Research Note: Effect of synbiotic supplementation on caecal *Clostridium perfringens* load in broiler chickens with different necrotic enteritis challenge models

R. Shanmugasundaram,[*,†] A. Markazi,[*] M. Mortada,[†] T. T. Ng,[†] T. J. Applegate,[†] L. R. Bielke,[*] B. Syed,[‡] C. M. Pender,[§] S. Curry,[§] G. R. Murugesan,[§] and R. K. Selvaraj[†,1]

*Department of Animal Sciences, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster 44691, USA; †Department of Poultry Sciences, University of Georgia, Athens 30602, USA; ‡BIOMIN Holding GmbH, Getzersdorf 3131, Austria; and §BIOMIN America Inc., Overland Park 66210, KS, USA

ABSTRACT Studies were conducted to determine the efficacy of synbiotic applications to combat the negative effects of necrotic enteritis (**NE**). An in vitro study was conducted to test the effect of probiotics species supernatants to decrease *Clostridium perfringens* (**CP**) proliferation. *Lactobacillus reuteri*, *Enterococcus faecium*, *Bifidobacterium animalis*, and *Pediococcus acidilactici* culture supernatants decreased the proliferation of CP at 1:1 supernatant-to-pathogen dilution in vitro. Two in vivo studies were conducted to determine the in vivo response of synbiotic supplementation containing the aforementioned probiotic strains on broiler production performance and caecal CP load in broilers induced with NE infection. In experiment 1, 75 broiler chicks were randomly allotted to 3 treatment groups, control (basal diet), ionophore (Salinomycin), and synbiotic (PoultryStar me), from day of hatch, and NE was induced in all birds. There were no significant treatment effects on BW, feed consumption, and feed gain ratio. However, at 35 D, ionophore or synbiotic supplementation increased ($P < 0.05$) villi height and decreased interleukin (**IL**)-1 mRNA abundance, while synbiotic supplementation increased ($P < 0.05$) IL-10 mRNA abundance compared with the control group, respectively. In experiment 2, 360 broiler chicks were randomly allotted to 3 treatments, an unchallenged negative control (control; basal diet), challenged positive control (NE; basal diet), or NE + synbiotic group (synbiotic). At both 21 and 42 D of age, NE birds had decreased ($P < 0.05$) BW, feed conversion, and jejunal villi height compared with control, while NE + synbiotic birds were not different from control groups. At 42 D of age, NE birds had 2.2 log/g increased CP in the ceca contents compared with control, while synbiotic birds had CP load that was not different than that of the control group. NE + synbiotic birds had significantly greater amounts of bile anti-CP IgA than the control and NE groups. It can be concluded that synbiotic supplementation decreased CP proliferation in vitro and caecal CP load in vivo while improving production parameters during an NE infection in broilers.

Key words: clostridium perfringens, *Eimeria maxima*, probiotic, Salinomycin, synbiotic

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INTRODUCTION

Clostridium perfringens (**CP**), the etiological agent of necrotic enteritis (**NE**) in poultry, is an important pathogen to the poultry industry. NE alone has been estimated to cause nearly $2 billion in increased costs to the poultry industry worldwide (Martin and Smyth, 2009). *C. perfringens* is a spore-forming gram-positive bacterium and is present in more than 50% of intensively reared broiler chickens with no apparent disease symptoms. *C. perfringens* caecal load ranges from 2 to 5.72 $\log_{10}$ CFU/g in intensively raised broiler birds, 2.78 to 5.46 $\log_{10}$ CFU/g in organic flocks, and 2.13 to 4.36 $\log_{10}$ CFU/g in free-range flocks (De Cesare et al., 2009). The major CP virulence factors include genes for the production of 17 different toxins (Bokori-Brown et al., 2011). Toxins produced by CP along with other predisposing factors such as coccidial infection, dietary factors, high stocking density, stress, and immunosuppression result in damaging the

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[1]Corresponding author: selvaraj@uga.edu

intestinal villi, reducing nutrient absorption, potentially creating negative energy balance, and resulting in excess nutrients in lumen available for opportunistic pathogens ultimately leading to loss in production (Wilson et al., 2005).

Traditional NE mitigation strategies have included, antibiotic growth promoters and ionophores. However, increasing pressure to reduce antibiotics use in poultry production has increased the incidence of NE in recent years (Cooper and Songer, 2009). Thus, alternative nutritional strategies have continued to be investigated to prevent or mitigate NE in birds. Probiotics are one such nutritional mitigation strategy and are defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host". Probiotics exhibit antimicrobial effects by secreting antimicrobial peptides (Smirnov et al., 2005), by competitive exclusion (La Ragione and Woodward, 2003), by enhancement of intestinal barrier (Yang et al., 2012), and by enhancement of host immunity (Yang et al., 2012). Probiotics could be alternatives to antibiotic growth promotors. Probiotics improve feed conversion and immune functions in chickens (Teo and Tan, 2005).

Probiotics have been applied to decrease the severity of NE (Caly et al., 2015). For example, *Lactobacillus fermentum* supplementation reduced CP-induced NE lesion score in poultry (Cao et al., 2012). Probiotics, in combination with coccidial vaccine, enhances host defense against a mixed Eimeria challenge (Ritzi et al., 2016). Considering that the gastrointestinal tract of poultry is a complex environment with multiple bacterial species and CP being an highly adaptable microorganism, it is essential to develop products in a rational manner (Caly et al., 2015).

Different experimental models that differ in route, dosage, timing, and frequency of the *Eimeria maxima* and CP dose have been reported in literature (McReynolds et al., 2004, 2007; Dahiya et al., 2007). The dosages of *C. perfringens* and coccidia vary between strains from each laboratory and from each batch of the inoculates with the laboratory. Hence, we studied the effects of probiotic supplementation on two different experimental models of NE that differed in NE severity (Latorre et al., 2018; Bortoluzzi et al., 2019).

The objective of this study is to identify whether a commercially available synbiotic that contains *Lactobacillus reuteri*, *Enterococcus faecium*, *Bifidobacterium animalis*, *Pediococcus acidilactici*, and a fructooligosaccharide can decrease the CP in vitro proliferation and decrease CP in vivo load during 2 different models of experimental NE challenge in broiler birds.

MATERIALS AND METHODS

All animal protocols were approved by the Institutional Animal Care and Use Committee at The Ohio State University.

In Vitro Study

Effect of Cell-Free Probiotic Supernatants on CP In Vitro Proliferation Single isolated colonies of *L. reuteri*, *E. faecium*, *B. animalis*, and *P. acidilactici* probiotic strains were inoculated in de Man, Rogosa and Sharpe agar (**MRS**) broth and incubated overnight. Overnight probiotic cultures (optical density [O.D] 600 = 0.9) were centrifuged at 4,500 × *g* for 10 min. The supernatant was filter-sterilized using 0.22-µm filter (EMD Millipore, Burlington, MA) to collect cell-free supernatant.

A volume of 10 µL of CP overnight culture (O.D 600 = 0.1) was incubated with 0:1, 1:1, 5:1, or 10:1 cell-free supernatant: pathogen dilutions in triplicates in 96-well plate. The total incubated volume was adjusted using MRS broth to 110 µL. Plates were incubated overnight at 37°C for 24 h. The absorbance was measured at 600 nm at 24 h, and the effect of probiotic culture supernatant on the inhibition of CP proliferation was reported as an O.D value.

In Vivo Studies

The in vivo study was conducted to identify whether synbiotic supplementation can decrease NE severity in the intestine and decrease CP load in the ceca.

Experiment 1

Birds and NE induction A total of 75 Cobb-500 broiler chicks were randomly allotted to 3 treatment groups, control (basal diet), ionophore (Salinomycin at 0.5 g/kg feed, SaCox; Huvepharma, Peachtree city, GA), and synbiotic (PoultryStar meUS at 0.5 g/kg feed; Biomin America Inc., Overland Park, KS) from day of hatch. The synbiotic contained four live strains isolated from adult chickens (*L. reuteri*, *E. faecium*, *B. animalis*, and *P. acidilactici*) with prebiotic, fructooligosaccharide. Each treatment was replicated in 5 floor pens of 5 chicks per replication (n = 5). The basal diet was based on a corn and soybean meal diet that met or exceeded the requirements for nutrients and energy based on NRC guidelines (NRC, 1994). BW and feed consumption was measured at weekly intervals, and BW gain and feed efficiency were calculated. At 18 D of age, all birds in experimental groups were inoculated orally with of 4×10^4 *E. maxima* oocysts per bird followed by CP 1×10^8 colony-forming units (**CFU**)/mL/bird/day at 22 and 23 D of age as described earlier (Latorre et al., 2018). The bacterial concentration (CFU/mL) of inoculum was calculated by plate counting using Brain Heart Infusion agar (Sigma-Aldrich, St. Louis, MO), followed by anaerobic incubation at 37°C for 16 h.

Experiment 2

Birds and NE induction A total of 360 Cobb-500 broiler chicks were randomly allotted to 3 treatment groups: Nonchallenged group fed the basal diet (control), challenged group fed the basal diet (NE), or

challenged group fed 0.05% synbiotic supplement (synbiotic + NE) from day of hatch through 42 D of age. Birds in the NE and synbiotic + NE groups were inoculated orally with 5×10^3 oocyst of *E. maxima* per bird on day 14 followed by 1×10^8 CFU/mL of CP on day 19, 20, and 21 to induce NE as described earlier (Bortoluzzi et al., 2019). Each treatment was replicated in 8 floor pens of 15 chicks per replication (n = 8). The basal diet was based on corn and soybean meal diet that met or exceeded nutrient and energy requirements based on NRC guide (NRC, 1994). BW and feed consumption was measured at weekly intervals, and BW gain and feed efficiency were calculated.

Effect of Synbiotic Supplementation on Jejunal Histological Parameters During an Experimental NE Infection Jejunal samples, cut from the end of the duodenal loop and proximal to the Meckel's diverticulum, were collected from one bird per each replication at day 35 in experiment 1 and at day 28 and 42 in experiment 2. Jejunal samples were processed at room temperature in a graded series of alcohols (15 min in 50% ethanol, 15 min in 70% ethanol, 15 min in 96% 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 1,020 tissue processor (GMI Inc., Ramsey, MN). Paraffin blocks were cut into 5-µm cross-sections and mounted on frosted slides. Slides were then stained with hematoxylin and eosin. Cross-sections were viewed using the cellSens Imaging software (Olympus America, Central Valley, PA) to measure villi length and crypt depth. Five villi and crypts per section and 5 sections per sample were analyzed.

Effect of Synbiotic Supplementation on Caecal Tonsils IL-1β and IL-10 mRNA Content During an Experimental NE Infection At 35 D of age, caecal tonsils were collected from one bird per pen in experiment 1 and analyzed for IL-10 and IL-1 mRNA content by real time PCR. Total RNA was collected from caecal tonsils and reverse transcribed into cDNA (Selvaraj and Klasing, 2006). IL-10 (5′-CATGCTGCTGGGCCTGAA-3′ and 5′-CGTCTCCTTGATCTGCTTGATG-3′) (Rothwell et al., 2004) and IL-1β (5′-CTACACCCGCTCACAGTCCT-3′ and 5′-TCACTTTCTGGCTGGAGGAG-3′) mRNAs were analyzed by real-time PCR (CFX96 Touch Real Time System; Bio-Rad, Hercules, CA) using SyBr green after normalizing for β-actin mRNA (5′-ACCGGACTGTTACCAACACC-3′ and 5′-GACTGCTGCTGACACCTTCA-3′) (Shan mugasundaram and Selvaraj, 2010). The annealing temperature for IL-10 was 55°C, 57.5°C for IL-1β, and 57°C for β-actin. Fold change from the reference was calculated as $2^{(\text{Ct Sample-housekeeping})}/2^{(\text{Ct Reference-housekeeping})}$, where Ct is the threshold cycle. Ct was determined by using the iQ5 software (Bio-Rad, Hercules, CA) when the fluorescence rises exponentially 2-fold above background. The reference group was the control group.

Effect of Synbiotic Supplementation on Caecal CP Load during an Experimental NE Infection Caecal contents were collected from one bird per replication at 28 and 42 D of age in experiment 2 and analyzed for CP load by real time PCR. Bacterial genomic DNA was isolated as described earlier (Shanmugasundaram et al., 2019). The DNA extracted from the different treatment groups was analyzed for CP load by real-time PCR using primers (5′- AAAGGAAGATTAATACCGCATAA- 3′ and 5′- ATCTTGCGACCGTACTCCCC = 3′) with an annealing temperature was 56°C. The threshold cycle (Cq) values were determined by using CFX software (Bio-Rad, Hercules, CA) when the fluorescence rises exponentially 2-fold above background. The copy numbers of CP was expressed in log 10 CFU/g digesta as described earlier (Shanmugasundaram et al., 2019).

Effect of Synbiotic Supplementation on Bile anti- CP IgA Content during an Experimental NE Infection Bile samples were collected from one bird per pen at 28 and 42 D of age in experiment 2 and analyzed for anti-CP IgA content using an enzyme-linked immunosorbent assay. The primary and secondary antibody concentrations were established using checkerboard titrations with dilutions of bile and antigens. CP antigen for coating was made by 3 consecutive freeze thaw cycles of pure culture of CP followed by mechanical lysing. The pure culture was lysed 2 times by glass beads size 425 to 600 µm (Sigma, St. Louis, MO) in a TissueLyser LT (Qiagen, Hilden, Germany) for 5 min at 50 Hz. The lysed cells were centrifuged at 10,000 × *g* for 10 min, and the resultant supernatant was collected and stored at −70°C until use. Flat-bottomed 96-well microtitration plates (Microlon 600 High Binding, Greiner, NC) were coated with 100 µL of 10 µg/mL of the antigen diluted in 0.1 M carbonate buffer and analyzed for anti–CP-specific IgA using enzyme-linked immunosorbent assay as described earlier (Markazi et al., 2018). IgA values were reported as the mean O.D.

Statistical Analysis

A one-way ANOVA was used to examine the effects of synbiotic supplementation on dependent variables, with pen being considered as the experimental unit. When the main effects were significant ($P < 0.05$), differences between means were analyzed by Tukey's least square means comparison.

RESULTS

In Vitro Experiment

All 4 probiotic culture supernatants decreased the proliferation of CP at 1:1 supernatant-to-pathogen dilution at 24 h (Figure 1). Increasing the supernatant ratio to either 5:1 or 10:1 in all the 4 probiotic culture supernatants further decreased the in vitro proliferation of CP.

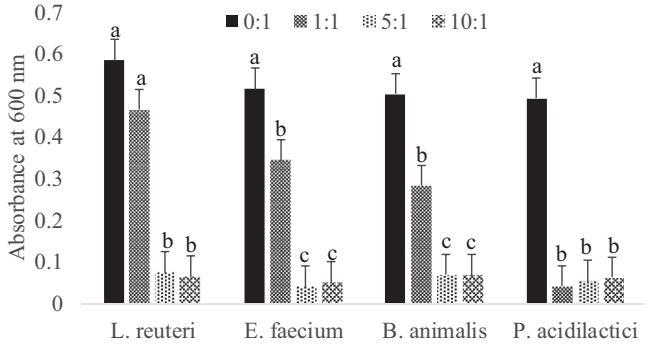

Figure 1. Effect of cell-free probiotic supernatants on *Clostridium perfringens* (CP) in vitro proliferation. Overnight culture of single isolated colonies of *Lactobacillus reuteri*, *Enterococcus faecium*, *Bifidobacterium animalis*, and *Pediococcus acidilactici* probiotic strains were centrifuged at 4,500 × *g* for 10 min to collect supernatants. The supernatant was filtered through 0.22-µm filter to collect cell-free supernatant. Ten microliters of *S. enteritidis* overnight culture was incubated with 0:1, 10:1, 5:1, or 1:1 cell-free supernatant-to-pathogen dilutions. The absorbance was measured at 600 nm at 24 h. n = 3. Bars (+SEM) with no common superscript differ significantly ($P < 0.05$). *P* values: *L. reuteri* $P = 0.01$; *E. faecium* $P < 0.01$; *B. animalis* $P = 0.01$; *P. acidilactici* $P < 0.01$.

C. perfringens is a gram-positive, anaerobic spore-forming bacteria and produces an array of more than 16 toxins (Uzal et al., 2014).

Experiment 1

Effect of Synbiotic Supplementation on Production Parameters during an Experimental NE Infection

There were no significant ($P > 0.05$) treatment effects on BW, feed consumption, and feed efficiency in experiment 1 (data not shown).

Effect of Synbiotic Supplementation on Jejunal Histological Parameters during an Experimental NE Infection

In experiment 1, synbiotic and ionophore supplementation had significant effects on jejunal villi height at 35 D of age (Table 1) in birds induced with NE. Ionophore and synbiotic supplementation increased villi height by 28 and 20% in birds induced with NE compared with the control group, respectively. There were no significant ($P > 0.05$) treatment effects on jejunal crypt depth and villus height: crypt depth in birds induced with NE.

Effect of Synbiotic Supplementation on Caecal IL-1β and IL-10 mRNA Content During an Experimental NE Infection

Synbiotic and ionophore supplementation had significant effects on caecal tonsils IL-1β ($P = 0.01$) and IL-10 ($P = 0.02$) mRNA content at 35 D of age (Figure 2) in birds induced with NE. Ionophore and synbiotic supplementation decreased IL-1β mRNA, while synbiotic supplementation increased IL-10 mRNA content in birds induced with NE compared with the control group.

Experiment 2

Effect of Synbiotic Supplementation on Production Parameters During an Experimental NE Infection

There were significant treatment effects on BW ($P < 0.01$; $P < 0.01$), feed consumption ($P = 0.01$; $P = 0.01$), and feed gain ($P = 0.01$; $P = 0.01$) at both 21 and 42 D of age, respectively (Table 2). Birds induced with NE had decreased BW and increased feed conversion compared with the control group with no induced NE. Birds in the synbiotic + NE group had significantly higher BW and lower feed gain than those in the NE group.

Effect of Synbiotic Supplementation on Jejunal Histological Parameters During an Experimental NE Infection

There were significant treatment effects on jejunal villi height at 28 ($P = 0.01$) and 42 ($P = 0.01$) D of age (Table 1). Birds induced with NE had 8.9 and 3.8% decrease in jejunal villi length compared with the control nonchallenge group at 28 and 42 D of age, respectively. Birds in the synbiotic + NE group had comparable villi height to the control group. There were significant treatment effects ($P = 0.03$) on jejunal villi height-to-crypt depth ratio at 28 D of age. Birds induced with NE

Table 1. Effect of synbiotic supplementation on jejunal histological parameters during an experimental necrotic enteritis (NE) infection.

Parameter	Treatments			SEM	*P* Value
Experiment 1 (35 D)	Control	Ionophore	Synbiotic		
Villi height (µm)	425.30[b]	545.97[a]	512.22[a]	19.03	0.01
Crypt depth (µm)	203.09	206.21	207.69	9.90	0.94
Villi height:crypt depth	2.09	2.65	2.54	0.16	0.08
Experiment 2	Control	NE	Synbiotic + NE		
28 D					
Villi height	630.13[a]	573.64[b]	625.03[a]	9.11	0.01
Crypt depth	187.40	184.11	192.87	30.1	0.14
Villi height:crypt depth	3.36[a]	3.12[b]	3.25[a,b]	0.06	0.03
42 D					
Villi height	689.51[a]	662.91[b]	690.00[a]	5.93	0.01
Crypt depth	174.41	171.32	173.79	1.94	0.50
Villi height:crypt depth	3.96	3.87	3.97	0.04	0.19

[a,b]Means with no common superscript within a row differ significantly ($P < 0.05$).

Experiment 1: Birds were fed either basal diet (control) or supplemented with 0.5 g/kg ionophore (Salinomycin), or 0.5 g/kg synbiotic from day of hatch through 0 to 35 D of age in 5 floor pens of 5 chicks per replication (n = 5). Experiment 2: Birds in control and NE groups were fed basal diet, and birds in NE + synbiotic groups were fed diets supplemented with 0.05% synbiotic from day of hatch through 0 to 42 D of age in 8 floor pens of 15 chicks per replication (n = 8). Birds were induced with NE as described in the text. Jejunal samples were analyzed for aforementioned parameters by histology.

had decreased jejunal villi height-to-crypt depth ratio, while birds in the synbiotic + NE group had comparable villi height-to-crypt depth to that in the nonchallenged control group. There were no significant ($P > 0.05$) treatment effects on jejunal crypt depth at 28 and 42 D of age.

Effect of Synbiotic Supplementation on Caecal CP Load During an Experimental NE Infection There were significant treatment effects on caecal CP load at 28 ($P < 0.01$) and 42 ($P < 0.01$) D of age (Figure 3). At 28 D of age, birds in the NE group had 4 log increase, while birds in the synbiotic + NE group had only 2.5 log increase in CP load compared with the control group with no NE. At 42 D of age, birds in the NE group had 2.2 log increase, while birds in the synbiotic + NE group had comparable CP load to that in the control group with no NE.

Effect of Synbiotic Supplementation on Bile Anti-CP IgA Content During an Experimental NE Infection There were significant treatment effects on bile IgA content at 42 ($P < 0.01$) D of age (Figure 3). Birds in the synbiotic + NE group had significantly higher amounts of bile anti-CP IgA than the control and NE groups.

DISCUSSION

Probiotic species outcompete pathogenic bacteria by secreting bacteriocins and reducing the pH of the intestine (Dobson et al., 2012). *C. perfringens* is highly sensitive to environmental pH where CP proliferation is greatly reduced at pH below 5.1 (Guo et al., 2017). Lactic acid bacteria have been shown to have bactericidal and bacteriostatic action through production of bacteriocins, and the optimal pH for lactic acid bacteria is 5.5 of which they produce lactic acid to further decrease the pH of the media (Hutkins and Nannen, 1993).

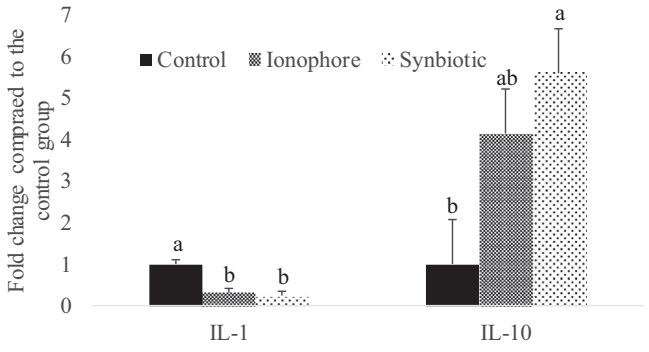

Figure 2. Effect of synbiotic supplementation on caecal IL-1 and IL-10 mRNA content during an experimental necrotic enteritis infection (experiment 1). Birds were fed either basal diet (control) or supplemented with 0.05% ionophore (Salinomycin), or 0.05% synbiotic from day of hatch through 0 to 35 D of age in 5 floor pens of 5 chicks per replication (n = 5). Birds were inoculated with 4×10^4 oocyst of *Eimeria maxima* on day 18 and 1×10^9 *Clostridium perfringens* on day 22 and 23. At 35 D of age, relative IL-10 and IL-1 mRNA content was analyzed after correcting for β-actin mRNA and normalizing to the mRNA content of the control group. Bars (+SEM) with no common superscript differ significantly ($P < 0.05$).

Table 2. Effect of synbiotic supplementation on production parameters during an experimental necrotic enteritis (NE) infection (experiment 2).

Parameter	Control	NE	Synbiotic + NE	SEM	*P* value
0–21 D					
Body weight (kg)	0.96[a]	0.78[b]	0.94[a]	0.01	<0.01
Feed consumption (kg)	1.33[a]	1.21[b]	1.45[a]	0.04	0.01
FCR	1.38[b]	1.55[a]	1.55[a]	0.02	0.01
0–42 D					
Body weight (kg)	2.70[a]	2.44[b]	2.76[a]	0.04	<0.01
Feed consumption (kg)	4.87[a]	4.62[b]	5.00[a]	0.03	0.01
FCR	1.80[a]	1.90[b]	1.81[a]	0.02	0.01

[a,b]Means with no common superscript within a column differ significantly ($P < 0.05$; n = 8).

Birds in control and NE groups were fed basal diet, and birds in NE + synbiotic groups were fed diets supplemented with 0.05% synbiotic from day of hatch through 0 to 42 D of age in 8 floor pens of 15 chicks per replication (n = 8). Birds in the NE and synbiotic + NE groups were inoculated with 5×10^3 oocyst of *Eimeria maxima* on day 14 and 1×10^9 *Clostridium perfringens* on day 19, 20, and 21 to induce NE.

Abbreviation: FCR, feed conversion ratio.

Supernatant from *L. reuteri* reduced the in vitro proliferation of CP in this study. An earlier report identified 2 different lactobacillus strains, *Lactobacillus acidophilus* and *L. fermentum*, reduced the in vitro proliferation of CP (Guo et al., 2017). This inhibitory reaction was confirmed by treating the *P. acidilactici* supernatants with proteolytic enzymes, which reversed the inhibitory effects of *P. acidilactici* (Jager and Harlander, 1992).

Lactobacillus probiotics not only decrease the production of alpha toxin by CP but also degraded the alpha toxin (Guo et al., 2017). Furthermore, in vitro preincubation of chicken intestinal cells with *L. acidophilus* decreases the attachment of CP to the intestinal cells. Alpha toxins and NetB produced by CP forms pores in the lipid bilayer and causes myonecrosis of the intestine (Keyburn et al., 2010). In experiment 1, birds infected with NE had decreased intestinal villi height, indicating that the NE infection caused damage to the intestinal structure. Probiotics reversing the NE-induced loss in villi height can either be compensatory mechanism to increase the absorptive capacity or decreasing the severity of the CP infection itself (Caly et al., 2015).

Bifidobacterium infantis reduces inflammatory scores by decreasing the production of proinflammatory cytokines, while maintaining the levels of anti-inflammatory IL-10 (McCarthy et al., 2003). Caco-2 cells infected with CP and treated with *Lactobacillus helveticus*, *Lactobacillus fermentum*, and *Streptococcus thermophilus* probiotics have elevated amount of transforming growth factor beta (TGFβ), suggesting that probiotic species induce protective effects against CP infections (Golic et al., 2017). Increase in IL-10 and decrease in inflammatory IL-1 in NE-induced birds can thus be attributed to the anti-inflammatory properties of probiotics.

Synbiotic supplementation increased anti–CP-specific IgA and decreased the load of CP in the chicken gut. Probiotics promote IgA maturation in formula-fed

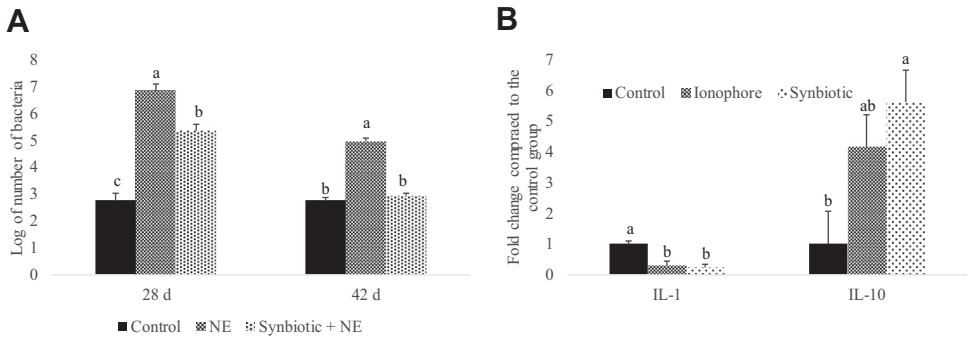

Figure 3. Effect of synbiotic supplementation on caecal *Clostridium perfringens* (CP) load (A) and bile anti-CP IgA (B) during an experimental necrotic enteritis infection (experiment 2). Birds were randomly allotted to negative control (control) or necrotic enteritis control (NE; basal diet) or NE + synbiotic group. Birds in control and NE groups were fed basal diet, and birds in NE + synbiotic groups were fed diets supplemented with 0.05% synbiotic from day of hatch through 0 to 42 D of age in eight floor pens of 15 chicks per replication (n = 8). Birds in the NE and synbiotic + NE groups were inoculated with 5,000 oocyst of *Eimeria maxima* on day 14 and 1×10^9 CP on day 19, 20, and 21 to induce necrotic enteritis (NE). At 28 and 42 D of age, caecal content was analyzed for CP load by real time PCR collected and expressed as log values. At 28 and 42 D of age, bile samples were analyzed for *Salmonella*-specific IgA content through ELISA, and results are reported as average optical density (OD) values. Bars (+SEM) with no common superscript differ significantly ($P < 0.05$).

infants by increasing the serum levels of soluble CD14 (Rautava et al., 2006). *P. acidilactici* supplementation has been shown to promote IL-6 and IL-10 production by dendritic cells to increase mucosal IgA content in humans (Kawashima et al., 2018). We observed that probiotic supplementation increased the caecal IL-10 content in this study. Increased antigen-specific IgA production by probiotics can be expected to decrease the load and severity of CP infection.

Synbiotic supplementation decreases NE severity by decreasing the CP load in the intestine and by increasing CP-specific antibodies in the mucosa. Synbiotic supplementation improved host defense against NE infection and thus can be a viable alternative to ionophores in controlling NE in poultry. Synbiotics can be used in conjunction with other NE control strategies to decrease the severity of NE in poultry.

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