Responses of broiler chickens to *Eimeria* challenge when fed a nucleotide-rich yeast extract[1]

H. Leung,[*,2] A. Yitbarek,[†] R. Snyder,[†] R. Patterson,[‡] J. R. Barta,[†] N. Karrow,[*] and E. Kiarie[*,3]

[*]*Department of Animal Biosciences, University of Guelph, Guelph, ON N1G 2W1, Canada;* [†]*Department of Pathobiology, University of Guelph, Guelph, ON N1G 2W1, Canada; and* [‡]*Canadian Bio-Systems Inc., Calgary, AL T2C 0J7, Canada*

ABSTRACT Nucleotide-rich yeast extract (**YN**) was investigated for effects on growth performance, jejunal physiology, and cecal microbial activity in *Eimeria*-challenged broiler chickens. A total of 360-day-old male chicks (Ross × Ross 708) were placed on floor pens and provided a corn–soybean meal-based diet without or with YN (500 g/MT; n = 12). On d 10, 6 replicates per diet were orally administered with 1 mL of *E. acervulina* and *E. maxima* sporulated oocysts and the rest (non-challenged control) were administered with 1 mL of distilled water. On d 15, 5 birds/pen were then necropsied for intestinal lesion scores, histomorphology and cecal digesta pH, short chain fatty acids (**SCFA**), and microbial community using Illumina Miseq platform. Supplemental YN improved ($P = 0.01$) Feed conversion ratio (FCR) during the prechallenge phase (d 0 to 10). In the postchallenge period (d 11 to 15), *Eimeria* depressed ($P < 0.05$) Body weight gain (BWG) relative to non-challenged birds, whereas YN-fed birds had a higher ($P = 0.05$) BWG compared to that of non-YN-fed birds. There was an interaction between YN and *Eimeria* on jejunal villi height (**VH**) ($P = 0.001$) and expression of cationic amino acid transporter 1(*CAT1*) ($P = 0.04$). Specifically, in the absence of *Eimeria*, YN-fed birds had a shorter VH (892 vs. 1,020 μm) relative to that of control but longer VH (533 vs. 447 μm) in the presence of *Eimeria*. With respect to *CAT1*, YN-fed birds had a higher (1.65 vs. 0.78) expression when subjected to *Eimeria* than when not challenged. Independently, *Eimeria* decreased ($P < 0.01$) the jejunal expression of maltase, Na glucose transporter 1 and occludin genes, ceca digesta abundance of genus *Clostridium* cluster XlVa and *Oscillibacter* but increased ($P < 0.01$) jejunal proliferating cell nuclear antigen and interleukin 10. Interaction between YN and *Eimeria* was observed for ceca digesta pH ($P = 0.04$) and total SCFA ($P = 0.01$) such that YN increased SCFA in the absence of *Eimeria* but reduced SCFA and increased pH in the presence of *Eimeria*. In summary, *Eimeria* impaired performance and gut function and shifted gut microbiome; YN improved performance independently, attenuated *Eimeria* damage on indices of gut function, and modulated cecal microbiome.

Key words: broiler chickens, *Eimeria*, gut health, growth performance, nucleotide-rich yeast extract

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INTRODUCTION

Coccidiosis, caused by the protozoan parasite *Eimeria*, is a global problem in the poultry industry, leading to losses in excess of $3 billion yearly due to intestinal damage, malabsorption of nutrients, and subsequently poor performance (Lillehoj and Lillehoj, 2000; Dalloul and Lillehoj, 2006). By extension, damaged intestines can lead to a shift in the gut microbiome due to a combination of changes in the immune response and the increased presence of undigested nutrients in the distal end of the intestine (Oakley et al., 2015). A healthy and balanced microbiome is critical in aiding the gut in recovering from inflammatory events through competitive exclusion and signaling of short-chain fatty acids (**SCFA**) (Kiarie et al., 2013; Oakley et al., 2015). To combat *Eimeria*, birds are typically given coccidiostats, but the shift to antibiotic and drug-free production due to legislative and public pressure has increased the usage of alternatives, such as vaccines and non-drug feed additives (Lillehoj and Lillehoj, 2000; Chapman et al., 2005). Unfortunately, *Eimeria* vaccines are live

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[2]Present address: Gowans Feed Consulting, New Hamburg, ON N3A 3H2, Canada.
[3]Corresponding author: ekiarie@uoguelph.ca

oocysts that are either attenuated or non-attenuated and both have drawbacks due to stimulation of a range of responses at the intestinal mucosal level, leading to small losses of performance (Chapman et al., 2005).

To attenuate the negative effects of coccidial vaccines, nucleotide supplementation has been proposed as they are conditionally essential and their requirement increases during intestinal repair (Jung, 2011). For example, in times of immune challenge, stress, and rapid growth, it has been suggested that the salvage pathway and de novo synthesis of nucleotides are insufficient for optimal growth as it is a high energy-consuming process (Sanchez-Pozo and Gil, 2002; Jung, 2011). The effects of nucleotide-rich yeast extracts (**YN**) on growth performance, immune function, and intestinal growth have been studied in broilers with variable responses (Jung, 2011; Pelicia et al., 2011; Alizadeh et al., 2016). When chickens were not under challenge, Pelícia et al. (2010) found no effect on the performance or carcass yield at d 42 with a supplementation level of 0.04 to 0.07% nucleotides. Similarly, Jung and Batal (2012) found no effect with 0.5% supplementation of Torula yeast RNA on the performance or intestinal weights in 1 experiment. In contrast, a second experiment in the same study reported that Torula yeast RNA or NuPro improved the performance and increased intestinal villi height (**VH**) and lymphocyte proliferation when birds were challenged with high stocking density (Jung and Batal, 2012). Jung (2011) reported 2 experiments in which supplemental nucleotides in the form of Torula yeast RNA or NuPro were fed to broiler chickens challenged with a high dose of Coccivac-B vaccine. Challenge depressed growth in 1 experiment, and supplemental nucleotides alleviated negative effects of vaccination in terms of improved growth and AMEn. However, vaccination did not affect growth performance in the second experiment, and subsequently, there were no effects of supplemental nucleotides. Collectively, these studies suggested that nucleotides may have a positive effect in stress-challenged birds and further investigation is required on the mode of action of nucleotides in *Eimeria*-challenged birds.

As aforementioned, nucleotides are essential during rapid intestinal growth or repair, which is the case in rapidly growing broilers and especially so when *Eimeria* causes damage in the gut (Grimble, 1994). Nucleotides may aid in attenuating adverse effects of *Eimeria* as it has been noted to have a beneficial effect on the absorption of nutrients and a subsequent cascading impact on cecal microbiota (Yu, 2002). Supplementation of nucleotides may also mitigate the negative effects of *Eimeria* on digestive enzymes, nutrient transporters, gut barrier, and immune function (Su et al., 2014; Kim et al., 2017). Alteration in nutrients available for fermentation in the cecum can lead to changes in the microbial population and metabolites that may impact gut growth and development (Kiarie et al., 2013). This is especially important when the impact of *Eimeria* on the microbiota is considered where growth of other bacteria such as *Lactobacillus* and *Bifidobacterium* is suppressed through change in the intestinal environment (e.g., increased flow of mucus in the ceca), predisposing the bird to necrotic enteritis through proliferation of mucolytic *Clostridium perfringens* (Williams, 2005; Hauck, 2017).

Yeast cell wall products have been shown to influence the species richness and diversity of intestinal microbiota (e.g., Roto et al., 2015). However, there is limited information on the effect of yeast nucleotides on intestinal microbiota of chickens. In a recent study, feeding dietary yeast nucleotides to specific pathogen-free chickens increased intestinal bacterial diversity and the abundance of *Lactobacillus* (Wu et al., 2018). In pigs, small or large intestine microbial populations were not influenced by dietary nucleotides (Saure et al., 2012). However, Waititu et al. (2017) showed that yeast nucleotides decreased cecal *Enterobacteriacea* and improved the proliferation of *Lactobacillus* spp. under clean conditions but increased the proliferation of cecal *Clostridium* cluster IV populations under unclean conditions. These studies indicated nucleotides could influence microbial community in poultry and pigs; however, the functional implication in the context of an enteric challenge such as *Eimeria* warrants further investigations. Moreover, differences in intestinal length and digesta transit time in poultry compared with the pig may result in different effects on microbial community and therefore lead to differences in production of SCFA and lumen pH (Moran, 1982; Jozefiak et al., 2004; Oakley et al., 2015). Therefore, the aim of the current study was to examine the effects of nucleotide-rich YN on growth performance, jejunal histomorphology, and expression of selected genes related to intestinal function and cecal microbial activity in broiler chickens challenged with *Eimeria*. It was hypothesized that dietary supplementation with nucleotides will promote gastrointestinal development and promote beneficial microbial activity and thereby attenuate the negative effects of an *Eimeria* challenge on growth performance and indices of gut health and function.

MATERIALS AND METHODS

The experimental protocol was reviewed and approved by the University of Guelph Animal Care Committee, and birds were cared for in accordance with the Canadian Council on Animal Care guidelines (CCAC, 2009).

Experimental Diets

A complete corn–soybean meal-based basal broiler diet (Table 1) was formulated to meet or exceed specifications for Ross 708 (Aviagen). The basal diet was split into 2 portions with 1 portion serving as control and the other mixed with 500 g of YN/mt. The YN contained cell wall polysaccharides (21.6%),

Table 1. Composition of the basal diet, *as fed*.

Ingredient, %	Amount
Corn	42.0
Soybean meal	26.4
Wheat	10.0
Soy oil	7.08
Pork meal	5.00
Canola meal	3.00
Vitamin-trace premix[1]	1.00
Bakery by-product	1.00
Limestone	0.62
Monocalcium phosphate	0.51
L-Lysine-HCl	1.77
DL-Methionine	0.39
L-Threonine	0.20
Choline Cl-60%	0.20
Sodium bicarbonate	0.19
Salt	0.18
Calculated provisions	
Metabolizable energy, mcal/kg	3.05
Crude protein, %	22.25
Calcium, %	0.92
Available phosphorus, %	0.46
Sodium, %	0.16
SID Lys, %	1.47
SID Met + Cys, %	0.91
SID Thr, %	0.82
SID Try, %	0.19

[1]Vitamin mineral premix provided per kilogram of premix: vitamin A, 880,000 IU; vitamin D3, 330,000 IU; vitamin E, 4,000 IU; vitamin B_{12}, 1,200 mcg; biotin, 22,000 mcg; menadione, 330 mg; thiamine, 400 mg; riboflavin, 800 mg; pantothenic acid, 1,500 mg; pyridoxine, 300 mg; niacin, 5000 mg; folic acid, 100 mg; choline, 60,000 mg; iron, 6,000 mg; and copper, 1,000 mg.

CP (32.7%), carbohydrates (14.3%), and a mixture of 5 nucleotides (1.1%; adenosine monophosphate, cytosine monophosphate, inosine monophosphate, uridine monophosphate, and guanosine monophosphate), with 1 g of the YN additive supplying approximately 0.1% of mixed nucleotides (Maxi-Gen Plus, Canadian Bio-Systems Inc., Calgary, AL, Canada). The diets were prepared in a crumble form.

Birds and Experimental Approach

A total of 360-day-old (male) Ross × Ross 708 broiler chicks were procured from a commercial hatchery (Maple Leaf Foods, New Hamburg, ON, Canada), weighed, and allocated to 24 floor pens (15 birds per pen) with fresh wood shavings. The pens were housed in 2 separate environmentally controlled rooms with 12 pens each (each pen provides 46 sq ft area). The room temperature was set to breeder recommendation of 32°C on d 0 and gradually decreased to 27°C by d 17 (Aviagen, Ross 708). Birds were exposed to fluorescent lighting in a 23 h of light (20+ lux) for the first 4 d and then a 16 L:8 D (10 to 15 lux) light cycle for the remainder of the experiment in accordance with Arkell Poultry Research Station standard operating procedures. The 2 diets, control and YN treated, were allocated to 12 floor pens in a completely randomized block (room) design. Body weight (**BW**) and feed intake (**FI**) were taken on d 10 for prechallenge growth performance. On d 10, all the birds in 6 pens of each diet received a 1 mL dose of coccidia culture administered manually with a syringe into the oral cavity. These pens were designated as the challenged group, and the birds in the non-challenged pens received a sham (i.e., distilled water) challenge of equal volume. In the challenged pens, 5 birds were challenged with a high dose (100,000 *E. acervullina* and 60,000 *E. maxima* sporulated oocysts) and 10 birds were challenged with a low dose (25,000 *E. acervulina* and 5,000 *E. maxima* sporulated oocysts) to impact duodenum and jejunum to examine consequences of altered nutrient digestion and absorption. To prevent cross contamination, non-challenged and challenged birds were in 2 separate but identical rooms in terms of pen size, temperature, and humidity regimen. *Eimeria* sp. parasites were propagated and purified as described previously (Shirley, 1995). High and low doses of mixed *Eimeria* spp. challenge were selected to provide macroscopic lesions (high dose) or modest impact on bird growth without serious lesions (low dose) based on dose titration trials conducted previously on these parasites (data not shown).

Birds were monitored for 7 d postchallenge for growth performance, lesion score, and oocyst shedding. On d 14 to 17 of age (i.e., d 4 and 7 postchallenge), fresh excreta samples were collected in a "W"-shaped route to cover as much as possible the whole pen for oocysts shedding count. On d 15, birds and feed were recorded for postchallenge growth performance and 5 birds (5 random birds per pen in the non-challenged and 5 birds with a high-dose challenge in challenged pens) were subsequently selected for necropsy. Two birds per pen (of the 5 selected per pen) were bled via wing vein puncture into sodium heparin tubes and immediately placed on ice and subsequently centrifuged at 2,000 × *g* for 20 min at 4°C. Plasma was then pipetted into a microcentrifuge tube and stored at −20°C until analysis for carotenoid. The 2 birds were then dissected for various samples as explained below. Jejunum was immediately located and excised at duodenal loop and 2 cm anterior to Meckel's diverticulum. Segments (∼3 cm) of mid-jejunum were excised and placed in buffered formalin for histomorphology analysis (Kiarie et al., 2007). Additional segments of mid-jejunum (∼1 cm) were placed in a 2 mL tube filled with 1.2 mL Ambion RNAlater (Life Technologies Inc., Burlington, ON, Canada). These samples were placed on ice and immediately transported to the laboratory and stored at −20°C until required for mRNA extraction for digestive enzymes, nutrients transporters, tight junction proteins, and cytokines expression. Cecal digesta was squeezed from excised ceca and pooled by pen. The pH of the cecal digesta was measured on fresh samples using an electronic pH meter (Accumet Basic, Fisher Scientific, Fairlawn, NJ) standardized with certified pH 4 and 7 buffer solutions. Cecal digesta samples were subsequently stored at −20°C until further analyses. The rest of intestinal samples from the 2 birds along with the intestinal samples of the other 3 necropsied birds per pen were evaluated for lesion scores. Briefly, intestinal lesion scores

(duodenum, jejunum, ileum, ceca, and colon) were assessed blindly as described by Price et al. (2014) using a scale of 0 (none) to 4 (high) (Johnson and Reid, 1970).

Sample Processing and Chemical Analysis

Oocyst Counts Oocyst per gram of excreta was analyzed with the method described by Chapman et al. (2016) with modifications (Price et al., 2014). Briefly, excreta samples (5 g) were mixed with 5 mL aqueous potassium dichromate and made up to 50 mL with deionized water and gently mixed with pipetting. A 1 mL aliquot was removed and placed in a 15 mL tube. The aliquot was then diluted with 9 mL of saturated salt solution as the floatation medium and gently mixed with pipetting. Aliquots were loaded onto a McMaster chamber slide, and the oocysts were counted using the 10× magnification on a compound microscope. Each sample was counted twice, and the mean count taken to provide a single count per pen. The mean was then divided by the weight of excreta in grams to measure the number of oocyst per gram; oocysts were not speciated.

Total Blood Carotenoid Concentration Total blood carotenoid concentration was analyzed using the method described by Donaldson (2012) with modifications. Acetone was used in place of hexane as it is more stable. Due to this change, the samples were measured at a wavelength of 478 nm, the optimal peak for beta-carotene extracted with acetone (Allen, 1986). Samples were analyzed under reduced light conditions. A 50 μL aliquot of the sample was taken and vortexed with 50 μL of ethanol for 5 s. Acetone (150 μL) was then added and the sample was homogenized by vortexing. The sample was then centrifuged for 1 min at 10,000 × *g*, and 145 μL of the acetone layer was pipetted into a 96-well plate, and the absorbance was measured using a UV spectrophotometer. A β-carotene standard curve was used to determine carotenoid concentration.

Jejunal Histomorphology Measurement Fixed jejunal tissues were cut into a longitudinal cross section and embedded in paraffin wax. The tissues were then sectioned (5 μm) and stained with hematoxylin and eosin for morphological measurements. A total of 5 villous-crypt structures were measured with a calibrated micrometer for each tissue using a Leica DMR microscope (Leica Microsystems, Wetzlay, Germany). Villous height and crypt depth ratio (**VH:CD**) was calculated.

Jejunal Expression of Selected Genes Jejunal tissue samples were stored at −20°C in RNA later until analysis. Approximately, 10 μg of jejunal tissue samples was used to extract total RNA using kit (Norgen, Biotek Corp., Thorold, ON, Canada). Quantity of RNA was analyzed using Nanodrop 8000 (Thermo Fisher Scientific, Waltham, MA), and the quality was checked using Agilent BioAnalyzer 2100 (Agilent, Santa Clara, CA). Only samples with a RNA integrity number >6.5 were used for further analyses; 2 samples (1 challenged without YN and 1 challenged with YN) failed to meet this criterion and were removed. A total of 0.5 μg RNA was used to synthesize the first strand of cDNA using the high-capacity cDNA reverse transcription kit (Applied Biosystems Inc., Foster City, CA) with RNase inhibitor as per the manufacturer's instructions. Samples were analyzed for maltase (*MGAM*), cationic amino acid transporter (*CAT1*), sodium glucose transporter 1 (*SGLT1*), occludin (*OCLN*), proliferating cell nuclear antigen (*PCNA*), interleukin-1 beta (*IL-1β*) and interleukin-10 (*IL-10*) genes. Primer sequences for *MGAM*, *CAT1*, *SGLT1*, *OCLN* and *PCNA* were taken from Kim et al. (2017) (Table 2), whereas primers for *IL-1β* and *IL-10* were designed using Primer Express 3.0 design software (Table 2). Primers were synthesized by Integrated DNA Technologies Inc. (Coralville, IA).

Quantitative real-time PCR was carried out on a StepOnePlus real-time PCR system (Applied Biosystems Inc., Foster City, CA). A 15 μL reaction mixture for RT-PCR was composed of 5 μL of cDNA, 1.9 μL of water, 0.6 μL of 10 mM forward and reverse primer each, and 7.5 μL RNA SYBR Green reagent master mix (Applied Biosystems Inc., Foster City, CA). The following conditions were used: denaturing for 30 s at 95°C, annealing for 30 s at 60°C, and repeating for 40 cycles. β-actin (*ActB*) and glyceraldehyde 3-phosphate dehydrogenase (*GADPH*) were used as housekeeping genes. A melting curve program was conducted to confirm the specificity of each product. All quantitative real-time PCR analyses were performed in duplicate. Quantitative real-time PCR efficiencies were found to be between 95 and 105%. The target gene expression was normalized with housekeeping genes and relative gene expression was determined by using the following equation: $R = 2^{(\mathrm{CT(reference)}-\mathrm{CT(test)})}$ (Kleta et al., 2004).

DNA Extraction and 16S rRNA Gene Sequencing Cecal samples were thawed and microbial genomic DNA extraction was performed using the method described by Yu and Morrison (2004). DNA concentrations were measured with Nanodrop 8000 (Thermo Fisher Scientific, Waltham, MA) and quality was assessed using Qubit (Thermo Fisher Scientific, Waltham, MA) before amplification. The V3–V4 hypervariable region of the *16S rRNA* gene was PCR-amplified and sequenced on Illumina MiSeq (Illumina, San Diego, CA) using a dual-indexing strategy for multiplexed sequencing developed at the University of Guelph's Genomics Facility, Advanced Analysis Centre (Guelph, ON, Canada), as described previously (Fadrosh et al., 2014). A subset of samples was run on a Bioanalyzer chip to ensure that amplification was successful on both indexes.

Sequence Processing and Bioinformatics Analysis Sequences were curated using Mothur v.1.37.5 as described in MiSeq SOP (Kozich et al., 2013). Briefly, contigs were generated followed by

Table 2. Forward and reverse primers used for real-time PCR.

Genes[1]	Forward primer (5′–3′)	Reverse primer (5′–3′)	GenBank Id
IL-1β (*IL-1*)	ACCAACCCGACCAGGTCAA	ACATACGAGATGGAAACCAGCAA	NM_204,524.1
IL-10 (*IL-10*)	CGACCTGGGCAACATGCT	CCTTGATCTGCTTGATGGCTTT	NM_0,010,04414
β-actin (*ActB*)	AATGGCTCCGGTATGTGCAA	GGCCCATACCAACCATCACA	NM_205,518.1
GADPH	ACTGTCAAGGCTGAGAACGG	CACCTGCATCTGCCCATTTG	NM_204,305
SGLT1	ATGCTGCGGACATCTCTGTT	TCCGTCCAGCCAGAAAGAAT	NM_0,012,93240.1
OCLN	ACGGCAGCACCTACCTCAA	GGGCGAAGAAGCAGATGAG	NM_205,128.1
CAT1	AACTGGGTTTCTGCCAGAGG	ACCCATGATGCAGGTGGAG	NM_0,011,45490.1
MGAM	AAGAACCTCTGCAACCTCCG	TCTCCGTCCACCCTATAGC	XM_01,527,3018.1
PCNA	GCCATGGGCGTCAACCTAAA	AGCCAACGTATCCGCATTGT	NM_204,170.2

[1]*GADPH*, glyceraldehyde 3-phosphate dehydrogenase; *SGLT1*, sodium glucose transporter 1; *OCLN*, occludin; *CAT1*, cationic amino acid transporter 1; *MGAM*, maltase; and *PCNA*, proliferating cell nuclear antigen.

screening to remove sequences with ambiguous base pairs and those with a length inconsistent with the target region using the "screen.seqs" command. Duplicate sequences were merged using the "unique.seqs" command followed by the alignment of the resulting non-redundant sequences to a trimmed references of SILVA 102 bacterial database using the "align.seqs" command (Quast et al., 2013).

Trimmed references of SILVA 102 bacterial database customized to our region of interest were created using the "pcr.seqs" command on an *E. coli* sequence with the primers followed by the alignment of the product to "silva.bacteria.fasta" and running the "summary.seqs" command on the aligned sequence to obtain the start and stop coordinates. Sequences that were aligned to the expected position were then kept for further processing and analyses. The "unique.seqs" command was then used to create non-redundant sequences of the aligned reads followed by the removal of chimeric sequences using the "chimera.uchime" and "remove.seqs" commands (Edgar et al., 2011). Lineages belonging to chloroplasts, mitochondria, Archaea, or eukaryotes were removed using the "remove.lineage" command. Sequences were binned into operational taxonomic units (OTUs) using the nearest neighbor algorithm with the "cluster.split" command, and were then used before conversion to ".shared" format using the "make.shared" command followed by generation of consensus taxonomy for each OTU using the "classify.otu" command. The "sub.sample" command in Mothur was then used to ensure 9,117 sequences for each sample. Taxonomy was also assigned to each sequence using the Ribosomal Database Project bacterial taxonomy classifier.

Short-Chain Fatty Acids The concentrations of SCFA (lactic, formic, acetic, propionic, isobutyric, and n-butyric) in the ceca digesta were assayed according to Leung et al. (2018). Briefly, the digesta was thawed and approximately 0.1 g was resuspended with 1 mL of 0.005 N H_2SO_4 (1:10 wt/vol) in a microcentrifuge tube. The tube was vortexed vigorously until the sample was completely dissolved, centrifuged at 11,000 x g for 15 min, and 400 μL of the supernatant was transferred into a high-pressure liquid chromatography vial and 400 μL of 0.005 N H_2SO_4 buffer was added. The resulting digesta fluid was then assayed for SCFA using high-pressure liquid chromatography (Hewlett Packard 1100, Germany) with a Rezex ROA-Organic Acid LC column, 300 × 7.8 mm from Phenomenex, and a refractive index detector at 40°C (Agilent 1260 Infinity RID; Agilent Technologies, Germany). Twenty microliter of the resulting sample was injected into the column, with a column temperature of 60°C and mobile phase of 0.005 N H_2SO_4 buffer at 0.5 mL/min isocratic for 35 min. The detector was heated to 40°C.

Calculation and Statistical Analysis

Pen was the experimental unit for this study. Prechallenge performance data were subjected to a one-way ANOVA of the GLM procedures. (SAS Inc., Cary, NC). Postchallenge data were subjected to a two-way ANOVA (with d 10 BW as a co-variate for performance data) using the GLM procedures (SAS Inc.) with diet, *Eimeria* challenge, and two-way interaction as fixed effects. There was no oocyst shedding detected in non-challenged birds; therefore, oocyst shedding was analyzed in challenged birds only. Oocyst shedding was analyzed for the effects of diet, time, and diet×time interactions using the PROC Mixed procedure (SAS Inc.). Intestinal lesions were detected in highly challenged birds only, and data were subsequently analyzed using a one-way ANOVA of the GLM procedure. All OTU-based analyses for alpha and beta diversities were performed in Mothur (University of Michigan, Ann Harbor, MI). In order to identify and visualize taxa with differential abundance in the control and YN-fed groups or *Eimeria* challenged and non-challenge groups, the linear discriminant analysis Effect Size (LEfSe) algorithm was used where treatment groups were assigned as comparison classes and LEfse identified features that were statistically different between the 2 treatments were then compared using the non-parametric factorial Kruskal-Wallis sum-rank test, and linear discriminant analysis > 2 (Segata et al., 2011). An α level of $P \leq 0.05$ was used as the criterion for assessing for statistical significance, and trends ($0.05 > P \leq 0.10$) were discussed.

Table 3. Growth performance of broiler chickens fed a corn–soybean meal diet supplemented with nucleotide-rich yeast extract (YN) before (d 0 to 10) *Eimeria* challenge.

	YN, g/mt			
	0	500	SEM	*P*-value
Initial BW, g	40.6	40.6	0.177	–
Final BW, g	303.7	303.9	3.043	0.949
BWG, g/bird	252.5	253.2	3.133	0.879
Feed intake, g/bird	291.4	285.7	2.696	0.139
FCR	1.154[b]	1.128[a]	0.007	0.005

Means assigned different letter superscripts within a row differ, $P \leq 0.05$.

Table 4. Growth performance in broiler chickens fed a corn–soybean meal diet supplemented with nucleotide-rich yeast extract (YN) and challenged (d 11 to 15) with *Eimeria*.

Eimeria	YN, g/mt	Final BW, g	BWG, g/bird	Feed intake, g/bird	FCR
No	0	411.8	168.8	193.8	1.154
No	500	417.3	177.5	189.2	1.064
Yes	0	389.3	147.0	202.0	1.417
Yes	500	409.3	163.0	182.3	1.119
	SEM	6.11	5.58	9.195	0.104
Main effect,	No	415.7[a]	172.8[a]	192.3	1.120
Eimeria	Yes	398.2[b]	155.3[b]	191.2	1.257
Main effect,	0	400.7[a]	157.9[b]	197.9	1.287
YN, g/mt	500	413.2[b]	170.3[a]	185.8	1.090
	SEM	3.99	3.95	6.5	0.074
Probabilities					
Eimeria		0.021	<0.01	0.943	0.142
YN		0.049	0.039	0.201	0.078
Eimeria×YN		0.249	0.519	0.424	0.329

Means assigned different letter superscripts within a response criterion differ, $P \leq 0.05$.

Table 5. Jejunal histomorphology and total blood carotenoid concentration in broiler chickens fed a corn–soybean meal diet supplemented with nucleotide-rich yeast extract (YN) and challenged with *Eimeria*, 5 d postchallenge.

Eimeria	YN, g/mt	Villi height, μm	Crypt depth, μm	VH:CD[1]	Carotenoid, μg/mL
No	0	1,020[a]	230.5	4.91	2.67
No	500	892.4[b]	192.6	4.93	1.95
Yes	0	447.2[d]	339.0	1.43	2.54
Yes	500	533.1[c]	323.0	1.79	1.95
	SEM	27.59	18.09	0.29	0.08
Main effect,	No	956.6[a]	211.6[b]	4.92[a]	2.60[a]
Eimeria	Yes	490.2[b]	331.0[a]	1.61[b]	1.95[b]
Main effect,	No	734.0	284.8	3.17	2.31
YN, g/mt	Yes	712.8	257.8	3.36	2.24
	SEM	19.51	12.77	0.21	0.059
Probabilities					
Eimeria		<0.01	<0.01	<0.01	<0.01
YN		0.452	0.152	0.516	0.440
Eimeria×YN		0.001	0.552	0.571	0.426

Means assigned different letter superscripts within a response criterion differ, $P \leq 0.05$.
[1]Villi height/crypt depth ratio.

RESULTS

Growth Performance

There were no significant ($P > 0.05$) diet effects on BWG and FI during the prechallenge phase (d 0 to 10; Table 3). YN-fed birds had significantly better ($P = 0.005$) FCR than that of control-fed birds during the prechallenge phase. There was no interaction between diet and *Eimeria* on BWG, FI, and FCR in postchallenge phase (d 11 to 15; $P > 0.05$; Table 4). The main effects were such that the *Eimeria* challenge significantly depressed BWG by 4.3% ($P < 0.01$), whereas YN-fed birds showed a significantly higher BWG ($P < 0.01$) and a tendency for improved FCR ($P = 0.08$; Table 5).

Oocyst Count and Intestinal Lesion Scores

There were no ($P > 0.05$) diet and sampling day or diet effects on oocyst shedding. The oocyst shedding from the low-dose birds was 189, 86,871, 103,339, and 69,783 (SEM = 17,546) oocysts/g excreta on d 4, 5, 6, and 7 postchallenge, respectively. Generally, oocyst shedding was higher on d 5, 6, and 7 postchallenge compared to that on d 4 ($P < 0.01$). The challenge with *E. acervulina* and *E. maxima* mainly affected the duodenum and jejunum, and there was no effect of YN or YN and *Eimeria* interactions (data not shown). The duodenum scores for high-dose birds were 2.87 and 2.83 (SEM = 0.13) for control and YN-supplemented birds, respectively. Corresponding scores in the high-dose birds for jejunum were 2.2 and 2.4 (SEM = 0.14), ileum were 0.3 and 0.43 (SEM = 0.09), ceca were 0.07 and 0.00 (SEM = 0.03), and colon were 0.03 and 0.00 (SEM = 0.02).

Total Blood Carotenoid Concentration and Jejunal Histomorphology and Expression of Selected Genes

There was a significant interaction ($P = 0.001$) effect between YN supplementation and *Eimeria* challenge on VH (Table 5) such that VH was shorter (892 vs. 1,021 μm) in YN-fed birds without a challenge but longer (533 vs. 447 μm) in *Eimeria*-challenged birds. *Eimeria* challenge significantly decreased VH (490 vs. 957 μm, $P < 0.01$), increased crypt depth (331 vs. 212 μm, $P < 0.01$), and decreased VH:CD ratio (1.61 vs. 4.92, $P < 0.01$) compared to non-challenged birds. Supplementation of YN had no ($P > 0.05$) effects on crypt depth or VH:CD ratio. *Eimeria* challenge significantly decreased total blood carotenoid concentrations from 2.6 μg/mL in non-challenged birds to 1.9 μg/mL in challenged birds ($P < 0.01$; Table 5).

The data for jejunal expression of digestive enzymes, nutrient transporters, tight junction protein, cytokines, and PCNA are presented in Table 6. Significant interaction between YN and *Eimeria* challenge was observed only for *CAT1* expression ($P = 0.04$) such that YN-fed birds had a higher (1.65 vs. 0.78) expression of *CAT1* when subjected to *Eimeria* than that

Table 6. Jejunal expression level of nutrient transporters, digestive enzymes, tight junction proteins, cytokines, and PCNA in broilers fed a corn–soybean meal-based diet supplemented with nucleotide-rich yeast extract (YN) and challenged with *Eimeria*, 5 d postchallenge.

Eimeria	YN, g/mt	*PCNA*	*CAT1*	*SGLT1*	*OCLN*	*MGAM*	*IL1β*	*IL10*
No	0	0.80	0.64^{c}	0.87	0.85	0.98	0.63	1.31
No	500	1.13	0.78^{c}	0.89	0.76	1.17	0.68	0.58
Yes	0	1.54	2.55^{a}	0.43	0.60	0.42	0.57	8.09
Yes	500	1.78	1.65^{a,b}	0.44	0.52	0.48	0.61	7.07
	SEM	0.216	0.236	0.116	0.062	0.113	0.119	1.118
Main effect, *Eimeria*	No	0.97^{b}	0.71^{b}	0.88^{a}	0.81^{a}	1.07^{a}	0.65^{b}	0.94^{b}
	Yes	1.66^{a}	2.10^{a}	0.44^{b}	0.56^{b}	0.41^{b}	0.59^{a}	7.58^{a}
Main effect, YN, g/mt	0	1.17	1.59	0.66	0.72	0.78	0.68	4.70
	500	1.46	1.21	0.65	0.64	0.78	0.65	3.82
	SEM	0.154	0.168	0.082	0.042	0.080	0.084	0.791
Probabilities								
Eimeria		<0.01	<0.01	<0.01	<0.01	<0.01	0.618	<0.01
YN		0.194	0.120	0.962	0.164	0.482	0.682	0.442
Eimeria×YN		0.827	0.037	0.899	0.976	0.351	0.982	0.898

PCNA, proliferating cell nuclear antigen; *CAT1*, cationic amino acid transporter 1, *SGLT1*, sodium glucose transporter 1; *OCLN*, occludin; *MGAM*, maltase; *IL1β*, interleukin-1 beta; *IL10*, interleukin 10.

Means assigned different letters within a response criterion differ, $P \leq 0.05$.

of non-challenged birds. Compared to non-challenged control, *Eimeria* significantly increased ($P < 0.01$) the expression of *PCNA* and *IL-10* by 1.7-fold and 7.6-fold, respectively, and decreased ($P < 0.01$) the expression of *SGLT1*, *OCLN*, and *MGAM* by 2.3-, 1.8-, and 2.4-fold, respectively (Table 6). Supplemental YN did not influence ($P > 0.05$) *PCNA*, *IL-1*, *IL-10*, *SGLT1*, *OCLN*, and *MGAM*.

Cecal Digesta Microbiota, pH, and Concentration of Short Chain Fatty Acids

There were no interaction ($P > 0.05$) between *Eimeria* challenge and YN on alpha diversity and relative abundance of microbial populations at phylum and genus levels (Table 7). *Eimeria* challenge had no ($P > 0.05$) effect on diversity measures (inverse-Simpson, Chao 1, and Shannon). Dietary supplementation of YN tended to decrease Chao1 (5,973 vs. 6,931, $P = 0.069$) compared to non-YN diet (Table 7). *Eimeria* challenge tended to decrease the abundance of phylum *Firmicutes* (88.7 vs. 83.8%, $P = 0.06$) and significantly decreased the abundance of genus *Clostridium* XIVa (9.94 vs. 5.37%, $P = 0.03$) and *Oscillibacter* (1.67 vs. 1.09%, $P = 0.05$). *Eimeria* challenge tended to increase the relative abundance of genus *Anaerostipes* (0.44 vs. 1.11%, $P = 0.06$). Supplemental YN significantly increased the abundance of genus *Anaerostipes* (0.28 vs. 1.21%, $P = 0.01$) and tended to increase the abundance of genus *Oscillibacter* (1.68 vs. 1.14%, $P = 0.06$) relative to non-supplemented diets. Birds fed the YN diet tended to show lower abundance of genus *Clostridium* XIVa (6.14 vs. 9.59%, $P = 0.06$) than birds fed the non-YN diets.

A significant interactive effect ($P < 0.05$) between *Eimeria* challenge and YN supplementation was observed for cecal digesta pH, propionic acid, and total SCFA concentrations (Table 8). In this context, supplemental YN significantly increased pH to more basic (6.85 vs. 6.21) and significantly decreased propionic (30.1 vs. 41.0 μmol/g) and total SCFA (219 vs. 251 μmol/g) compared with non-supplemented birds in *Eimeria*-challenged birds. However, in absence of *Eimeria* challenge, YN-fed birds exhibited significantly higher cecal digesta concentration of total SCFA (253 vs. 216 μmol/g) than non-supplemented birds. *Eimeria* challenge significantly increased N-butyric acid concentration ($P = 0.01$) by 10% and decreased lactic acid concentration ($P = 0.10$, 33.0 vs. 36.1 μmol/g; Table 8).

DISCUSSION

Concerns on antibiotic resistance have led to decreased antibiotic usage for growth promotion and increased focus on usage of vaccines and antibiotic alternatives that are not without their drawbacks. In the case of coccidiosis vaccines, negative effects on growth performance and intestinal lesions are a concern (Chapman et al., 2013). To attenuate these negative effects, dietary YN has been proposed. In a healthy adult animal, de novo synthesis and salvage pathways are considered to be sufficient (Sanchez-Pozo and Gil, 2002). However, there is a higher demand for nucleotides when birds are undergoing a disease challenge or subjected to other forms of stress (Sanchez-Pozo and Gil, 2002; Jung, 2011). For example, supplementation of dietary nucleotides has been shown to improve pig growth (Waititu et al., 2016, 2017); however, some previous studies on broilers have not shown improvement in growth performance, which was attributed to nucleotides being conditionally essential in birds not subjected to health or stress challenge (Jung, 2011; Hess and Greenberg, 2012; Alizadeh et al., 2016). We observed improved FCR during the prechallenge phase (d 0 to 10), and independently of *Eimeria* challenge, YN improved BWG and final BW by 8 and 3%, respectively, during the postchallenge phase in low-dose

Table 7. Alpha diversity and relative bacterial abundance (%) at the phylum and genus levels in broiler chickens fed a corn–soybean meal diet supplemented with nucleotide-rich yeast extract (YN) and challenged with *Eimeria*, 5 d postchallenge.

	Main effect, *Eimeria*			Main effect, YN, g/mt			Probabilities	
	No	Yes	SEM	No	Yes	SEM	*Eimeria*	YN
Alpha diversity								
Coverage[1]	0.98	0.98	0.004	0.98	0.98	0.004	0.580	0.613
Inverse-simpson[2]	31.5	28.5	0.193	31.2	29.2	0.193	0.521	0.674
Chao1[3]	6474	6426	241	6931	5973	123	0.925	0.069
Shannon[4]	4.49	4.53	0.01	4.54	4.48	0.01	0.774	0.601
Phylum								
Bacteroidetes	8.65	12.3	1.52	10.8	9.79	1.63	0.130	0.650
Firmicutes	88.7	83.8	1.78	86.4	86.5	1.57	0.064	0.971
Actinobacteria	2.29	3.62	0.67	2.41	3.38	0.67	0.930	0.323
Genus								
Faecalibacterium	27.0	23.6	3.22	24.8	26.0	3.23	0.484	0.795
Alistipes	18.6	25.1	3.05	23.0	20.1	3.24	0.174	0.542
Clostridium_IV	9.84	8.77	0.87	9.62	9.09	0.87	0.415	0.682
Clostridium_XlVa	9.94[a]	5.37[b]	1.37	9.59	6.14	1.49	0.034	0.098
Acetanaerobacterium	5.17	5.86	0.43	5.75	5.22	0.44	0.283	0.402
Bifidobacterium	4.35	6.32	1.44	4.29	6.19	1.43	0.361	0.375
Subdoligranulum	4.24	5.79	2.13	3.45	6.44	2.08	0.615	0.333
Butyricicoccus	5.03	4.13	0.58	4.58	4.67	0.60	0.282	0.917
Flavonifractor	3.40	3.27	0.61	2.66	4.03	0.66	0.900	0.193
Blautia	3.19	3.00	0.68	3.87	2.34	0.64	0.859	0.169
Clostridium_XlVb	2.52	2.45	0.39	2.37	2.60	0.39	0.900	0.694
Oscillibacter	1.67[a]	1.09[b]	0.17	1.14	1.68	0.21	0.050	0.062
Ruminococcus2	1.35	1.17	0.27	1.48	1.05	0.27	0.658	0.293
Pseudoflavonifractor	1.10	1.12	0.16	1.08	1.14	0.16	0.915	0.829
Anaerostipes	0.44	1.11	0.27	0.28[b]	1.21[a]	0.21	0.063	0.013

[1]Good's coverage represents the percentage of the total species represented in a sample.
[2]Indicator of richness in a microbial community with uniform richness with the same level of diversity.
[3]Estimator of diversity from abundance data.
[4]Characterizes species diversity considering abundance and evenness of species present.
Means assigned different letter superscripts within a response criterion differ, $P \leq 0.05$.

Table 8. Ceca digesta pH and concentration of short chain fatty acids (SCFA) in broiler chickens fed a corn–soybean meal diet supplemented with nucleotide-rich yeast extract (YN) and challenged with *Eimeria*, 5 d postchallenge.

			SCFA, μmol/g of ceca digesta						
Eimeria	YN, g/MT	Ceca pH	Lactic	Formic	Acetic	Propionic	Iso-butyric	n-Butyric	Total SCFA[1]
No	0	6.78[a,b]	36.1	9.0	72.0	31.6[a]	16.8	42.3	215.9[b]
No	500	6.76[a,b]	34.8	10.4	86.3	33.2[a,b]	16.8	42.8	253.1[a]
Yes	0	6.21[b]	36.1	10.9	78.2	41.0[a]	16.3	48.3	250.5[a]
Yes	500	6.85[a]	31.1	8.4	72.7	30.1[b]	16.1	45.9	219.4[b]
	SEM	0.15	1.81	1.58	5.85	2.17	0.62	1.60	10.90
Main effect, *Eimeria*	No	6.77	36.1	9.7	79.2	32.4	16.9	42.5[b]	234.5
	Yes	6.53	33.0	9.7	75.5	35.5	16.2	47.1[a]	235.0
Main effect, YN, g/mt	0	6.49[b]	35.5	9.9	75.1	36.3	16.5	45.3	233.2
	500	6.81[a]	33.6	9.4	79.5	31.6	16.5	44.4	236.2
	SEM	0.11	1.28	1.01	4.14	1.54	0.44	1.13	7.71
Probabilities	*Eimeria*	0.131	0.100	0.976	0.537	0.163	0.318	0.011	0.968
	YN	0.056	0.307	0.730	0.457	0.043	0.906	0.572	0.782
	Eimeria×YN	0.040	0.313	0.196	0.108	<0.01	0.845	0.386	<0.01

Means assigned different letter superscripts within a response criterion differ, $P \leq 0.05$.
[1]Summation of lactic, formic, acetic, propionic, iso-butyric, and n-butyric acids.

birds. Moreover, it is noteworthy that even without significant interaction effects, YN improvement in BWG and FCR was numerically higher in *Eimeria*-challenged compared to that of non-challenged birds. Differences in performance effects compared to studies by other authors can be attributed to differences in the level of immune challenge and the environmental conditions under which the birds were raised (Jung, 2011).

Eimeria is known for its negative effects on growth performance, causing a loss in BWG in the current study. *Eimeria* targets mainly the intestines for its reproduction and is the cause of the avian disease coccidiosis (Chapman et al., 2016). *Eimeria* replicates within the intestinal wall of the chicken causing lesions (Chapman, 2014). Locations of lesions vary depending on the species, but for the species used in the current

study, *E. acervulina* and *E. maxima*, they mainly targeted the duodenum and jejunum, respectively, with lesions extending toward the distal end of the intestine in cases of severe infection (Chapman, 2014). Depending on the severity of the infection, *E. acervulina* can also cause inflammation of the intestine resulting in dehydration and malabsorption of nutrients, whereas *E. maxima* causes small hemorrhages and damage to intestinal epithelia, with both species in extreme cases capable of causing death (Chapman, 2014). Further investigations on the effects of *Eimeria*, noted lower protein levels in the intestinal wall, causing decreased carotene absorption, and a concomitant decrease in vitamin A levels in the blood (Kouwenhoven and Van der Horst, 1969). In line with these long-known features, challenge with the 2 *Eimeria* species (*E. acervulina* and *E. maxima*) in the current study resulted in lesions located mostly in the duodenum and jejunum. Lesions found after Meckel's diverticulum (ileum, cecum and colon) likely resulted from oocyst colonization extending distally from the jejunum. *Eimeria acervulina* was reported to decrease carotene absorption resulting in decreased vitamin A levels in the blood (Kouwenhoven and Van der Horst, 1969). This may explain decreased levels of total blood carotenoid concentration in the current study. An increase in oocyst shedding on d 5 postchallenge was attributed to increased shedding of *E. acervulina*, and the increase in oocyst count on d 6 and 7 postchallenge occurred from the increased shedding of *E. maxima.* Collectively, the oocysts and lesion score data indicated the development of coccidiosis and corroborate with our previous study in cage housing (Kim et al., 2017). Supplemental YN had no effects on oocyst shedding, lesion scores, and blood carotenoid concentrations, indicating that YN does not have an effect on the replication of *Eimeria* in the gut. Further indication of damaged intestinal surface was demonstrated by decreased VH and increased crypt depth in response to *Eimeria* challenge. The interactive effect of YN and *Eimeria* was also observed by Jung (2011) and Alizadeh et al. (2016) with YN increasing VH. The improved VH noted in the current study may have contributed to the improved growth performance noted in YN-fed birds (Xu et al., 2003). It is of note that the nucleotide product used is not a pure source of nucleotide, and as such, effects may also be attributed to other components of the product such as yeast cell wall polysaccharides (Waititu et al., 2017).

In the current study, *Eimeria* infection decreased the expression level of *MGAM* and *SGLT1*, genes associated with carbohydrates digestion and nutrient transport, respectively, depicting adverse changes in both nutrient digestion and absorption (Su et al., 2014). This was in agreement with research demonstrating *E. acervulina and E. maxima* infection down-regulated the expression of digestive enzymes and nutrients transporters in broiler chickens (Su et al., 2014, 2015; Kim et al., 2017). The interactive effect between *Eimeria* and YN on *CAT1*, an amino acid transporter, suggests that dietary YN may attenuate the negative effects of *Eimeria* (Su et al., 2014). Expression of *CAT1* is known to increase with limited amino acid availability in the intestinal wall to allow survival of cells under stress conditions and allow cells to resume growth as soon as amino acids are available again (Hatzoglou et al., 2004). The lack of change in FI concomitant with reduced BWG indicates alterations in digestibility and nutrient absorption (Wen et al., 2015). *Eimeria* also altered gene expression levels of *OCLN*, *PCNA*, and *IL-10*. Decrease in *OCLN* expression levels indicated increased gut permeability, as tight junction protein OCLN along with claudins and cadherins is required for the intestinal epithelial barrier to function properly (Al-Sadi et al., 2011). Decreased expression of *OCLN* has been noted in human patients with intestinal permeability disorders, especially in regard to macromolecules (Al-Sadi et al., 2011). This decrease in OCLN may further predispose the broiler to other intestinal health challenges. Increased *PCNA* gene expression is another indication of sloughed intestinal epithelial cells, as PCNA plays a vital role in cell reparation and DNA repair (Kelman, 1997; Kim et al., 2017).

Changes in absorption and digestibility of nutrients as well as intestinal permeability and environment by *Eimeria* may also affect intestinal microbial populations (Williams, 2005; Hauck, 2017). The increased mucogenesis and nutrients flowing into the cecum can cause changes in relative populations of bacteria, due to unique preferences for organic substrate and ability to respond to availability of highly digestible nutrients (Kiarie et al., 2013; Pan and Yu, 2014; Hauck, 2017). This can result in increased proliferation of pathogens such as *C. perfringens* that thrive in this type of environment, particularly high levels of nitrogen-rich mucin (Hauck, 2017). Additionally, the microbiome has been closely correlated with immune health, nutrient absorption, and growth performance in birds (Clavijo and Florez, 2018). Interestingly, despite associations between increased species diversity and improved bird performance, YN tended to decrease Chao1, a measurement of species richness, with no changes to other indicators of alpha diversity measured (inverse-Simpson, Shannon, and Good's coverage). This result indicated no effect on the abundance of species despite the tendency for a decrease in species diversity, which may not be beneficial to the bird as increased microbial diversity is associated with improved gut health (Gotelli and Chao, 2013; Stanley et al., 2014; Clavijo and Florez, 2018). This is similar to the effect of antimicrobials on alpha diversity where addition of an antibiotic growth promoter decreased alpha diversity in broilers (Salaheen et al., 2017). The use of the antibiotic growth promoter has also been shown to increase the *Firmicutes* to *Bacteriodetes* ratio in obese mice and broilers (Turnbaugh et al., 2006; Salaheen et al., 2017). *Firmicutes*, such as *Clostridium* XIVa and IV, and *Bacteriodetes*, such as Alistipes, are both considered

important for the breakdown of indigestible polysaccharides in the gut, and the ratio between the two is correlated with obesity in humans and mice due to changes in energy absorption (Turnbaugh et al., 2006). In chickens, increases in the *Firmicute* population are linked with obesity and increased fat deposition, as seen in differences between obese and lean chicken lines (Torok et al., 2011; Hou et al., 2016). This may imply changes in fat deposition in *Eimeria*-challenged birds as *Eimeria* decreased *Firmicute* abundance and decreased BWG and FCR—the benchmarks of commercial broiler performance.

Additional effects from microbial populations and associated metabolites can occur with metabolites from microbial fermentation of complex polysaccharides such as butyric acid utilized by enterocytes for energy (Bedford and Gong, 2017). Around 8% of the maintenance energy required by a broiler can be provided by SCFA and can signal the broiler intestine as an immune stimulation (Jozefiak et al., 2004). Another effect of SCFA was demonstrated by Panda et al. (2009), where butyrate supplementation increased villus height due to reduced inflammatory reactions. Similar to butyrate but with a weaker effect, propionate has been observed to be a stimulator of the gut to signal a decrease in inflammation in rats and induce cell proliferation in crypt cells (Hosseini et al., 2011; Vinolo et al., 2011). In the current study, as seen with the interactive effect in VH, expression of *CAT1*, cecal pH, and propionic acid YN acted beneficially when birds were challenged with *Eimeria*, but not in unchallenged birds, leading to the proposition that YN may be beneficial in immuno-challenged birds, but acts as a negative stimulant in healthy birds. This was not shown when gene expressions of IL-1β, a pro-inflammatory cytokine, and IL-10, an anti-inflammatory cytokine, were examined as YN had no effect on either and *Eimeria* increased expression of IL-10. To account for lack of effect, relative expression of other genes such as those in the TNF family, IFN-γ, and TGF-β or in other immune organs such as the spleen and thymus may be more responsive to *Eimeria* challenge (Choi et al., 1999; Wigley and Kaiser., 2003). The lack of effect in interleukin expression at the RNA level may also be alternately due to changes at the RNA translation level instead (Mazumder et al., 2010). Inflammation in the gut can also decrease as a result of signaling by microbial populations through the use of butyrate production as butyrate has been reported to inhibit the production of pro-inflammatory cytokines (Vinolo et al., 2011). Interestingly, *Eimeria* increased n-butyric acid concentrations by 10.5% despite the decrease of the relative abundance of bacteria of the phylum *Firmicutes*, specifically bacteria from the genus *Clostridia* cluster *XIVa* and *Oscillibacter*, which are both dominant producers of butyric acids (Onrust et al., 2015). Although *Anaerostipes* are also butyrate producers, the increase in the relative population does not necessarily compensate for the loss of butyrate production with the decrease in *Clostridium* cluster *XIVa* and *Oscillibacter* (Eeckhaut et al., 2010).

Immune health in *Eimeria*-challenged broilers may also be affected by competitive exclusion with a more acidic cecal pH. Although a more acidic pH is beneficial due to competitive exclusion of colonization by pathogenic bacteria such as *Salmonella*, this may not be the case in the cecum compared to other parts of the intestine (Corrier et al., 1990). Yeast nucleotides caused a more basic cecal pH, but only when broilers were challenged with *Eimeria*, in relation to the concentration of total SCFA. Corrier et al. (1990) noted that changes in SCFA concentrations were associated with cecal pH as pH indicates the percentage of SCFA dissociated into its acid form.

Eimeria is prevalent world-wide and care can only be taken to mitigate its multifaceted negative effects ranging from intestinal damage to changes in microbiome populations and intestinal development (Kouwenhoven and Van der Horst, 1969; Chapman, 2014). In the current study, dietary supplementation with YN had benefits dependent and independent of *Eimeria*. Dietary YN was effective in increasing growth performance regardless of *Eimeria*. Supplemental YN attenuated effects of *Eimeria* in the case of villus development and cecal pH; however, it negatively affected villus development in the absence of an *Eimeria* challenge. Although both YN and *Eimeria* can affect microbial populations, effects on populations are independent of each other and effects on SCFA concentrations and cecal pH are interactive. However, further research is required to elucidate effects of YN and *Eimeria* on nutrient digestibility due to effects seen on intestinal development and microbial population.

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