Immune responses to dietary β-glucan in broiler chicks during an *Eimeria* challenge

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ABSTRACT Escalating consumer concerns regarding pathogen resistance have placed the poultry industry under mounting pressure to eliminate the use of chemotherapeutic agents as feed additives. One possible alternative receiving increased attention is the use of immunomodulators such as β-glucan. A study was conducted to investigate the effects of a yeast-derived β-glucan (Auxoferm YGT) on broiler chick performance, lesion scores, and immune-related gene expression during a mixed *Eimeria* infection. Day-old chicks were fed diets containing 0, 0.02, or 0.1% YGT. On d 8 posthatch, one-half of the replicate pens were challenged with a mixed inoculum of *Eimeria acervulina*, *Eimeria maxima*, and *Eimeria tenella*. Measurements were taken and samples collected on d 4, 10, 14, and 21 posthatch. Dietary supplementation had no effect on performance or mortality. On d 14, 3 birds per pen (n = 24/treatment) were scored for intestinal coccidia lesions. Gross lesion severity was significantly reduced in birds supplemented with 0.1% YGT. On d 10, inducible nitric oxide synthase (iNOS) expression was downregulated in the jejunum of challenged birds fed 0.1% YGT. Expression of iNOS in the ileum was downregulated in the nonchallenged birds, but upregulated in the challenged birds fed 0.1% YGT on d 14. Interleukin (IL)-18 was upregulated in the jejunum of 0.1% YGT-treated birds. Interferon (IFN)-γ expression was decreased in challenged and nonchallenged birds fed 0.1% YGT. The IL-4 expression was downregulated in the nonchallenged birds with 0.1% YGT diet supplementation. The IL-13 and mucin-1 levels were also reduced due to β-glucan supplementation. Mucin-2 expression was increased in the nonchallenged birds, but decreased in the infected birds fed 0.1% YGT. These results suggest that although Auxoferm YGT at doses of 0.02 and 0.1% does not influence performance, it significantly reduces lesion severity and is capable of altering immune-related gene expression profiles, favoring an enhanced T helper type-1 cell response during coccidiosis.

Key words: β-glucan, broiler, coccidiosis, cytokine, immunity

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INTRODUCTION

Parasitism of the intestinal tract is a major stress factor leading to decreased nutrient utilization and reduced performance in livestock and poultry. Coccidiosis is an intestinal infection brought about by intracellular protozoan parasites belonging to the genus *Eimeria* (Dalloul and Lillehoj, 2005). *Eimeria* species invade the intestinal lining and disrupt the enterocyte layer, resulting in gross lesions, nutrient malabsorption, and reduced performance (Brake et al., 1997). Coccidiosis is regarded as the parasitic disease that has the greatest economic impact on the poultry industry, with total losses estimated at a devastating $3 billion annually worldwide (Dalloul and Lillehoj, 2006).

Currently, the poultry industry relies heavily on the use of anticoccidial feed additives to prevent coccidiosis outbreaks. Recently, consumers have become exceedingly concerned about antimicrobial use and increased pathogen resistance. The controversy and potential risk have resulted in the ban of some antibiotics as feed additives by the European Union as of January 1, 2006. Unfortunately, this ban has led to a decline in animal health and greater variability in carcass size and meat characteristics (Castanon, 2007).

The need to move away from chemotherapeutic control of coccidiosis has prompted an intense search for alternatives capable of maintaining animal health without negatively affecting performance. β-Glucans have received increasing interest due to their immunomodulating capabilities and better acceptance by consumers. β-Glucans are glucose polymers that are structural

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components of the cell wall of many bacteria, fungi, algae, and yeast, as well as cereal grains such as oats and barley. Large variation exists in the structure of β-glucans from these different sources that ultimately results in differences in their physiological functions (Volman et al., 2008). Those β-glucans derived from yeast and fungi are termed 1,3/1,6-β-D-glucans and are considered to be the most effective type of β-glucans in terms of stimulating the immune system due to their highly branched structure (Vetvicka and Vetvickova, 2007; Harada and Ohno, 2008).

The effects of β-glucans as biologically active immunomodulators have been well noted in mammalian species; however, only recently has research been published concerning the effects of β-glucans in poultry. Exposure to β-glucan enhanced proliferation and phagocytizing efficiency of avian macrophages (Guo et al., 2003) and heterophils (Lowry et al., 2005). Similar to mammals, broilers supplemented with β-glucans had amplified humoral (Guo et al., 2003; Zhang et al., 2008) and cell-mediated immune responses (Chen et al., 2003; Chae et al., 2006). The immune-enhancing capabilities of β-glucans have resulted in the clearance of several economically important pathogens such as *Salmonella enterica* and *Escherichia coli*, further asserting their potential use as an antibiotic alternative (Lowry et al., 2005; Huff et al., 2010).

The objective of this study was to determine the effects of dietary supplementation of a β-glucan derived from the yeast *Saccharomyces cerevisiae* on performance, lesion scores, and intestinal immune-related gene expression in broiler chicks with or without an *Eimeria* species challenge.

MATERIALS AND METHODS

Birds, Diets, and Eimeria Challenge

This project was approved and conducted under the guidelines of the Virginia Tech Institutional Animal Care and Use Committee. On the day of hatch, 1,440 straight-run Cobb 500 broiler chicks were picked up from a commercial hatchery (Pilgrim's Pride, Broadway, VA) and transported to the Virginia Tech Turkey Research Farm. Chicks were weighed by pen for equal weight distribution and placed into 48 floor pens (16 replicate pens/treatment) consisting of concrete floors and pine shavings with 30 chicks per pen (0.1 m^2/bird). Chicks had ad libitum access to water and a nonmedicated corn/soy-based starter diet in mash form containing 0, 0.02, or 0.1% YGT Auxoferm (β-glucan extracted from *S. cerevisiae*, AB Vista, Marlborough, UK). The corn-soybean basal diet was formulated to meet or exceed the nutrient requirements for broilers as recommended by the NRC (1994). On d 8 posthatch, one-half of the replicate pens were orally gavaged with 1 mL of a mixed inoculum containing 50,000 *Eimeria acervulina*, 10,000 *Eimeria maxima*, and 2,500 *Eimeria tenella* sporulated oocysts.

Performance Parameters

Birds were weighed before placement on the day of hatch and on d 4, 10, 14, and 21 with birds in each pen being weighed as a group. Mean BW for each treatment group was calculated from the pen replicates for each weigh day. Average BW gain (**BWG**) was calculated for each period and cumulatively based on pen weights. Feed intake (**FI**) of each group was measured at the same time periods as BW (d 4, 10, 14, and 21) with cumulative averages calculated. Feed conversion ratio (**FCR**) was calculated using BW and FI and adjusted for mortality.

Lesion Scoring

On d 14, 3 birds per pen (n = 24/treatment) were randomly selected and killed for scoring of lesions from the intestinal *Eimeria* challenge. Lesions were scored by personnel blinded to the treatment in the duodenum, jejunum, and ceca according to the method of Johnson and Reid (1970) based on score range from 0 (no gross lesion) to 4 (most severe lesion).

Tissue Sampling for Gene Expression Analysis

Eight birds per treatment (1 bird/replicate) were sampled on d 4, 10, 14, and 21 posthatch. Sampled chicks were killed by cervical dislocation, and the small intestinal segments (duodenum, jejunum, and ileum) were aseptically excised, rinsed in cold PBS, minced, snap-frozen in liquid nitrogen, and stored at –80°C until analysis.

Total RNA Extraction and Reverse Transcription

Total RNA was extracted from individual intestinal tissues using the RNeasy kit (Kim et al., 2010) according to the animal tissue protocol (Qiagen, Germantown, MD). Intestinal samples were removed from –80°C and placed on dry ice. A 20- to 30-mg aliquot of each sample was weighed, placed into a 2-mL microcentrifuge tube, and kept on dry ice until homogenization according to manufacturer's recommendation. Following extraction, RNA was eluted by rinsing the column membrane twice with 25 μL of RNase-free water. Total RNA concentration was determined at optical density (**OD**) 260 (NanoDrop-1000, Thermo Fisher Scientific, Waltham, MA), and RNA purity was verified by evaluating the ratio of OD 260 to OD 280. Total RNA was diluted to 0.2 μg/μL in nuclease-free water. Reverse transcription was accomplished using the high capacity cDNA Reverse Transcription kit (Applied Biosystems, Carlsbad, CA) following the manufacturer's protocol (Kim et al., 2010), and the cDNA was stored at –20°C.

Quantitative Real-Time PCR

Quantitative real-time PCR (**qRT-PCR**) was performed using an ABI 7500 Fast Real-Time PCR System (Applied Biosystems). The cDNA was diluted 1:30 in nuclease-free water, and 1 μL of the diluted cDNA was added to each well of a 96-well plate. Next, 9 μL of real-time PCR master mix containing 5 μL of Fast SYBR Green Master Mix (Applied Biosystems), 0.5 μL each of 2 μM forward and reverse primers, and 3 μL of sterile nuclease-free water per reaction were added to each well for a final volume of 10 μL. During the PCR reaction, samples were subjected to an initial denaturation phase at 95°C for 20 s followed by 40 cycles of denaturation at 95°C for 3 s and annealing and extension at 60°C for 30 s. Gene expression for IL-4, IL-8, IL-13, IL-18, interferon (**IFN**)-γ, inducible nitric oxide synthase (**iNOS**), mucin-1 (**Muc-1**), and mucin-2 (**Muc-2**) was analyzed using glyceraldehyde-3-phosphate dehydrogenase (**GAPDH**) as an endogenous control. Each reaction was run in duplicate. Primers were designed (Table 1) using the Primer Express 3.0 software (Applied Biosystems) and synthesized by MWG Operon (Huntsville, AL).

Quantitative Real-Time PCR Analysis

Results from qRT-PCR were analyzed using the 7500 Real-Time PCR software (Applied Biosystems). Average gene expression relative to the GAPDH endogenous control for each sample was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). The calibrator for each gene was the average ΔCt value from the duodenum of the dietary control group on d 4 (first sampling).

Statistical Analysis

Data were analyzed using the PROC GLIMMIX procedure of SAS (SAS Institute Inc., Cary, NC). For performance data, the model included dietary treatment, challenge, and age, and all possible interactions as fixed effects with pen representing the experimental unit. For analysis of gene expression data, intestinal segment was also placed in the model. Following ANOVA, differences in experimental treatments were compared by performing slices within significant effects followed by the adjustment of *P*-values using Tukey's. Values were considered statistically different at $P < 0.05$. Results are reported as least squares (**LS**) means.

RESULTS

Performance Parameters

Following the *Eimeria* infection, age and challenge presented a 2-way interaction ($P < 0.0001$) with the challenged birds having an expectedly decreased BW compared with the nonchallenged birds on d 14 and 21 (data not shown). The *Eimeria* challenge also resulted in decreased BWG regardless of dietary treatment from d 10 to 21 ($P < 0.0001$; Table 2). Dietary treatment had no effect on BW or BWG following the *Eimeria* infection.

β-Glucan supplementation did not influence FI or FCR at any time point during the study. *Eimeria*-chal-

Table 1. Primers used for relative real-time PCR[1]

Target[2]	Accession no.	Nucleotide sequence (5′ → 3′)
GAPDH[3]_F	NM_204305	CCTAGGATACACAGAGGACCAGGTT
GAPDH_R		GGTGGAGGAATGGCTGTCA
IL[4]-4_F	NM_001007079	GCTCTCAGTGCCGCTGATG
IL-4_R		GAAACCTCTCCCTGGATGTCAT
IL-8_F	NM_205498	TCCTGGTTTCAGCTGCTCTGT
IL-8_R		CGCAGCTCATTCCCCATCT
IL-13_F	NM_001007085	CATGACCGACTGCAAGAAGGA
IL-13_R		CCGTGCAGGCTCTTCAGACT
IL-18_F	NM_204608	AGGTGAAATCTGGCAGTGGAAT
IL-18_R		TGAAGGCGCGGTGGTTT
IFN[5]-γ_F	NM_205149	GCTCCCGATGAACGACTTGA
IFN-γ_R		TGTAAGATGCTGAAGAGTTCATTCG
iNOS[6]_F	D85422	CCTGTACTGAAGGTGGCTATTGG
iNOS_R		AGGCCTGTGAGAGTGTGCAA
Muc[7]-1_F	XM_430395	CAGAGATGTGGTGGCAAAAGC
Muc-1_R		CCCTTATCACCACTTGCAGGAA
Muc-2_F	XM_421035	TTCATGATGCCTGCTCTTGTG
Muc-2_F		CCTGAGCCTTGGTACATTCTTGT

[1]Primers designed by Primer Express software (Applied Biosystems, Foster City, CA).

[2]F = forward, R = reverse.

[3]Glyceraldehyde-3-phosphate dehydrogenase.

[4]IL = interleukin.

[5]INF = interferon.

[6]iNOS = inducible nitric oxide synthase.

[7]Muc = mucin.

lenged birds resulted in a decrease in FI ($P < 0.0001$) and increase in FCR ($P = 0.005$; Table 2). When compared with the nonchallenged controls, mortality was increased due to the *Eimeria* challenge ($P < 0.0001$) with no observed effect of β-glucan on mortality (data not shown).

Coccidial Lesion Scores

Dietary treatment reduced lesion scores in the duodenum ($P = 0.04$) and jejunum ($P = 0.02$) of *Eimeria*-infected chicks on d 14. Gross lesion severity was significantly reduced in the duodenum and jejunum of birds supplemented with 0.1% YGT when compared with controls (Figure 1). Although a similar trend was observed, there were no significant differences among dietary treatment groups in the ceca ($P = 0.18$).

Intestinal Gene Expression

Dietary β-glucan supplementation did not alter any of the immune-related genes on d 4, and thus those data are not reported. Following the *Eimeria* challenge, IL-8 expression was not affected by dietary treatment on any day in any intestinal section. Age and challenge demonstrated a 2-way interaction on IL-8 expression ($P = 0.03$). On d 14, the *Eimeria*-challenged birds had higher IL-8 expression than the nonchallenged birds ($P = 0.004$). There were no differences in IL-8 expression among the challenged and nonchallenged birds on d 10 or 21.

Age, intestinal segment, diet, and challenge presented a 4-way interaction for iNOS gene expression from d 10 to 21 ($P < 0.0001$; Figures 2a,b,c). There were no differences observed among groups in the duodenum at any time point (Figure 2a). On d 10, iNOS expression was downregulated in the jejunum of challenged birds fed 0.1% YGT when compared with the challenged birds fed the control diet (Figure 2b). On d 14, expression of iNOS was downregulated in the ileum of nonchallenged birds fed the 0.1% YGT diet when compared with the nonchallenged, 0.0% YGT-fed birds ($P = 0.02$). In the challenged birds, however, iNOS expression was upregulated in the ileum of 0.1% YGT-fed birds when compared with the controls ($P = 0.001$; Figure 2c). A similar trend was seen in the jejunum though this was not found to be significant. There were no differences in iNOS gene expression seen among groups on d 21 (Figure 2a,b,c).

Intestinal section and dietary treatment produced a 2-way interaction for IL-18 expression from d 10 to 21 ($P = 0.005$; Figure 3). Interleukin-18 expression was upregulated due to β-glucan supplementation in the jejunum when compared with the control-fed birds ($P = 0.004$). Dietary treatment did not modulate IL-18 expression in the duodenum or ileum. Though not statistically significant, there was a strong trend for a diet × challenge interaction where IL-18 expression tended to

Table 2. Interactive effects of dietary β-glucan treatment and *Eimeria* challenge on BW gain, feed intake, and feed conversion ratios from d 10 to 21

	BW gain (g)				Feed intake (g/bird)				Feed conversion ratio			
Diet[1]	Not Chall[2]	Chall[3]*	SEM	*P*-value	Not Chall	Chall†	SEM	*P*-value	Not Chall	Chall‡	SEM	*P*-value
0.0% YGT	575.5	398.4	±25.7	0.78	847.9	657.5	±37.8	0.73	1.47	1.66	±0.12	0.85
0.02% YGT	565.2	367.3			796.0	611.0			1.41	1.70		
0.1% YGT	574.3	383.5			820.8	687.5			1.43	1.80		

[1]YGT = Auxoferm YGT, *Saccharomyces cerevisiae*-derived β-glucan.
[2]Not challenged.
[3]Challenged with *Eimeria*.
* $P < 0.0001$; † $P < 0.0001$; ‡ $P = 0.005$.

be upregulated in the challenged birds due to β-glucan exposure ($P = 0.07$; data not shown).

Intestinal segment, dietary treatment and *Eimeria* challenge resulted in a 3-way interaction for IFN-γ expression ($P = 0.002$; Figure 4). Interferon-γ expression was decreased in the jejunum and ileum due to dietary supplementation in the nonchallenged birds ($P = 0.003$ and $P = 0.04$ respectively). Interferon-γ was also downregulated in the duodenum and ileum of challenged birds fed 0.1% YGT when compared with the controls ($P = 0.006$ and $P = 0.04$, respectively). A similar trend was seen in the jejunum, but this was not significant. Age and challenge also demonstrated a 2-way interaction with IFN-γ expression being greater in the *Eimeria*-challenged group than the nonchallenged group on d 14 ($P < 0.001$).

After administration of the *Eimeria* oocysts, dietary treatment and challenge presented a 2-way interaction in the intestine for IL-4 expression from d 10 to 21 ($P = 0.005$) where nonchallenged birds fed 0.1% β-glucan had a decreased level of IL-4 expression when compared with the nonchallenged, control-fed birds ($P = 0.0002$; Figure 5). There was a main effect of dietary treatment on IL-13 expression ($P = 0.03$). Birds fed 0.1% YGT had less intestinal IL-13 expression than the control-fed birds (fold change of 1 for 0.0% YGT vs. 0.75 for 0.1% YGT). There was also a 2-way interaction of age and challenge for IL-13 expression ($P = 0.003$). Interleukin-13 expression was significantly upregulated due to the *Eimeria* challenge on d 14 and 21 ($P = 0.0001$ and $P = 0.03$, respectively).

Following the *Eimeria* challenge, there was main effect of dietary treatment on intestinal Muc-1 expression where levels were significantly downregulated in the 0.1% YGT-fed birds (fold change of 1 for 0% YGT vs. 0.72 for 0.1% YGT; $P = 0.008$). Age and challenge also produced a 2-way interaction for Muc-1 expression ($P = 0.004$). A host response to the challenge was observed on d 14 and 21 where infected birds had significantly greater Muc-1 expression than the nonchallenged birds ($P = 0.002$ and $P = 0.01$, respectively). There were no differences based on challenge observed on d 10.

Age, dietary treatment, challenge, and intestinal section presented a 4-way interaction for Muc-2 expression ($P = 0.04$; Figures 6a,b,c). Differences among groups were not discerned in the duodenum or the ileum at any time point (Figures 6a and 6c, respectively). On d 14, Muc-2 expression was upregulated in the nonchallenged, 0.1% YGT-fed birds when compared with the nonchallenged, control-fed birds ($P = 0.02$). In the jejunum of the challenged birds, however, Muc-2 levels were decreased in the 0.1% YGT-fed birds ($P = 0.03$). On d 21, Muc-2 was upregulated in the jejunum of challenged birds fed 0.1% YGT when compared with the challenged controls ($P = 0.02$; Figure 6b). A similar trend was seen on d 10, but this was not found to be significant.

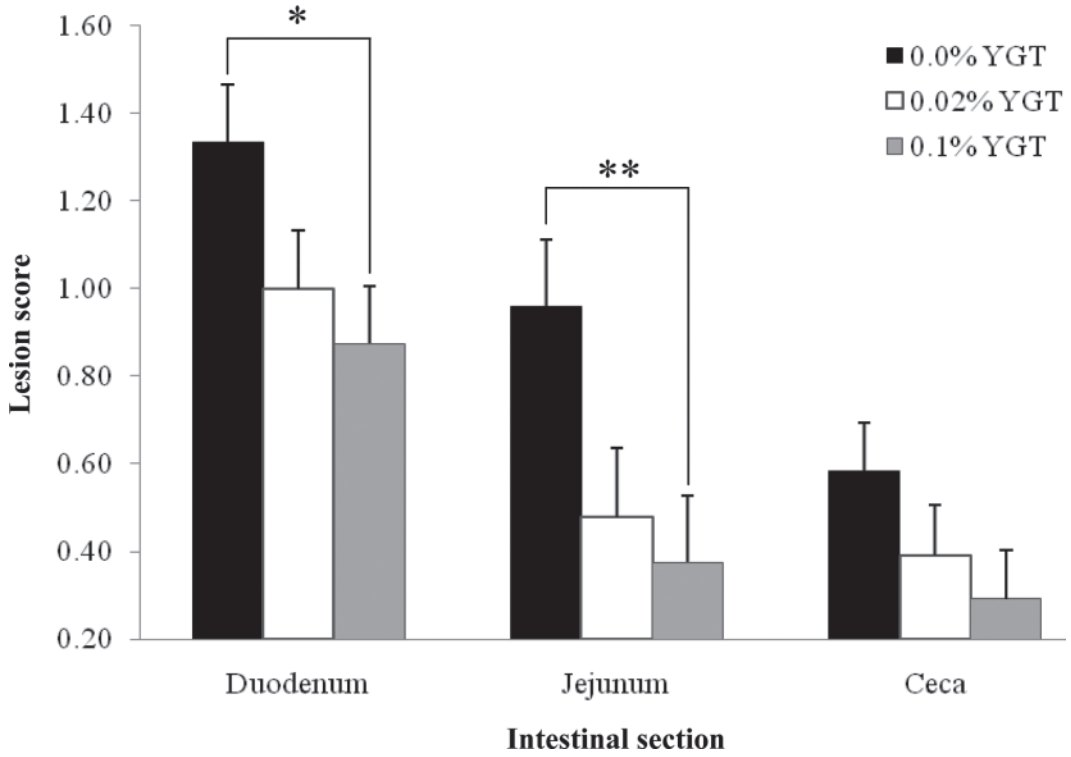

Figure 1. Effect of β-glucan supplementation on gross intestinal lesions scores of Cobb 500 broiler chicks on d 14 (6 d post *Eimeria* infection). Data are represented as least squares means + SEM. YGT = Auxoferm YGT, *Saccharomyces cerevisiae*-derived β-glucan. There was a significant effect of dietary treatment in the duodenum (*$P = 0.04$) and jejunum (**$P = 0.02$).

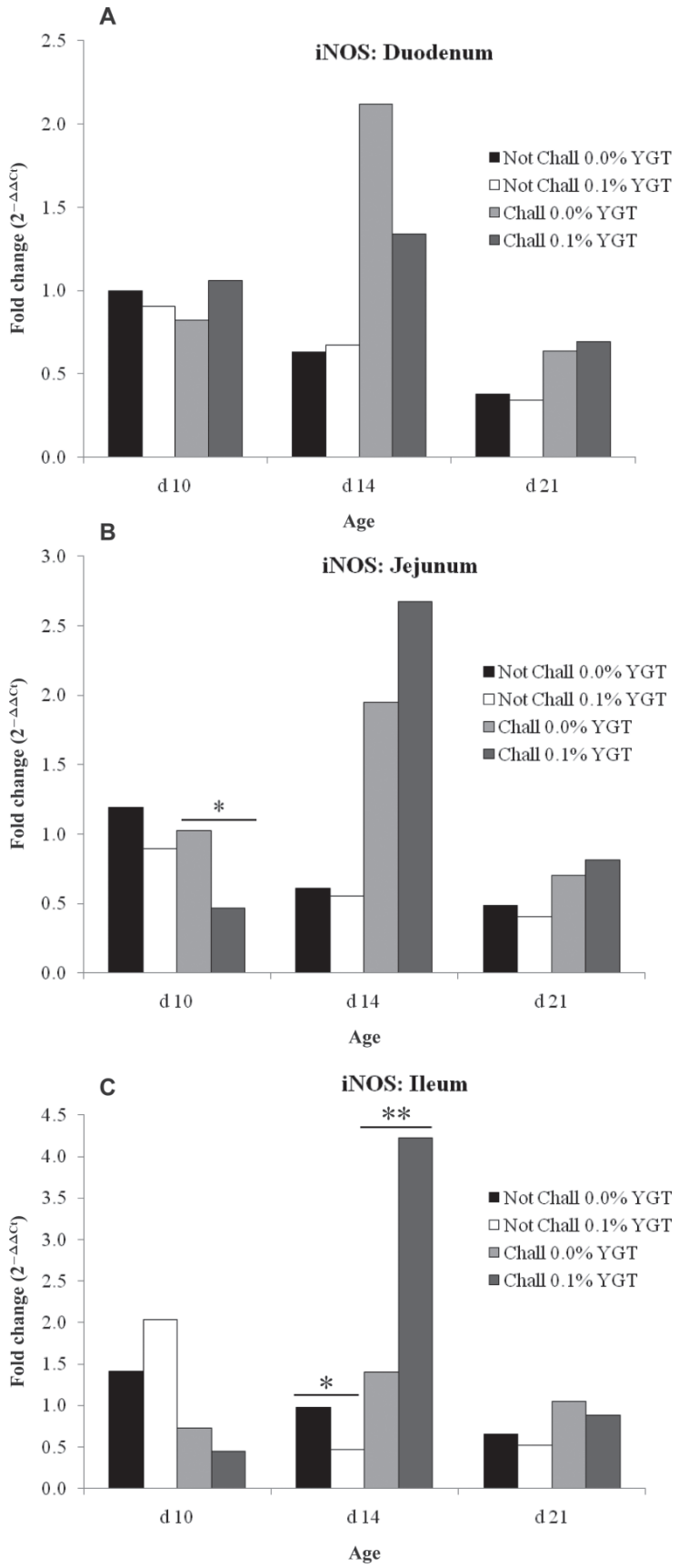

Figure 2. Effect of age, β-glucan supplementation, and *Eimeria* challenge on relative inducible nitric oxide synthase (iNOS) expression in the duodenum (A), jejunum (B), and ileum (C) of Cobb 500 broiler chicks from d 10 to 21. Relative gene expression ($2^{-\Delta\Delta Ct}$) was calculated using the ΔΔCt method with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the endogenous control and the average ΔCt value for the duodenum of the nonchallenged, 0.0% YGT-fed birds on d 10 as the calibrator. Data are represented as least squares means. Not Chall = not challenged; Chall = challenged with *Eimeria*; YGT = Auxoferm YGT, *Saccharomyces cerevisiae*-derived β-glucan. There was a significant 4-way interaction ($P < 0.0001$) of age, dietary treatment, challenge, and intestinal section. Panel B: $^{*}P = 0.01$; panel C: $^{*}P = 0.02$, $^{**}P = 0.001$.

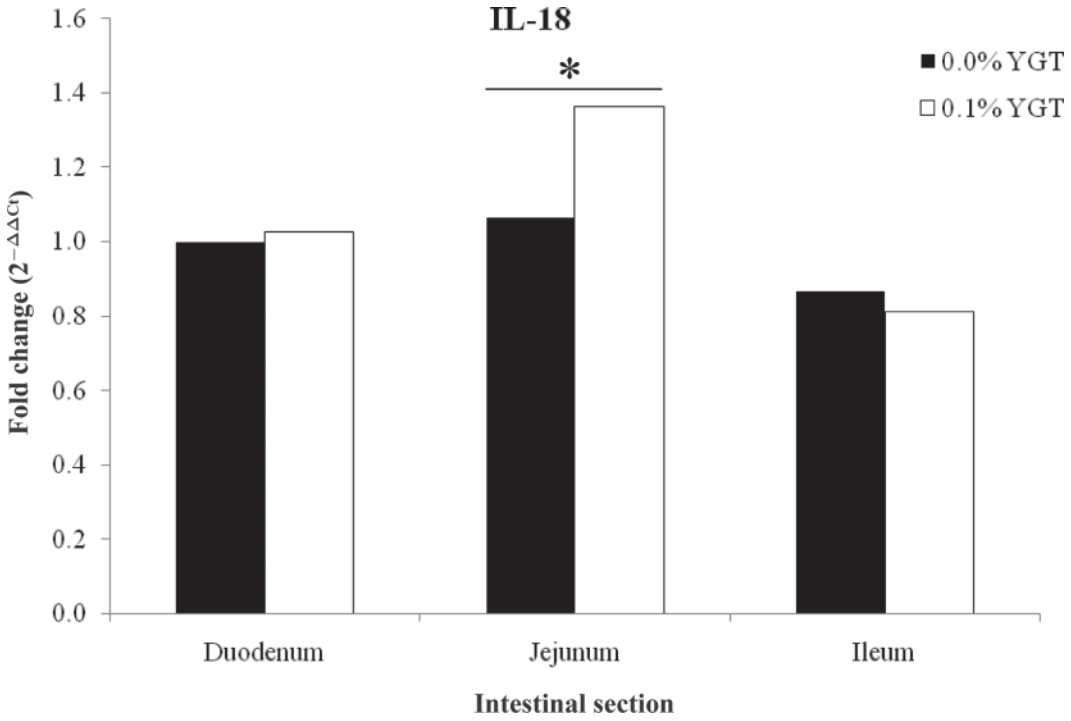

Figure 3. Effect of β-glucan supplementation and intestinal section on relative IL-18 expression in the small intestine of Cobb 500 broiler chicks from d 10 to 21. Relative gene expression ($2^{-\Delta\Delta Ct}$) was calculated using the ΔΔCt method with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the endogenous control and the average ΔCt value for the duodenum of the 0.0% YGT-fed birds as the calibrator. Data are represented as least squares means. YGT = Auxoferm YGT, *Saccharomyces cerevisiae*-derived β-glucan. There was a significant 2-way interaction ($P = 0.005$) of diet and intestinal section. *$P = 0.004$. IL = interleukin.

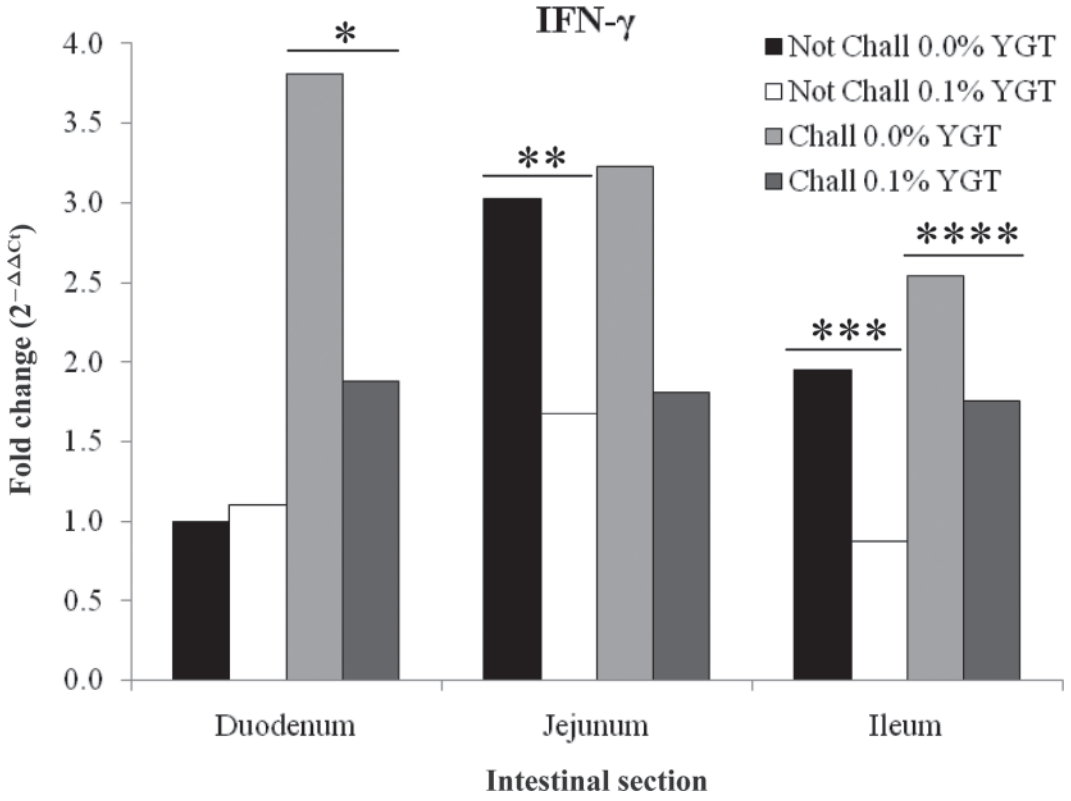

Figure 4. Effect of β-glucan supplementation, *Eimeria* challenge, and intestinal section on relative interferon (IFN)-γ expression in the small intestine of Cobb 500 broiler chicks from d 10 to 21. Relative gene expression ($2^{-\Delta\Delta Ct}$) was calculated using the ΔΔCt method with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the endogenous control and the average ΔCt value for the duodenum of the nonchallenged, 0.0% YGT-fed birds as the calibrator. Data are represented as least squares means. Not Chall = not challenged; Chall = challenged with *Eimeria*; YGT = Auxoferm YGT, *Saccharomyces cerevisiae*-derived β-glucan. There was a significant 3-way interaction ($P = 0.002$) of diet, challenge, and intestinal section. *$P = 0.006$, **$P = 0.003$, ***$P = 0.04$, ****$P = 0.04$.

DISCUSSION

Although 1,3/1,6-β-D-glucans are well known to activate the innate and adaptive immune systems of mammals, their effect on the avian immune system still remains elusive. In this study, the influence of dietary β-glucan supplementation on broiler chicks with and without an *Eimeria* challenge was investigated. In terms of performance, no significant differences were found among treatment groups. These data confirm our previous work where β-glucan supplementation did not affect performance (Cox et al., 2010). Zhang et al. (2008) found that β-glucans derived from the yeast *S. cerevisiae* significantly increased BW and BWG of broilers at 50 and 75 mg/kg in the diet. In a comparable study, Rathgeber et al. (2008) observed enhanced BW in broiler chicks during the grower phase of production. Conversely, Huff et al. (2006) reported a decrease in performance after β-glucan supplementation in chickens. These results could be due to reallocation of energy toward immune development resulting in inefficient nutrient utilization for growth. Huff et al. (2006) found, however, that broiler chicks fed β-glucan for 7 d before an *E. coli* infection had higher BW than challenged birds fed a control diet. Correlating with our findings, several studies have noted no significant effects of β-glucans on growth performance, suggesting that β-glucans do not negatively affect performance in nonchallenge (Cheng et al., 2004; Chae et al., 2006; Morales-López et al., 2009) or challenge settings (Chen et al., 2006, 2008). The variable results of those studies could be due to a variety of reasons such as differences in the source and preparation of the β-glucan or the presence and type of challenge utilized. Further research is required to define the optimal source and dosage of β-glucans to achieve consistently favorable results in poultry.

The reduction in BW, BWG, and FI, and increase in FCR and mortality due to the *Eimeria* inoculation were not surprising because coccidial infections are known to cause disruption to the intestinal mucosa, resulting in nutrient malabsorption and reduced performance (Dalloul and Lillehoj, 2005). Furthermore, parasitic infections elicit nutrient-demanding immune responses, which may have also contributed to the impaired growth. Coccidia parasites are known to cause substantial intestinal damage as they destroy enterocytes during the progression of their life cycle (Allen and Fetterer, 2002). Even though the *Eimeria* challenge only produced a mild infection (based on lesion scores), dietary β-glucan supplementation at 0.1% reduced lesion severity in the duodenum and jejunum. Though not statistically significant, a similar trend was observed in the ceca. These results indicate that dietary β-glucan shows immunoprotective properties and can enhance host defenses against *E. acervulina* and *E. maxima* in the duodenum and jejunum, respectively.

Numerous cytokines are involved in directing innate and adaptive immunity. When exposed to antigens or chemotactic agents, macrophages will begin to produce iNOS. This enzyme leads to the production of nitric oxide, which will subsequently react with superoxide anions to generate toxic derivatives, allowing macrophages to proficiently kill several types of pathogens (Tizard, 2009). On d 14, iNOS expression in the ileum was downregulated in the nonchallenged birds supplemented with β-glucan. In the challenged birds, however, iNOS was enhanced in 0.1% YGT-fed birds. Jejunal iNOS expression was downregulated in the challenged birds on d 10. However, at d 10 the *Eimeria* infection may not have been fully established in the gut, thus causing the challenged birds to follow a similar pattern as the nonchallenged birds. These results suggest that in the absence of a challenge, β-glucans possess an anti-inflammatory function, but in the presence of an intracellular pathogen, such as coccidia, they are capable of enhancing the innate immune response. These results are supported by mammalian studies where β-glucan exposure augmented iNOS expression and nitric oxide production (Ljungman et al., 1998; Mucksová et al., 2001). The data presented by this study demonstrate the ability of β-glucans to induce the expression of iNOS mRNA, thus implying subsequent nitric oxide production. Because nitric oxide is a major mediator of the nonspecific immune response against a wide scope of invading microbes, the ability to stimulate nitric oxide production is important for host defense (Ljungman et al., 1998).

Interleukin-18 is a pro-inflammatory cytokine that is also primarily produced by macrophages. Interleukin-18 works in connection with IL-12 to induce a cell-mediat-

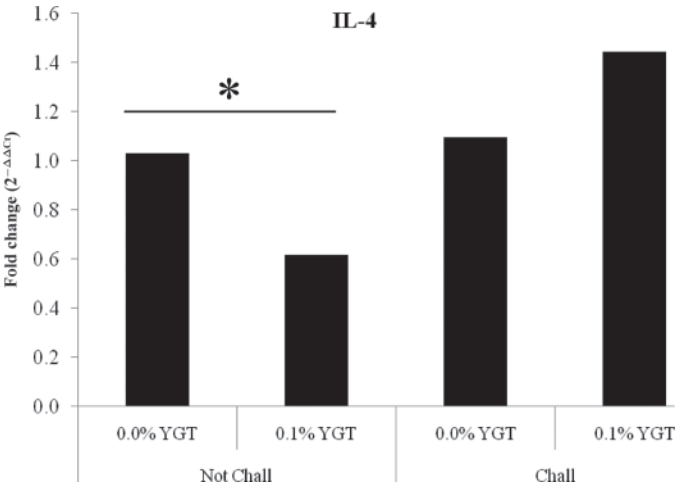

Figure 5. Effect of β-glucan supplementation and *Eimeria* challenge on relative IL-4 expression in the small intestine of Cobb 500 broiler chicks from d 10 to 21. Relative gene expression ($2^{-\Delta\Delta Ct}$) was calculated using the ΔΔCt method with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the endogenous control and the average ΔCt value for the nonchallenged, 0.0% YGT-fed birds as the calibrator. Data are represented as least squares means. YGT = Auxoferm YGT, *Saccharomyces cerevisiae*-derived β-glucan; Not Chall = not challenged; Chall = challenged with *Eimeria*. There was a significant 2-way interaction ($P = 0.005$) of dietary treatment and challenge. *$P = 0.0002$.

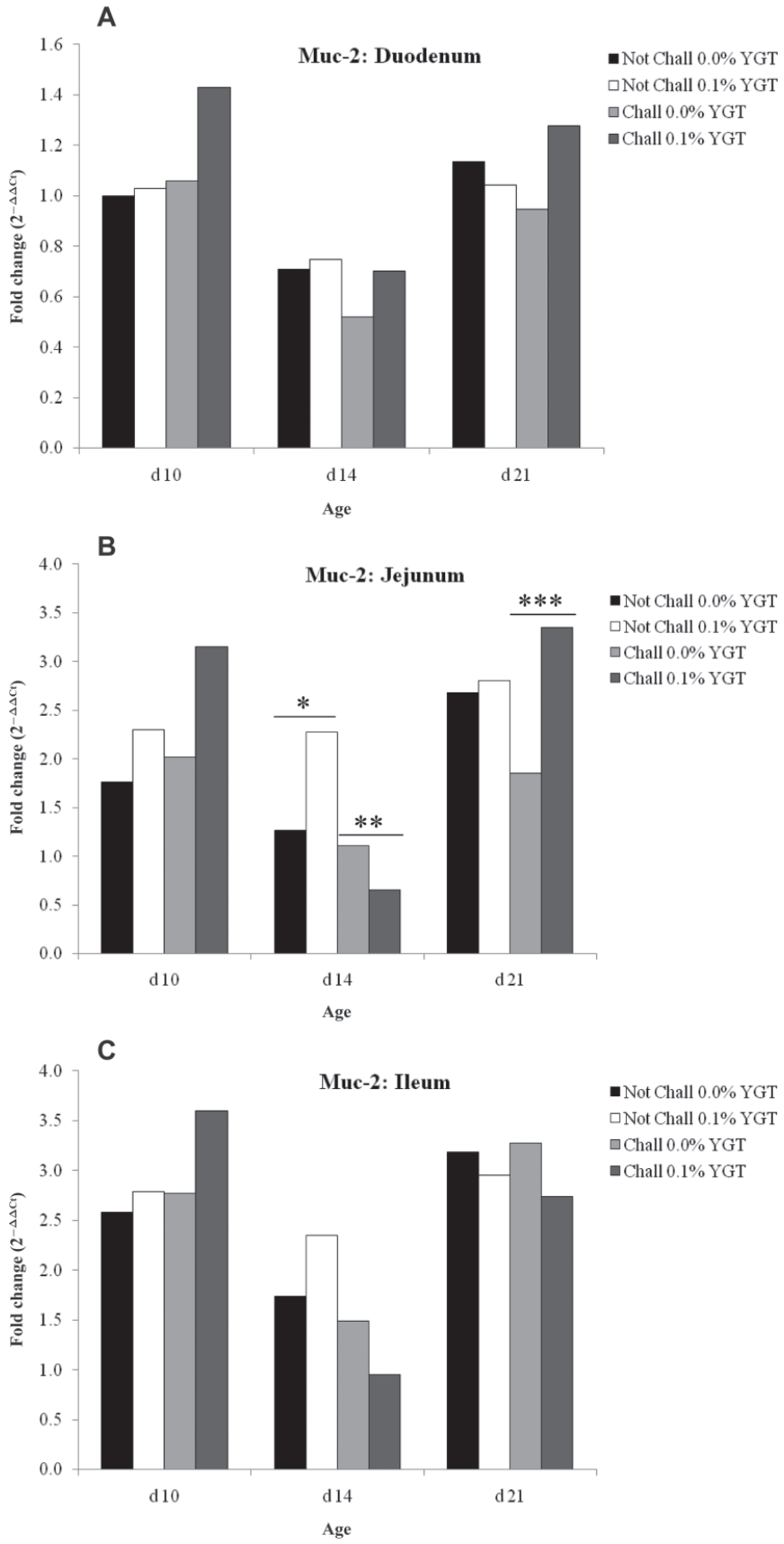

Figure 6. Effect of age, β-glucan supplementation, and *Eimeria* challenge on relative mucin (Muc)-2 expression in the duodenum (A), jejunum (B), and ileum (C) of Cobb 500 broiler chicks from d 10 to 21. Relative gene expression ($2^{-\Delta\Delta Ct}$) was calculated using the ΔΔCt method with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the endogenous control and the average ΔCt value for the duodenum of the nonchallenged, 0.0% YGT-fed birds on d 10 as the calibrator. Data are represented as least squares means. Not Chall = no challenge; Chall = challenged with *Eimeria*; YGT = Auxoferm YGT, *Saccharomyces cerevisiae*-derived β-glucan. There was a significant 4-way interaction ($P = 0.04$) of age, diet, challenge, and intestinal section. Panel B: $^{*}P = 0.02$, $^{**}P = 0.03$, $^{***}P = 0.02$. IL = interleukin.

ed immune response following exposure to a pathogen. Its major targets are T helper type-1 (**Th1**) cells that subsequently secrete IFN-γ, which plays an essential role in activating macrophages (Göbel et al., 2003). We found that IL-18 expression was enhanced in the presence of Auxoferm YGT in the jejunum. When looking at IFN-γ, β-glucan supplementation reduced IFN-γ expression levels in challenged and nonchallenged birds. These results support our previous findings in which IFN-γ expression was downregulated due to β-glucan exposure in 7-d-old chicks (Cox et al., 2010). This cytokine is secreted by several cells including Th1 and cytotoxic T lymphocytes and is responsible for activating and enhancing antigen presentation by macrophages (Tizard, 2009).

Interleukin-4, a representative of T helper type-2 (**Th2**) cytokines, plays a key role in the stimulation of B lymphocytes, T lymphocyte proliferation and the differentiation of CD4+ T cells into Th2 cells (Fietta and Delsante, 2009). During this study, IL-4 expression was downregulated in nonchallenged birds supplemented with 0.1% β-glucan. The functions of IL-13, also characterized as a Th2 cytokine, overlap considerably with those of IL-4. Like IL-4, IL-13 expression also decreased due to β-glucan supplementation. Taken together, the downregulation of these 2 cytokines implies that β-glucan supplementation does not support a Th2 cell-mediated response.

Mucin-1 and Mucin-2 are 2 genes responsible for encoding mucin production. Mucin is made up of glycoproteins and serves a protective function by binding to pathogens, preventing their adhesion to the intestinal surface. The T lymphocytes and Th2 cytokines are mediators shown to promote mucin production by upregulating mucin gene expression (Beum et al., 2005). The downregulation in Muc-1 and Muc-2 expression seen following the *Eimeria* challenge due to β-glucan supplementation implies a waning Th2 response, further suggesting a bias of β-glucan modulation toward a Th1-mediated response. In absence of the challenge, however, β-glucan supplementation appeared to upregulate mucin production and, thus, may offer some additional protection from invading pathogens.

The small intestine is not only part of the digestive system, but it is one of the most important organs of the immune system. In this study, we demonstrate the ability of yeast-derived β-glucan to modulate the Th1/Th2 balance within the small intestine. The addition of β-glucan to the diet of broiler chicks subjected to an *Eimeria* infection enhanced the host's innate and Th1-mediated immune responses and consequently downregulated the Th2-mediated immunity. This response to β-glucan exposure has also been reported in human and mice studies (Kirmaz et al., 2005; Baran et al., 2007). The augmented pro-inflammatory response enhanced protection against the challenge as seen by the decreased lesion scores. The overall peak in immune response seen on d 14 and decline by d 21 further suggests that β-glucan supplementation does not result in a chronic inflammatory response, but instead primes the immune system and even provides an anti-inflammatory effect. Moreover, the addition of β-glucan showed no signs of negatively affecting performance parameters, further suggesting that the immune system was not stimulated enough to be detrimental to growth.

In conclusion, our data suggest that dietary inclusion of yeast-derived β-glucan does not affect performance. When looking at the immune response, β-glucans modulate cytokine profiles, resulting in an enhanced innate and Th1-mediated immune response during an *Eimeria* infection. β-Glucan acts as an immunoprotective agent by upregulating the inflammatory response leading to enhanced protection against intracellular pathogens. Because the present study was carried out to 3 wk of age, it remains to be seen if the beneficial effects of dietary β-glucan supplementation would be more pronounced as the birds grow to market age. Also, this work involved an intracellular pathogen as a challenge model, and it would be interesting to monitor the Th1/Th2 balance in response to extracellular pathogens. To our knowledge, this is the first study to evaluate the effects of dietary β-glucan on disease status and immune-related gene expression during an *Eimeria* challenge in poultry, thus providing new evidence for the potential use of β-glucans for the prevention or amelioration of coccidial infections.

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REFERENCES

Allen, P. C., and R. H. Fetterer. 2002. Recent advances in biology and immunobiology of *Eimeria* species and in diagnosis and control of infection with these coccidian parasites of poultry. Clin. Microbiol. Rev. 15:58–65.

Baran, J., D. J. Allendorf, F. Hong, and G. D. Ross. 2007. Oral beta-glucan adjuvant therapy converts nonprotective Th2 response to protective Th1 cell-mediated immune response in mammary tumor-bearing mice. Folia Histochem. Cytobiol. 45:107–114.

Beum, P. V., H. Basma, D. R. Bastola, and P. W. Cheng. 2005. Mucin biosynthesis: Upregulation of core 2 beta 1,6 *N*-acetylglucosaminyltransferase by retinoic acid and Th2 cytokines in a human airway epithelial cell line. Am. J. Physiol. Lung Cell. Mol. Physiol. 288:L116–L124.

Brake, D. A., G. Strang, J. E. Lineberger, C. H. Fedor, R. Clare, T. A. Banas, and T. Miller. 1997. Immunogenic characterization of a tissue culture-derived vaccine that affords partial protection against avian coccidiosis. Poult. Sci. 76:974–983.

Castanon, J. I. 2007. History of the use of antibiotic as growth promoters in European poultry feeds. Poult. Sci. 86:2466–2471.

Chae, B. J., J. D. Lohakare, W. K. Moon, S. L. Lee, Y. H. Park, and T. W. Hahn. 2006. Effects of supplementation of beta-glucan on the growth performance and immunity in broilers. Res. Vet. Sci. 80:291–298.

Chen, H. L., D. F. Li, B. Y. Chang, L. M. Gong, X. S. Piao, G. F. Yi, and J. X. Zhang. 2003. Effects of lentinan on broiler spleno-

cyte proliferation, interleukin-2 production, and signal transduction. Poult. Sci. 82:760–766.
Chen, K. L., B. C. Weng, M. T. Chang, Y. H. Liao, T. T. Chen, and C. Chu. 2008. Direct enhancement of the phagocytic and bactericidal capability of abdominal macrophage of chicks by beta-1,3–1,6-glucan. Poult. Sci. 87:2242–2249.
Chen, T. T., S. M. Tsay, C. Y. Yu, B. C. Weng, and K. L. Chen. 2006. Effects of dietary β-glucan supplementation against *Eimeria tenella* infection and immune parameter in male leghorn chicks. Chin. Soc. Anim. Sci. 35:101–108.
Cheng, Y., D. Lee, C. Wen, and C. Weng. 2004. Effects of dietary supplementation on lymphocyte proliferation, macrophage chemotaxis and specific immune response in broilers. Asian-australas. J. Anim. Sci. 17:1145–1149.
Cox, C. M., L. H. Stuard, S. Kim, A. P. McElroy, M. R. Bedford, and R. A. Dalloul. 2010. Performancce and immune responses to dietary beta-glucan in broiler chicks. Poult. Sci. 89:1924–1933.
Dalloul, R. A., and H. S. Lillehoj. 2005. Recent advances in immunomodulation and vaccination strategies against coccidiosis. Avian Dis. 49:1–8.
Dalloul, R. A., and H. S. Lillehoj. 2006. Poultry coccidiosis: recent advancements in control measures and vaccine development. Expert Rev. Vaccines 5:143–163.
Fietta, P., and G. Delsante. 2009. The effector T helper cell triade. Riv. Biol. 102:61–74.
Göbel, T. W., K. Schneider, B. Schaerer, I. Mejri, F. Puehler, S. Weigend, P. Staeheli, and B. Kaspers. 2003. IL-18 stimulates the proliferation and IFN-gamma release of CD4+ T cells in the chicken: Conservation of a Th1-like system in a non mammalian species. J. Immunol. 171:1809–1815.
Guo, Y., R. A. Ali, and M. A. Qureshi. 2003. The influence of beta-glucan on immune responses in broiler chicks. Immunopharmacol. Immunotoxicol. 25:461–472.
Harada, T., and N. Ohno. 2008. Contribution of dectin-1 and granulocyte macrophage-colony stimulating factor (GM-CSF) to immunomodulating actions of beta-glucan. Int. Immunopharmacol. 8:556–566.
Huff, G. R., W. E. Huff, M. B. Farnell, N. C. Rath, F. Solis de Los Santos, and A. M. Donoghue. 2010. Bacterial clearance, heterophil function, and hematological parameters of transport-stressed turkey poults supplemented with dietary yeast extract. Poult. Sci. 89:447–456.
Huff, G. R., W. E. Huff, N. C. Rath, and G. Tellez. 2006. Limited treatment with beta-1,3/1,6-glucan improves production values of broiler chickens challenged with *Escherichia coli*. Poult. Sci. 85:613–618.
Johnson, J., and W. M. Reid. 1970. Anticoccidial drugs: Lesion scoring techniques in battery and floor-pen experiments with chickens. Exp. Parasitol. 28:30–36.
Kim, S., K. B. Miska, M. C. Jenkins, R. H. Fetterer, C. M. Cox, L. H. Stuard, and R. A. Dalloul. 2010. Molecular cloning and functional characterization of the avian macrophage migration inhibitory factor (MIF). Dev. Comp. Immunol. 34:1021–1032.
Kirmaz, C., P. Bayrak, O. Yilmaz, and H. Yuksel. 2005. Effects of glucan treatment on the Th1/Th2 balance in patients with allergic rhinitis: A double-blind placebo-controlled study. Eur. Cytokine Netw. 16:128–134.
Livak, K. J., and T. D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-delta delta C(T)) method. Methods 25:402–408.
Ljungman, A. G., P. Leanderson, and C. Tagesson. 1998. (1–3)-b-D-Glucan stimulates nitric oxide generation and cytokine mRNA expression in macrophages. Environ. Toxicol. Pharmacol. 5:273–281.
Lowry, V. K., M. B. Farnell, P. J. Ferro, C. L. Swaggerty, A. Bahl, and M. H. Kogut. 2005. Purified beta-glucan as an abiotic feed additive up-regulates the innate immune response in immature chickens against *Salmonella enterica* serovar Enteritidis. Int. J. Food Microbiol. 98:309–318.
Morales-López, R., E. Auclair, F. Garcia, E. Esteve-Garcia, and J. Brufau. 2009. Use of yeast cell walls; beta-1,3/1,6-glucans; and mannoproteins in broiler chicken diets. Poult. Sci. 88:601–607.
Mucksová, J., K. Babicek, and M. Pospisil. 2001. Particulate 1,3-beta-D-glucan, carboxymethylglucan and sulfoethylglucan—Influence of their oral or intraperitoneal administration on immunological respondence of mice. Folia Microbiol. (Praha) 46:559–563.
National Research Council. 1994. Nutrient Requirements of Poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.
Rathgeber, B. M., K. L. Budgell, J. L. MacIsaac, M. A. Mirza, and K. L. Doncaster. 2008. Growth performance and spleen and bursa weight of broilers fed yeast beta-glucan. Can. J. Anim. Sci. 88:469–473.
Tizard, I. R. 2009. Veterinary Immunology: An Introduction. 8th ed. Saunders Elsevier, St. Louis, MO.
Vetvicka, V., and J. Vetvickova. 2007. Physiological effects of different types of beta-glucan. Biomed. Pap. Med. Fac. Univ. Palacky Olomouc Czech Repub. 151:225–231.
Volman, J. J., J. D. Ramakers, and J. Plat. 2008. Dietary modulation of immune function by beta-glucans. Physiol. Behav. 94:276–284.
Zhang, B., Y. Guo, and Z. Wang. 2008. The modulating effect of β-1,3/1,6-glucan supplementation in the diet on performance and immunological responses of broiler chickens. Asian-australas. J. Anim. Sci. 21:237–244.