Effect of yeast cell product (CitriStim) supplementation on broiler performance and intestinal immune cell parameters during an experimental coccidial infection[1]

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ABSTRACT This experiment studied the effects of whole yeast cell product supplementation on broiler production parameters, fecal coccidial oocyst counts, and local and systemic immune parameters following an experimental coccidial infection. Birds were fed 0, 0.1, or 0.2% whole yeast cell product (CitriStim). At 21 d of age, birds were challenged with live coccidial oocysts. Supplementation with whole yeast cell product increased BW gain between 0 and 12 d ($P = 0.01$) postcoccidial challenge. Birds supplemented with 0.2% Citristim had better ($P = 0.01$) feed efficiency between 0 and 12 d postcoccidial infection. Supplementation with whole yeast cell product decreased ($P = 0.01$) the fecal coccidial oocyst count at 7 d postcoccidial challenge. Citristim supplementation at 0.2% increased ($P < 0.01$) macrophage nitric oxide production by 93 and 193% at 5 and 12 d postcoccidial challenge. Supplementation with whole yeast cell product at 0.2% increased cecal tonsil interleukin-1 mRNA amounts approximately 4.5- and 3.7-fold at 5 and 12 d postcoccidial challenge, respectively, over the group with no whole yeast cell product supplementation. Citristim supplementation downregulated cecal tonsil interleukin-10 mRNA amounts compared with the unsupplemented groups at both 5 ($P = 0.01$) and 12 d ($P < 0.01$) postcoccidial challenge. Supplementation with whole yeast cell product did not alter ($P > 0.05$) serum anticoccidial IgG contents or cecal tonsil $CD4^+$ and $CD8^+$ cell percentages at 5 and 12 d postcoccidial infection. It could be concluded that supplementing whole yeast cell product (CitriStim) to broiler diets can improve production parameters, decrease fecal oocyst count, and increase inflammatory cytokine production postcoccidial infection.

Key words: prebiotic, whole yeast cell, CitriStim, immunity, coccidiosis, gut health

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INTRODUCTION

Whole yeast cell products are derived from several species of yeast such as *Saccharomyces cerevisiae* or *Pichia guilliermondii*. Most yeast cell products are rich in mannan oligosaccharides, β-glucan, D-mannose, α-methyl-D-mannoside, and several other compounds (Shanmugasundaram and Selvaraj, 2012a). Many of the compounds have immunomodulatory effects and are supplemented either individually or as a mixture with other compounds as immune stimulators in several species including poultry. Immune stimulators such as β-glucan (Cox et al., 2010) and mannose oligosaccharides (Yitbarek et al., 2012) are promoted as alternatives in the poultry production industry to antibiotics or other chemotherapeutic agents.

Coccidiosis is a costly protozoal disease of the poultry industry. Although live coccidial vaccines decrease the number of incidences and severity of coccidiosis, live vaccines can decrease production performance (Al-Sheikhly and Al-Saieg, 1980). Identifying alternatives to anticoccidial ionophores or live vaccines that can improve production performance following coccidial infection will be beneficial to the poultry production industry. β-Glucans resemble pathogen-associated molecular patterns and can stimulate innate immunity. When β-glucans engage the pathogen-associated molecular pattern recognizing receptors, namely Dectin-1, lactosylceramide, scavenger receptors, and Toll-like receptors 2 and 6, in immune cells, the immune cells become more active in engulfing, killing, and digesting pathogens and secreting inflammatory cytokines such as interleukin (**IL**)-1 (Soltanian et al., 2009). Similarly, the prebiotic effects of mannose oligosaccharides reduce the number of gut pathogens and severity of infections (Yitbarek et al., 2012). In addition, several other components of whole yeast cell products such as D-mannose (Faber et al., 2012) have immune-stimulating effects.

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CitriStim is a commercial killed whole yeast cell (ADM, Quincy, IL) that is considered a good source of mannan oligosaccharides and β-glucans and contains a proprietary mixture of partially fermented yeast (*Pichia guilliermondii*) that is left following citric acid extraction from the yeast culture. The product provides the whole yeast cell and all its components. We previously studied the effect of CitriStim supplementation in the absence of any inflammatory challenge and identified that CitriStim supplementation upregulates IL-10 and decreases IL-1 mRNA in the cecal tonsils in the absence of any experimental inflammatory challenge (Shanmugasundaram and Selvaraj, 2012a). Decreased inflammatory cytokine mRNA might adversely affect the host's ability to fight infection, and hence the present studies were conducted to identify the effect of Citristim supplementation during an experimental intestinal coccidial infection. The present experiment was conducted to study the effects of whole yeast cell product supplementation on broiler production performance, jejunal villi height and crypt depth, splenic macrophage nitric oxide production, spleen and cecal tonsil IL-1 and IL-10 mRNA amounts, spleen and cecal tonsil $CD4^+$ and $CD8^+$ cell percentages, and fecal oocyst count following an experimental coccidial infection in broiler birds unvaccinated against coccidia.

MATERIALS AND METHODS

Two experiments were conducted to study the effect of yeast product supplementation on production parameters and intestinal immune parameters during an experimental coccidial infection model. All animal protocols were approved by the Institutional Animal Care and Use Committee at The Ohio State University.

Experiment I

Birds. A total of ninety 1-d-old chicks (Ross × Ross; Orville Chick Hatchery, Orville, OH) were randomly distributed to 1 of 3 dietary treatments with 0, 0.1, and 0.2% whole yeast cell products (CitriStim, ADM, Quincy, IL). Each treatment was replicated in 5 pens (n = 5) of 6 chicks per replication. The basal diet was based on corn and soybean meal (Table 1). At 21 d of age, the birds were weighed individually to identify birds of similar sizes that will be used in the coccidial challenge studies. Two birds per pen were selected based on BW at 21 d so there were no significant differences in BW of birds across the treatment groups at the start of coccidial infection. The 10 birds in each treatment group for the coccidial challenge experiment were housed in a total of 5 battery cages (n = 5) with 2 birds per battery cage.

Coccidial Infection and Production Parameters Postcoccidial Infection. At 21 d of age, 10 birds from each treatment group were orally challenged with 1.2×10^6 live coccidial oocysts (Inovocox, Pfizer Animal Health, NY) in 200 μL of PBS, as described previously (Annamalai and Selvaraj, 2012), to induce a coccidial infection. The birds were weighed on a cage basis at 0, 5, and 12 d postcoccidial challenge to analyze BW gain of different treatment groups. Feed consumption was measured at 5 and 12 d postcoccidial challenge to calculate feed efficiency.

Table 1. Composition of experimental diets[1]

Ingredient	g/kg of diet
Corn	582.5
Soybean meal	338.0
Oil	41.0
Dicalcium phosphate	15.0
Limestone	11.0
Vitamin and mineral mix	5.0
Salt	4.4
DL-Methionine	2.1
Choline chloride (60%)	1.0

[1]Vitamins and minerals were provided in the form and amount described in the NRC Standard Reference Diet for chickens.

Experiment II

Birds. The experimental design, animal husbandry, and coccidial challenge protocols were similar to experiment I. The average BW (±SD) of birds in 0, 0.1, and 0.2% whole yeast product supplemented groups were 838 ± 111, 860 ± 118, 830 ± 43 g, respectively.

Effect of Whole Yeast Cell Product Supplementation on Fecal Coccidial Oocyst Shedding Postcoccidial Challenge. At 3, 7, and 12 d postcoccidial challenge, feces were collected from 5 battery cages per treatment group (n = 5), enriched for coccidial oocysts using a salt flotation technique, and analyzed for total number of coccidial oocysts using a McMaster chamber as described previously (Annamalai and Selvaraj, 2012).

Effect of Whole Yeast Cell Product Supplementation on Jejunum Villi Length and Crypt Depth. The jejunums from 4 samples per treatment group were dehydrated at room temperature in a graded series of alcohols (15 min in 50% ethanol, 15 min in 70% ethanol, 15 min in 95% ethanol, 30 min in 100% ethanol with one change at 15 min), cleared in Pro-par (Anatech, Battle Creek, MI) for 45 min with 2 changes at 15 and 30 min and infiltrated with paraffin at 60°C overnight with one change at 15 min using a Leica TP 1020 tissue processor (GMI Inc., Ramsey, MN). Paraffin blocks were cross sectioned at 5 μm using a microtome. The sections were mounted on frosted slides (Fisher Scientific, Pittsburgh, PA) warmed to 37°C and stained with hematoxylin and eosin (Velleman et al., 1998). The cross sections were viewed and photographed using an Olympus IX81 microscope and analyzed using CellSens Imaging software (Olympus America, Central Valley, PA) to determine the villi height and crypt depth. Five villi per section and 5 sections per sample were analyzed.

Effect of Whole Yeast Cell Product Supplementation on Macrophage Nitric Oxide Production Post-

coccidial Challenge. At 5 and 12 d postcoccidial challenge, macrophages were collected from the spleen of one bird in each of the 5 battery cages per treatment group (n = 5) as described previously (Annamalai and Selvaraj, 2012). Briefly, a single cell suspension from the spleen was enriched for macrophages by density centrifugation over Histopaque (1.077 g/mL, Sigma-Aldrich, St. Louis, MO) for 15 min at 400 × *g* at room temperature. The macrophage-enriched populations were cultured (2×10^8 cells per well) in 6-well plates in 2 mL of RPMI-1640 medium supplemented with 5% chicken serum and 1% penicillin plus streptomycin (media) in a 5% CO_2 incubator at 37°C. After 24 h incubation, the nonadherent cells were removed by washing with PBS. The adherent cells were detached by trypsinization, washed, and reseeded in 96-well plates (2×10^5 cells per well) in 200 μL of media supplemented with 15 μg/mL of coccidial antigen for 48 h. At 48 h of culture, the plates were centrifuged at 400 × *g* for 10 min, and the supernatant was removed. The nitrite content of the supernatant was determined using a sulfanilamide/*N*-(1-Naphthyl) ethylenediamine dihydrochloride solution (#R2233500, Ricca Chemical Company, Arlington, TX) following manufacturer's instructions. Nitrite concentrations were determined from a standard curve drawn with different concentrations of sodium nitrite solution.

Effect of Whole Yeast Cell Product Supplementation on Cecal Tonsil and Spleen IL-1 and IL-10 mRNA Contents. At 5 and 12 d postcoccidial challenge, total RNA was collected from the spleen and cecal tonsils from one bird in each of the 5 battery cages per treatment group (n = 5) and reverse transcribed into cDNA (Selvaraj and Klasing, 2006). The mRNA was analyzed for IL-10 (5′-caatccagggacgatgaact-3′ and 5′-ggcaggacctcatctgtgtag-3′) and IL-1β (5′-tcctccagccagaaagtga-3′ and 5′-caggcggtagaagatgaagc-3′) mRNA by real-time PCR (iCycler, BioRad, Hercules, CA) using SyBr green after normalizing for β-actin mRNA (5′-accggactgttaccaacacc-3′ and 5′-gactgctgctgacaccttca-3′; Shanmugasundaram and Selvaraj, 2010). The annealing temperature for IL-10 was 55°C, for IL-1β was 55°C and for β-actin was 57°C. Fold change from the reference was calculated as $2^{(\text{Ct Sample-housekeeping})}/2^{(\text{Ct Reference-housekeeping})}$, where Ct is the threshold cycle (Schmittgen and Livak, 2008). The Ct was determined by iQ5 software (BioRad) when the fluorescence rises exponentially 2-fold above the background. The reference group was the 0% whole yeast cell product-supplemented group.

Effect of Whole Yeast Cell Product Supplementation on CD4⁺ and CD8⁺ Cell Percentages in the Spleen and Cecal Tonsil Postcoccidial Challenge. At 5 and 12 d postcoccidial challenge, the spleen and cecal tonsils were collected from 1 bird in each of the 5 battery cages per treatment group (n = 5). Single-cell suspensions from the spleens and cecal tonsils were enriched for lymphocytes by density centrifugation over Histopaque (1.077 g/mL, Sigma-Aldrich) for 15 min at 400 × *g* at room temperature. The percentages of $CD4^+$ and $CD8^+$ cells were analyzed using a flow cytometer (Guava Easycyte, Millipore, Billerica, MA) as described previously (Shanmugasundaram and Selvaraj, 2012a).

Effect of Whole Yeast Cell Product Supplementation on Serum Anticoccidial IgG Content. At 5 and 12 d postcoccidial challenge, serum was collected from 1 bird in each of the 5 battery cages per treatment group (n = 5) and stored at −20°C until further use. Serum anticoccidial IgG content was determined by ELISA as described previously (Annamalai and Selvaraj, 2012). The IgG values were reported as the mean optical density.

Statistical Analysis

A one-way ANOVA (JMP, SAS Institute Inc., Cary, NC) was used to examine the effect of CitriStim supplementation on dependent variables. When main effects were significant ($P < 0.05$), differences between means were analyzed by Tukey's least squares means comparison.

RESULTS

Experiment I: Effect of Whole Yeast Cell Product Supplementation on Production Parameters Pre- and Postcoccidial Challenge

Supplementation of whole yeast product did not significantly increase the BW gain, feed consumption and feed efficiency parameters between 0 and 21 d of age (Table 2). Because the BW of birds in the 0.1 and 0.2% Citristim-fed groups were numerically higher, birds of similar sizes were chosen for the experiment with coccidiosis in such a way that there were no significant differences in BW of birds across the treatment groups at the start of coccidial infection. Supplementation of whole yeast cell product increased BW gain between 0 and 12 d ($P = 0.01$) postcoccidial challenge (Table 2). Feed consumption was not significantly different between the experimental groups at 5 and 12 d postcoccidial challenge. Birds supplemented with 0.1 and 0.2% whole yeast cell product had a 12% better ($P = 0.01$) feed efficiency between 0 and 12 d postcoccidial infection. Expressed as a percentage of 21 d BW, birds in the 0.1 and 0.2% yeast cell wall product-supplemented group had gained 92 and 99%, whereas the birds in the control group had gained 82% during the 0 to 12 d postcoccidial infection.

Experiment II: Effect of Whole Yeast Cell Product Supplementation on Fecal Oocyst Count Postcoccidial Challenge

Supplementation with whole yeast cell product decreased ($P = 0.01$) the fecal coccidial oocyst count at 7

Table 2. Body weight and feed consumption of broiler birds fed experimental diets[1]

	CitriStim				
Parameter	0%	0.1%	0.2%	SEM	*P*-value
Precoccidial challenge (0 to 21 d)					
BW gain (g)	853	904	901	36	0.47
Feed consumption (g)	1,362	1,401	1,391	40	0.51
Feed efficiency	1.591	1.542	1.547	0.021	0.36
Postcoccidial challenge (21 to 33 d)					
BW (g) 21 d	848	861	852	29	0.95
BW gain (g)					
0 to 5 d postcoccidial challenge	370	389	422	39	0.67
5 to 12 d postcoccidial challenge	324	400	419	49	0.38
0 to 12 d postcoccidial challenge	694[b]	789[a]	841[a]	28	0.01
Feed consumption (g)					
0 to 5 d postcoccidial challenge	536	515	554	31	0.68
5 to 12 d postcoccidial challenge	577	617	594	40	0.78
0 to 12 d postcoccidial challenge	1,113	1,133	1,148	19	0.44
Feed efficiency					
0 to 5 d postcoccidial challenge	1.497	1.368	1.327	0.093	0.43
5 to 12 d postcoccidial challenge	2.053	1.619	1.460	0.271	0.31
0 to 12 d postcoccidial challenge	1.614[a]	1.439[b]	1.367[b]	0.034	0.01

[a,b]Means without a common superscript differ significantly within a row ($P < 0.05$).

[1]Chicks were fed feed supplemented with 0, 0.1, or 0.2% CitriStim (ADM, Quincy, IL) from day of hatch. At 21 d of age, birds were challenged with 1.2×10^6 live coccidial oocysts. At 0, 5, and 12 d postcoccidial challenge, BW and feed consumption were measured. n = 5.

d postcoccidial challenge (Figure 1). There were no significant differences in the fecal oocyst counts between the experimental groups at 3 ($P = 0.28$) and 12 ($P = 0.49$) d postcoccidial challenge.

Effect of Whole Yeast Cell Product Supplementation on Jejunum Villi Length and Crypt Depth

Supplementation with whole yeast cell product increased the jejunum villi length to crypt depth ratio by 23% at 5 d postcoccidial challenge ($P = 0.01$; Table 3).

Effect of Whole Yeast Cell Product Supplementation on Macrophage Nitric Oxide Production Postcoccidial Challenge

Supplementation with whole yeast cell product increased ($P < 0.01$) the macrophage nitric oxide production at both 5 and 12 d postcoccidial challenge (Figure 2). Whole yeast cell product supplementation at 0.2% increased macrophage nitric oxide production by 93 and 193% at 5 and 12 d postcoccidial challenge, respectively.

Effect of Whole Yeast Cell Product Supplementation on IL-1 and IL-10 mRNA Contents Postcoccidial Challenge

Supplementation with whole yeast cell product at 0.2% increased ($P = 0.01$) the amounts of IL-1 mRNA at 5 and 12 d postcoccidial challenge (Figure 3). Supplementation with whole yeast cell product at 0.2% increased IL-1 mRNA amounts approximately 4.5- and 3.7-fold at 5 and 12 d postcoccidial challenge, respectively, compared with the group with no whole yeast cell product supplementation. Supplementation with whole yeast cell product downregulated IL-10 mRNA amounts compared with the unsupplemented groups at both 5 ($P = 0.01$) and 12 d ($P < 0.01$) postcoccidial challenge (Figure 4).

Effect of Whole Yeast Cell Product Supplementation on Serum Anticoccidial IgG Content and $CD4^+$ and $CD8^+$ Cell Percentages in the Cecal Tonsils

Supplementation with whole yeast cell product did not alter ($P > 0.05$) serum anticoccidial IgG contents

Table 3. Jejunal villus height to crypt depth ratio of broiler birds fed experimental diets[1]

	CitriStim				
Parameter	0%	0.1%	0.2%	SEM	*P*-value
0 d postcoccidial challenge	2.8	3.1	3.1	0.2	0.09
5 d postcoccidial challenge	2.6[b]	3.2[a]	3.2[a]	0.1	0.01
12 d postcoccidial challenge	4.1	4.5	4.7	0.4	0.67

[a,b]Means without a common superscript differ significantly within a row ($P < 0.05$).

[1]Chicks were fed feed supplemented with 0, 0.1, or 0.2% CitriStim (ADM, Quincy, IL) from day of hatch. At 21 d of age, birds were challenged with 1.2×10^6 live coccidial oocysts. At 0, 5, and 12 d postcoccidial challenge, villi height to crypt depth ratio was measured as a ratio between jejunum villus height and crypt depth. n = 4.

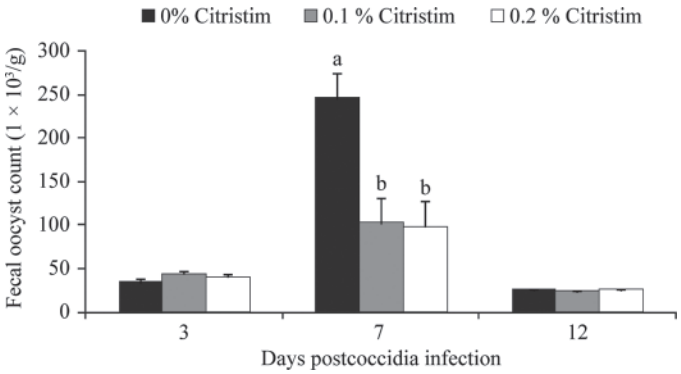

Figure 1. Effect of whole yeast cell product supplementation on fecal oocyst count postcoccidial challenge. Chicks were fed feed supplemented with 0, 0.1, or 0.2% CitriStim (ADM, Quincy, IL) from the day of hatch. At 21 d of age, birds were challenged with 1.2×10^6 live coccidial oocysts. At 3, 7, and 10 d postcoccidial challenge, fecal oocysts were counted under a microscope. Bars (+ SEM) without a common letter (a,b) differ significantly within a day ($P < 0.05$). *P*-values: 3 d, $P = 0.28$; 5 d, $P = 0.01$; 12 d, $P = 0.49$. n = 5.

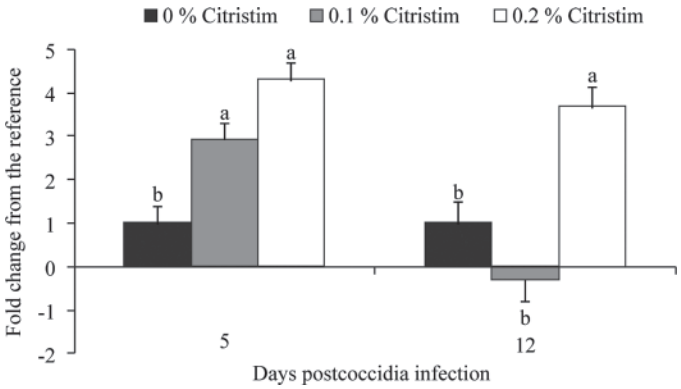

Figure 3. Interleukin (IL)-1 mRNA contents of the cecal tonsils from birds fed different experimental diets. Chicks were fed feed supplemented with 0, 0.1, or 0.2% CitriStim (ADM, Quincy, IL) from day of hatch. At 21 d of age, birds were challenged with 1.2×10^6 live coccidial oocysts. At 5 and 12 d postcoccidial challenge, IL-1 mRNA content was analyzed after correcting for β-actin mRNA content and normalizing to the mRNA content of the 0% CitriStim group, so all bars represent fold change compared with the 0% CitriStim group. Bars (+ SEM) without a common letter (a,b) differ significantly within a day ($P < 0.01$). *P*-values: 5 d, $P = 0.01$; 12 d, $P = 0.01$. n = 5.

or cecal tonsil $CD4^+$ and $CD8^+$ cell percentages at 5 and 12 d postcoccidial infection.

DISCUSSION

This experiment studied the effects of whole yeast cell product supplementation on broiler production parameters, fecal coccidial oocyst count, and immune parameters following an experimental coccidial infection in broiler birds that were not vaccinated against coccidia. Whole yeast cell product supplementation improved the production performance, increased the macrophage nitric oxide production and cecal tonsil IL-1 mRNA content, and decreased the fecal oocyst count and IL-10 mRNA content postcoccidial infection.

Macrophages are cells of innate immunity and express several pathogen-associated molecular pattern recognizing receptors. Lectins, mannose, and β-glucans activate receptors of innate immune cells (Wismar et al., 2010). Activated immune cells produce an array of inflammatory mediators to clear a pathogen, among which IL-1 plays an important role. Interleukin-1 is a proinflammatory cytokine and is produced by activated macrophages. Whole yeast cell product supplementation increased the amount of IL-1 mRNA in the cecal tonsils. In addition, whole yeast cell product supplementation increased macrophage nitric oxide production in vitro following coccidial stimulation. Increased production of nitric oxide and amount of IL-1 can be expected to increase the coccidiosis clearance and decrease the pathogenesis of infection (Lillehoj et al., 2005). Whole yeast cell product supplementation decreased the fecal

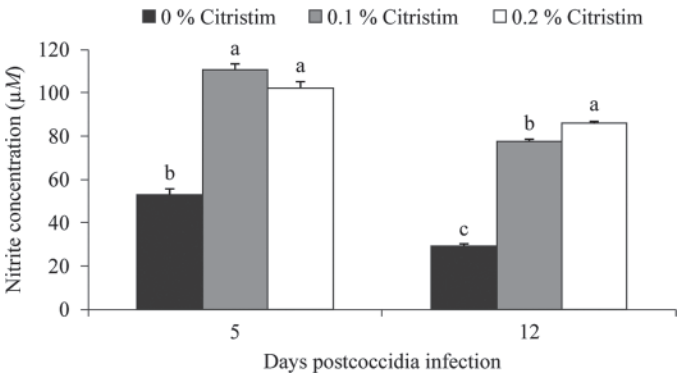

Figure 2. Effect of whole yeast cell product supplementation on macrophage nitric oxide production postcoccidial challenge. Chicks were fed feed supplemented with 0, 0.1, or 0.2% CitriStim (ADM, Quincy, IL) from the day of hatch. At 21 d of age, birds were challenged with 1.2×10^6 live coccidial oocysts. At 5 and 12 d postcoccidial challenge, macrophage nitric oxide production was measured. Bars (+ SEM) without a common letter (a–c) differ significantly within a day ($P < 0.01$). *P*-values: 5 d, $P < 0.01$; 12 d, $P < 0.01$. n = 5.

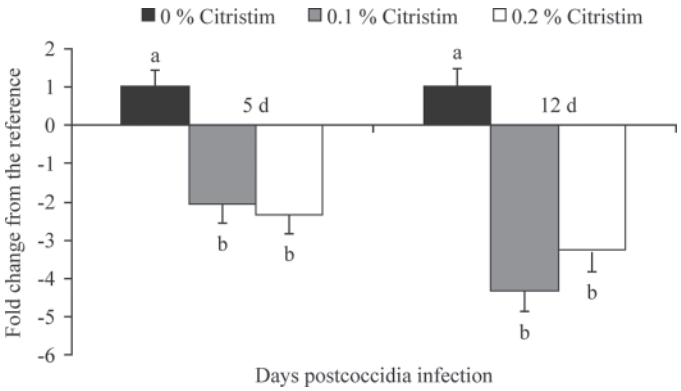

Figure 4. Interleukin (IL)-10 mRNA contents of the cecal tonsils from birds fed different experimental diets. Chicks were fed feed supplemented with 0, 0.1, or 0.2% CitriStim (ADM, Quincy, IL) from day of hatch. At 21 d of age, birds were challenged with 1.2×10^6 live coccidial oocysts. At 5 and 12 d postcoccidial challenge, IL-10 mRNA content was analyzed after correcting for β-actin mRNA content and normalizing to the mRNA content of the 0% CitriStim group, so all bars represent fold change compared with the 0% CitriStim group. Bars (+ SEM) without a common letter (a,b) differ significantly within a day ($P < 0.05$). *P*-values: 5 d, $P = 0.01$; 12 d, $P < 0.01$. n = 5.

coccidial oocyst count at 7 d postcoccidial infection and thus accelerated the clearance of coccidia.

Mannose oligosaccharide supplementation increasing the villi height to crypt depth ratio has been studied (Solis de los Santos et al., 2007). Mannose oligosaccharide supplementation increases the lactobacillus and bifidobacteria colonization in broilers (Baurhoo et al., 2007) by acting like a prebiotic. An increase in beneficial bacterial population presumably increases the villi height to crypt depth ratio (Baurhoo et al., 2007). In this study, whole yeast cell product supplementation increased the jejunal villi height to crypt depth ratio. Increased villi height to crypt depth ratio can be expected to improve nutrient absorption and to improve production parameters. Whole yeast cell product supplementation improved BW gain and feed efficiency postcoccidial infection.

Supplementation of whole yeast cell products, in the absence of any pathogen challenge, increases the IL-10 mRNA amount and Treg population in the cecal tonsils of birds (Shanmugasundaram and Selvaraj, 2012a). Treg percentage and IL-10 production in gut-associated lymphoid tissues are very sensitive to gut microbiota. In the absence of infection, higher IL-10 production and Treg numbers in the gut will facilitate immune tolerance. In the presence of pathogenic bacteria, an inflammatory response mediated by Th17 cells predominates (Ivanov et al., 2009) and Tregs lose their suppressive properties and IL-10 production (Shanmugasundaram and Selvaraj, 2011; Shanmugasundaram and Selvaraj, 2012b). Thus, during coccidiosis, the intestinal physiology can be expected to downregulate the IL-10 mRNA to facilitate an effective anticoccidial immune response. Supplementation of whole yeast cell product decreased the IL-10 mRNA content of the cecal tonsils compared with the control group, which might facilitate a better inflammatory immune response. Increasing inflammatory cytokines can be expected to increase coccidial clearance as previous research has shown increased coccidial clearance rate with in ovo IL-1 and interferon-γ plasmid vaccination (Min et al., 2001).

It could be concluded that supplementing whole yeast cell products (CitriStim) to broiler diets can improve production parameters, decrease fecal oocyst count, and increase inflammatory cytokine production postcoccidial infection.

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