	
MOS	728	198	3.85	
β -glucan	758	209	3.67	
MOS + β -glucan	815	223	3.73	
SEM	15.87	6.52	0.13	
MOS	-	762	215	3.62
+	772	211	3.79	
β -glucan	-	747	209	3.71
+	787	216	3.70	
<i>P</i> value				
MOS	0.759	0.746	0.511	
β -glucan	0.217	0.592	0.964	
MOS \times β -glucan	0.139	0.189	0.679	

N = 8; Data is present as the average of 8 replicates per treatment.

¹VH, villi height; CD, crypt depth; VH:CD, ratio of villi height to crypt depth; MOS, supplementation of 0.04% of mannan-oligosaccharides in diets; β -glucan, supplementation of 0.002% β -glucan in diet; MOS + β -glucan, supplementation of 0.04% of mannan-oligosaccharides and 0.002% β -glucan in diet.

perfringens, Coliforms, and *Salmonella* in the intestine of chickens (Wexler, 2007; Yang et al., 2008; Baurhoo et al., 2009; Pourabedin et al., 2014). Furthermore, the increase of *Lactobacillus* coupled with the decrease of pathogens would provide a more robust intestinal environment which supports the proliferation of enterocytes, increases mucin production, and strengthens intestinal immunity (Teng and Kim, 2018).

Besides improving intestinal morphology and intestinal microbiome, MOS and β -glucan also regulate immune responses in broiler chickens. Once toll-like receptors recognized MOS, it triggers the proinflammatory cytokines' cascade, including upregulating interleukin-12 and IFN- γ which further stimulate proliferation of T cells, natural killer cells and macrophages (Yitbarek et al., 2012; Teng and Kim, 2018). MOS not

Table 6. Effects of combination of mannan-oligosaccharides (MOS) and β -glucan on immune responses in the ileum of broiler chickens (d 21 and 35).

Items ¹	d 21			d 35		
	Il-6	Il-10	IFN- γ	Il-6	Il-10	IFN- γ
Control	1.12	1.14	1.15	1.05	1.03	1.08
MOS	1.27	1.39	1.78	1.61	1.43	1.22
β -glucan	1.31	1.43	1.95	1.87	1.31	1.06
MOS + β -glucan	1.54	2.16	2.1	1.94	1.37	1.14
SEM	0.09	0.14	0.24	0.20	0.12	0.10
MOS	-	1.22	1.29	1.55	1.46	1.07
+	1.41	1.78	1.94	1.78	1.40	1.18
β -glucan	-	1.20	1.27	1.47	1.33	1.15
+	1.43	1.80	2.03	1.91	1.34	1.10
	<i>P</i> value					
MOS	0.279	0.057	0.438	0.439	0.205	0.621
β -glucan	0.203	0.079	0.272	0.165	0.403	0.820
MOS \times β -glucan	0.837	0.368	0.630	0.548	0.522	0.881

N = 8. Data is present as the average of 8 replicates per treatment.

¹Il-6, interleukin-6; Il-10, interleukin-10; IFN γ , interferon- γ ; MOS, supplementation of 0.04% of mannan-oligosaccharides in diets; β -glucan, supplementation of 0.002% β -glucan in diet; MOS + β -glucan, supplementation of 0.04% of mannan-oligosaccharides and 0.002% β -glucan in diet.

Table 7. Effects of combination of mannan-oligosaccharides (MOS) and β -glucan on immune responses in the cecal tonsils of broiler chickens (d 21 and 35).

Items	d 21			d 35		
	Il-6	Il-10	IFN- γ	Il-6	Il-10	IFN- γ
Control	1.08	1.09	1.10	1.04	1.10	1.06
MOS	1.97	1.81	2.54	1.15	1.09	1.05
β -glucan	1.88	1.57	2.48	1.13	1.43	1.23
MOS + β -glucan	2.21	2.01	2.44	1.43	1.66	1.05
SEM	0.19	0.17	0.23	0.16	0.14	0.09
MOS	-	1.48	1.33	1.79	1.09	1.15
+	2.09	1.91	2.49	1.29	1.38	1.05
β -glucan	-	1.53	1.45	1.82	1.10	1.06
+	2.05	1.79	2.46	1.28	1.55	1.14
	<i>P</i> value					
MOS	0.164	0.091	0.108	0.542	0.689	0.627
β -glucan	0.103	0.310	0.142	0.589	0.110	0.654
MOS \times β -glucan	0.444	0.662	0.091	0.766	0.658	0.670

Il-6, interleukin-6; Il-10, interleukin-10; IFN γ , interferon- γ ; MOS, supplementation of 0.04% of mannan-oligosaccharides in diets; β -glucan, supplementation of 0.002% β -glucan in diet; MOS + β -glucan, supplementation of 0.04% of mannan-oligosaccharides and 0.002% β -glucan in diet.

N = 8. Data is present as the average of 8 replicates per treatment.

only regulated innate immunity, but also strengthened antibody titers against several virus infection, such as bursal disease virus, Newcastle disease virus, and avian influenza virus (Shashidhara and Devegowda, 2003; Tohid et al., 2010; Salehimanesh et al., 2016). On the other hand, β -glucan can be recognized by macrophages which produce inducible nitric oxide synthase and several interleukins and lead to the increase of cytotoxic T cells and recruitment of heterophils (Cox et al., 2010a; Teng and Kim, 2018). The immune responses triggered by β -glucan could inhibit pathogens proliferation in the intestine, establishing a better microbiome structure in the intestine of birds (Ricke et al., 2020). However, even though neither MOS nor β -glucan presented significant immune responses in the current study, there was a trend suggesting that Il-10 and IFN- γ was increased in the cecal tonsils and ileum on d 21 by application of prebiotics (Tables 6 and 7, $P < 0.1$). The inconsistent results to previous studies may be attributed to the

different dosages provided, different ages of the birds used, different samples determined, or numerous resources of the β -glucan extracted. Moreover, regardless of pathogen challenge, MOS and β -glucan themselves might have tremendous impacts on the immunity regulation. It has been reported that supplementation of β -glucan or MOS regulated immune responses of chickens infected with *Salmonella* or *Clostridium* (Yitbarek et al., 2012; Shao et al., 2016).

In conclusion, MOS and β -glucan increased growth performance in the starter phase, and the combination of the 2 prebiotics improved intestinal morphology. Furthermore, both MOS and β -glucan presented a trend of upregulation of immune responses in the ileum and cecal tonsil. Application of MOS and β -glucan has potential to improve growth performance, intestinal development, and immunity which might provide better protection from pathogens infection as well as establish a stronger intestinal ecosystem in broiler chickens. In the future, it is important

to evaluate the prebiotic combination on growth performance, immunity, and gut health in broilers under stress conditions, such as heat stress and pathogens infection.

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DISCLOSURES

The authors declare no conflicts of interest.

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