2-Nitro-1-propanol improved nutrient digestibility and oocyst shedding but not growth performance of *Eimeria*-challenged broilers

Po-Yun Teng ,* Sudhir Yadav,* Tatiane Souza dos Santos,† Alberta Lorraine Fuller,* and Woo Kyun Kim*,[1]

*Department of Poultry Science, University of Georgia, Athens, GA, USA; and †College of Veterinary Medicine and Animal Science, Sao Paulo State University, Botucatu, SP, Brazil

ABSTRACT A 2 × 3 factorial arrangement study was conducted to evaluate 3 dosages of 2-nitro-1-propanol (**NP**; 0, 150, and 200 ppm) on intestinal health of birds with or without *Eimeria* challenge. A total of 432 thirteen-day-old male broiler chickens were randomly allocated to 6 treatments with 8 replicate cages of 9 birds per cage. All birds were fed with treatment diets from day 13 to 21. Birds in the challenge groups were gavaged with *Eimeria maxima* (50,000 oocysts per bird), *Eimeria tenella* (50,000 oocysts per bird), and *Eimeria acervulina* (250,000 oocysts per bird) on day 15. Growth performance was evaluated from day 13 to 21, and gut permeability was measured by fluorescein isothiocyanate dextran on day 20. The intestinal lesion, intestinal morphology, and oocysts shedding were determined at the end of the trial. The linear and quadratic orthogonal polynomial contrasts were used to evaluate the effects of increasing NP doses in responses to *Eimeria* challenge. The results showed that NP was not able to maintain efficient growth performance but improved gut leakage during *Eimeria* infection period. On the other hand, *Eimeria* infection increased gut permeability ($P < 0.0001$) and reduced ileal digestible energy (**IDE**) and apparent ileal digestibility (**AID**) of nitrogen. However, the increase of NP linearly enhanced IDE and AID of nitrogen ($P < 0.01$). Moreover, an interaction between challenge and linear dosage effects was observed for IDE ($P = 0.0066$) and AID of nitrogen ($P = 0.0462$). The results indicated that NP improved nutrient digestibility and reduced total oocysts shedding in birds challenged with *Eimeria* spp. Besides, higher NP doses numerically improved villi height in the intestine. In summary, NP was not able to maintain growth performance of birds but presented positive outcomes on nutrient digestibility and reduced oocysts shedding during mixed *Eimeria* infection.

Key words: 2-nitro-1-propanol, nitro-compound, Eimeria, coccidiosis, digestibility

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INTRODUCTION

Nitro-compounds consist of nitro functional groups bound to the carbon of alcohol, such as 2-nitro-1-propanol (**NP**, $C_3H_7NO_3$) (Olender et al., 2018). The NP and nitro-ethanol could inhibit food-born pathogens, including *Salmonella*, *Enterococcus*, *Campylobacter*, and *Eimeria coli* (Jung et al., 2003, 2004a; Dimitrijevic et al., 2006; Horrocks et al., 2007). In addition, it was reported that the microbial inhibitory ability of nitro-compounds was stronger than that of their acid and alcohol counterparts (Kim et al., 2006). Nitro-compounds were first tested in a ruminant study to replace dietary supplementation of nitrate because of nitrate toxicity to animals when it is metabolized to nitrite in the rumen (Anderson et al., 2003). Nitro-compounds could act as methane inhibitors and decreased 94% of methane production in ruminal fluid (Anderson et al., 2003). Furthermore, NP exhibited microbial inhibitory effects on broiler chickens and laying hens by reducing *Salmonella typhimurium* in the intestine, as well as upregulated immune responses against pathogens invasion (Jung et al., 2004b; Adhikari et al., 2017).

A previous study reported that NP and NE also inhibited development of *Eimeria* spp. both in vitro and in vivo (Teng et al., 2020a). The numbers of sporozoites of *Eimeria tenella* were reduced by 0.5 mM of NP

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[1]Corresponding author: wkkim@uga.edu

and NE in the Madin-Darby bovine kidney cells. The NP also improved nutrient digestibility and reduced intestinal lesion scores of broiler chickens challenged with *Eimeria acervulina*, *E. tenella*, and *Eimeria maxima*. However, dietary supplementation of 200 ppm NP would reduce body weight gain (**BWG**) (Teng et al., 2020a). There were limited studies indicating toxicity of NP to broiler chickens. A previous study reported that birds gavaged with 13 mg of NP had no effects on growth performance (Jung et al., 2004b); however, it did not investigate the maximum tolerable dosage of NP applied in broiler diets. Based on the results from previous experiments (Teng et al., 2020a), 200 ppm might exceed the safe levels of NP for 21-day-old chickens, but 100 ppm NP had no effects on inhibiting parasites. Instead, 150 ppm might be an appropriate dosage which could inhibit pathogens but maintain growth performance of birds. Furthermore, previous studies have not reported how nitro-compounds impact on birds raised in a nonchallenged environment. It is hypothesized that NP might show some beneficial effects similar to other coccidiostats by inhibiting development of sporozoites in epithelial cells. Thus, the objective of the study was to evaluate the effects of 150 ppm and 200 ppm of NP on growth performance, intestinal leakage, nutrient digestibility, oocysts shedding, and intestinal lesion score of birds challenged with or without *E. acervulina*, *E. tenella*, and *E. maxima*.

MATERIALS AND METHODS

Experimental Design, Diet Formulation, and Eimeria Challenge

The study was approved by the Institutional Animal Care and Use Committee at the University of Georgia, Athens, GA. The completely randomized design was used in the study. A total of 432 thirteen-day-old male Cobb 500 chicks (males from the Cobb 500 female line) were randomly distributed in a factorial arrangement 2 × 3 to evaluate the effects of 3 doses of NP, 0, 150, and 200 ppm in nonchallenged or *Eimeria*-challenged broilers. Each treatment had 8 replicate cages with 9 birds per cage. The experimental unit in the present study is cage. The six treatments included a nonchallenged control (**NCC**: no NP), 2 groups of NP supplementation (150 ppm or 200 ppm) without *Eimeria* challenge (**NCNP150; NCNP200**), a challenge control (**CC**: no NP), and 2 groups of NP supplementation (150 ppm or 200 ppm) with *Eimeria* challenge (**CNP150; CNP200**). On day 15, birds in the challenged groups (CC, CNP150, and CNP200) were gavaged with a mix of 3 *Eimeria* spp. that contained *E. maxima* (50,000 oocysts per bird), *E. tenella* (50,000 oocysts per bird), and *E. acervulina* (250,000 oocysts per bird). The NP treatment diets were formulated by mixing 150 or 200 ppm of NP in the corn-soybean meal–based basal diets which contained 0.3% chromium dioxide as an indicator for measuring apparent ileal digestibility (**AID**). Feed and water were provided ad libitum, and the environmental temperature program was followed to the recommendation of Cobb Broiler Management Guide (Cobb. 2013).

Growth Performance and Sample Collection

Body weight (**BW**) and feed intake (**FI**) of the birds were weighed on the first day of the experiment and 6 D after infection (**DPI**). BWG and feed conversion ratio (**FCR**) were calculated from day 13 to 21. Mortalities were removed and recorded to adjust FCR. On 6 DPI, 6 birds from each cage were sacrificed by cervical dislocation. The gross intestinal lesion was scored according to the four-score scale described by Johnson and Reid (1970). Ileal digesta were collected from the one-third section of the ileum from the ileo-cecal-colic junction to the Meckel's diverticulum. Feces samples were collected on 6 DPI from each cage to count oocysts shedding. Intestinal tissue was collected from one bird per pen for measuring intestinal morphology. Three-centimeter sections of the duodenum, jejunum, and ileum were cut and rinsed with phosphate buffer saline and fixed in 10% formalin immediately for further analyses.

Gut Permeability

The gut permeability was determined at 5 DPI by the method followed in the previous study (Teng et al., 2020b). Briefly, one bird from each cage was randomly selected and gavaged with 2.2-mg/mL fluorescein isothiocyanate dextran (**FITC-d**; MW 4000; Sigma-Aldrich, Ontario, Canada). Two hours after inoculation, the blood was collected from birds and kept in a dark container in room temperature. The clotted blood was centrifuged at 1,000 *g* for 15 min to separate serum. A serial dilution of the standard solution was made from the FITC-d stock (2.2 mg/mL) with a pool of the serum from 10 extra unchallenged birds. The serum samples and standard solution were loaded into 96-well plates. The concentration of FITC-d was measured by using a spectrophotometer (SpectraMax M5; Molecular Devices, San Jose, CA) at an excitation wavelength of 485 nm and an emission wavelength of 528 nm.

IDE and AID of Macrominerals

Feed and ileal digesta were dried in an oven before analyses. The dried digesta were ground, and the gross energy of the samples was determined by using a calorimeter (IKA Calorimeter C1; IKA Works Inc., Wilmington, NC). The digesta and feed samples were sent to the Soil Laboratory in the University of Georgia for measurement of macrominerals. The chromic oxide was analyzed according to the methods described by Dansky and Hill (1952). Briefly, 0.3 g of sample was ashed in a nickel crucible at 600°C overnight. Then, 5.8 g of fusion mixture (190 g KNO_3–100 g Na_2CO_3) and 5.6 g of NaOH were added and heated for 2 additional hours. The chromite was dissolved in 150 mL of distilled water with 1 mL of H_2O_2. The solution was filtered and diluted by adding distilled water to

250 mL. The samples and standard solution were measured at 400 nm by using a spectrophotometer (SpectraMax M5). The calculations of ileal digestible energy (**IDE**) as well as AID of minerals and nitrogen were performed by using the following equations:

$$\text{IDE} = GE_{\text{Diet}} - \left(GE_{\text{Digesta}} \times Cr_2O_{3\text{Diet}}\right) / Cr_2O_{3\text{Digesta}}$$

$$\text{AID, } \% = \frac{1-\left(\text{Nutrient}_{\text{Digesta}}\right)\times\left(Cr_2O_{3\ \text{Diet}}\right)\times 100}{\left(\text{Nutrient}_{\text{Diet}}\right)\times\left(Cr_2O_{3\ \text{Digesta}}\right)}$$

Oocysts Shedding

Approximately 200 g of fresh fecal materials were collected from each pen on 6 DPI and mixed thoroughly in a sample bag. Five grams from each sample were added in a 50-mL centrifuge tube containing 45 mL of saturated salt solution. After well homogenization, fecal samples were loaded in a Mcmaster chamber (Jorgensen Laboratories, Loveland, CO). Standing in room temperature for 5 min, the oocysts were counted under a microscope.

Intestinal Morphology

The method of morphometric analysis of the small intestine was followed by Teng et al. (2017). The intestinal tissue was first embedded in paraffin and cut at 4-μm thickness to fix on a slide. The samples were later stained by the hematoxylin and eosin method (Feldman and Wolfe, 2014). The slide pictures were captured by using a light microscope with a camera (Leica DC500 camera; Leica Microsystems Inc., Buffalo Groove, IL) at 1.6× (for duodenum and jejunum) or 5× (for ileum) magnification. Five representative villi or crypt per slide were randomly selected and measured by using the LAS v4.8 software (Leica Microsystems Inc., Buffalo Groove, IL). The villi height to crypt depth ratio was calculated from each set of villi and crypt in the duodenum, jejunum, and ileum.

Statistical Analyses

The PROC GLM program of SAS software (SAS Institute Inc., Cary, NC) was used to analyze data in the present study. The linear and quadratic orthogonal polynomial contrasts were used to evaluate the effects of increasing NP doses on growth performance and other gut health biomarkers. In addition, the interaction effects of challenge by linear dosage and challenge by quadratic dosage were analyzed to determine if graded NP could improve gut health and growth performance in *Eimeria*-challenged birds. The log transformation was applied for the analyses of oocysts shedding. The Kruskal-Wallis nonparametric analysis described by Elliott and Hynan (2011) was used to compare the intestinal lesion scores between different treatments. Statistical significance of all analyses was set at $P < 0.05$.

RESULTS

Growth Performance

Eimeria challenge significantly reduced growth performance of broilers ($P < 0.0001$) (Table 1). Birds challenged with *Eimeria* spp. reduced 35% of BWG compared with the nonchallenge groups. Furthermore, the FCR for challenged birds was significantly increased from 1.40 to 1.86, reaching 32.9%. On the other hand, dietary supplementation of NP significantly decreased BW, BWG, and FI ($P < 0.0001$). A linear effect was observed on growth performance (BW, BWG, and FI; $P < 0.0001$) in response to the increase of NP, whereas there was no linear or quadratic effect on FCR by the graded NP doses ($P > 0.05$). In addition, there was no significant interaction between *Eimeria* challenge and NP addition on growth performance. Overall results indicated that addition of 150- or 200-ppm NP was not effective to improve growth performance in both challenged and nonchallenged birds.

IDE and AID of Nitrogen and Macrominerals

The results in Table 2 showed that AID significantly reduced on 6 DPI because of *Eimeria* infection. Birds challenged with *Eimeria* had lower IDE than nonchallenge groups ($P < 0.0001$). AID of nitrogen and macrominerals, except iron, significantly decreased in the challenged birds, indicating that *Eimeria* infection showed negative impacts on nutrient digestibility and absorption. Furthermore, there were linear effects on IDE as well as AID of nitrogen and sodium in response to the increase of NP in broiler diets. A significant interaction of challenge by linear dosage effect was also observed for IDE ($P = 0.0066$) and AID of nitrogen ($P = 0.0462$). The results indicated that higher NP supplementation improved digestibility of energy and protein of only infected broilers.

Intestinal Morphology and Gut Permeability

Villi height, crypt depth, and the ratio of villi height to crypt depth were significantly reduced in the duodenum, jejunum, and ileum of the challenged broilers compared with the nonchallenge groups ($P < 0.01$) (Table 3), except jejunal crypt depth. There was no significant dosage effect ($P > 0.05$), but villi height in the duodenum showed a trend of interaction between NP and *Eimeria* challenge ($P < 0.1$). Moreover, there was a trend of interaction on the ratio of villi height to crypt depth in the ileum ($P < 0.1$).

The results of gut permeability are presented in Table 4. There was no significant dose effect and interaction of the 2 main factors on gut permeability ($P > 0.05$), but *Eimeria* challenge significantly increased FITC-d levels in the serum ($P < 0.0001$), indicating that severe gut leakage occurred at DPI 5 when birds were challenged with mixed *Eimeria* spp.

Table 1. Effects of 2-nitro-1-propanol (NP) on growth performance of *Eimeria*-challenged broilers (D13-21).

Item			BW	BWG	FI	FCR
Treatment	Dosage (ppm)	Challenge				
NCC	0	None	785.9	444.1	610.3	1.37
NCNP150	150	None	761.1	421.2	576.9	1.37
NCNP200	200	None	720.0	381.9	556.7	1.46
CC	0	Yes	621.4	285.1	521.0	1.84
CNP150	150	Yes	610.1	268.6	499.8	1.87
CNP200	200	Yes	597.6	256.7	481.1	1.87
	0	-	703.7	364.6	565.6	1.61
	150	-	685.6	344.9	538.3	1.62
	200	-	658.8	319.3	518.9	1.67
	-	None	755.7	415.7	581.3	1.40
	-	Yes	609.7	270.1	500.6	1.86
P value						
Source of variation						
Dosage effect			<0.001	<0.001	<0.001	0.144
	Linear		<0.001	<0.001	<0.001	0.058
	Quadratic		0.615	0.730	0.618	0.612
Challenge effect			<0.001	<0.001	<0.001	<0.001
Dosage*Challenge			0.111	0.194	0.712	0.372
	Challenge*Linear dosage		0.121	0.232	0.753	0.724
	Challenge*Quadratic dosage		0.908	0.825	0.944	0.509

N = 8.

Birds were fed with different treatment diets on day 13 and challenged with *Eimeria maxima* (50,000 oocysts per bird), *Eimeria tenella* (50,000 oocysts per bird), and *Eimeria acervulina* (250,000 oocysts per bird) on day 15.

Abbreviations: BW, body weight; BWG, body weight gain; CC, challenge control; CNP150, *Eimeria* challenge and 150-ppm NP supplementation; CNP200, *Eimeria* challenge and 200-ppm NP supplementation; FCR, feed conversion rate; FI, feed intake; NCC, nonchallenge control; NCNP150, nonchallenge and 150-ppm NP supplementation; NCNP200, nonchallenge and 200-ppm NP supplementation.

Oocysts Shedding and Intestinal Lesion Scores

Supplementation of graded NP linearly reduced oocysts shedding of *E. acervulina* and total oocysts shedding ($P < 0.05$) (Table 5), whereas NP numerically reduced oocysts shedding of *E. tenella*. The results of lesion scores showed no difference among treatments in the upper small intestine, middle small intestine, and ceca (Figure 1; $P > 0.05$).

DISCUSSION

Both *Eimeria* spp. and NP addition reduced growth performance of broilers in the present study. The reduction of growth performance was strongly associated with the severity of *Eimeria* infection (Conway et al., 1993; Zhu et al., 2000; Teng et al., 2020b). However, the toxicity of NP and its impact on growth performance in broiler chickens or laying hens remain unclear. The LD_{50} of NP for broiler chicken might be above 1,300 mg/kg (Jung et al., 2004b), which is higher than the dosages tested in the present study. Previous studies reported that adding 100-ppm NP in broiler diet did not cause negative outcomes on laying hens or *Eimeria*-challenged broilers (Adhikari et al., 2017; Teng et al., 2020a). However, it should be noted that high doses of NP (150 or 200 ppm) decreased BW, BWG, and FI of broiler chickens. Therefore, the supplementation level of NP should be somewhat less than 150 ppm to inhibit parasites or pathogens as well as maintain growth performance of birds in further studies.

The reduction of growth performance might be attributed to poor digestion and absorption during *Eimeria* infection. Once *Eimeria* sporozoites penetrated and damaged intestinal epithelial cells, the parasites reduced villi height in the duodenum, jejunum, and ileum, leading to the decrease of AID of amino acids and IDE (Rochell et al., 2016; Teng et al., 2020b). The present study indicated that *Eimeria* challenge significantly decreased IDE and AID of nitrogen and macrominerals, except iron ($P < 0.01$). Interestingly, NP improved digestibility of nitrogen and IDE in the challenged birds. The interaction of *Eimeria* challenge and dosage linear effects was observed, revealing that increasing NP doses only improved digestibility when birds were challenged. The IDE and AID of nitrogen and sodium in challenged birds were significantly enhanced in response to the increase of NP supplementation. The regulation of nutrient digestibility by NP might be associated with the ileal sodium levels. Sodium plays an important role on maintaining osmotic force in the gut and in nutrient absorption. Most of the amino acids and single hexose sugars are absorbed by the sodium-dependent cotransporters in the jejunum and ileum. However, *Eimeria* infection downregulated gene expression of sodium-dependent amino acid transporters and glucose transporters in the intestine (Su et al., 2014). The mechanisms of action that cotransporters inhibited by *Eimeria* infection are not clear. It might be related to the damage of epithelial cells and abnormal sodium concentration in

Table 2. Effects of 2-nitro-1-propanol (NP) on ileal digestible energy (IDE) and apparent ileal digestibility (AID) of nutrient of *Eimeria*-challenged broilers (day 21).

Item			IDE	Nitrogen	Na	K	Ca	Mg	P	Cu	Fe	Zn
Treatment	Dosage (ppm)	Challenge										
NCC	0	None	2,754	80%	8%	91%	21%	35%	46%	−23%	44%	23%
NCNP150	150	None	2,783	81%	14%	91%	33%	37%	54%	−20%	47%	20%
NCNP200	200	None	2,728	80%	11%	89%	23%	30%	49%	−37%	43%	11%
CC	0	Yes	2,258	68%	−38%	78%	50%	37%	50%	−8%	35%	21%
CNP150	150	Yes	2,532	77%	−16%	82%	49%	49%	60%	−5%	48%	24%
CNP200	200	Yes	2,569	76%	8%	82%	52%	47%	59%	5%	46%	33%
	0		2,506	74%	−15%	84%	35%	36%	48%	−16%	40%	22%
	150		2,658	79%	−1%	87%	41%	43%	57%	−12%	48%	22%
	200		2,649	78%	10%	85%	37%	39%	54%	−16%	45%	22%
		None	2,755	80%	11%	90%	26%	34%	50%	−27%	45%	18%
		Yes	2,453	73%	−15%	81%	50%	45%	56%	−3%	43%	26%
P value												
Source of variation												
Dosage effect			0.009	0.010	0.054	0.206	0.348	0.150	0.010	0.8615	0.139	0.980
	Linear		0.007	0.023	0.017	0.32	0.546	0.478	0.008	0.927	0.213	0.928
	Quadratic		0.072	0.037	0.849	0.139	0.187	0.070	0.005	0.5926	0.124	0.860
Challenge effect			<0.001	<0.001	0.002	<0.001	<0.001	<0.001	<0.001	<0.001	0.574	0.004
Dosage*Challenge			0.004	0.019	0.104	0.063	0.146	0.123	0.4995	0.146	0.216	0.001
	Challenge*Linear dosage		0.007	0.046	0.109	0.081	0.993	0.125	0.4693	0.226	0.276	0.002
	Challenge*Quadratic dosage		0.683	0.350	0.953	0.754	0.147	0.979	0.9564	0.628	0.724	0.582

N = 8.

Birds were fed with different treatment diets on day 13 and challenged with *Eimeria maxima* (50,000 oocysts per bird), *Eimeria tenella* (50,000 oocysts per bird), and *Eimeria acervulina* (250,000 oocysts per bird) on day 15. IDE and AID were measured on 6 D after infection.

Abbreviations: CC, challenge control; CNP150, *Eimeria* challenge and 150-ppm NP supplementation; CNP200, *Eimeria* challenge and 200-ppm NP supplementation; NCC, nonchallenge control; NCNP150, nonchallenge and 150-ppm NP supplementation; NCNP200, nonchallenge and 200-ppm NP supplementation.

Table 3. Effects of 2-nitro-1-propanol (NP) on intestinal morphology of *Eimeria*-challenged broilers (day 21).

Items			Duodenum			Jejunum			Ileum		
			VH	CD	VH:CD	VH	CD	VH:CD	VH	CD	VH:CD
Treatments	Dosage (ppm)	Challenge									
NCC	0	None	2,380	263	9.2	1,312	309	4.5	928	209	4.6
NCNP150	150	None	2,368	277	8.8	1,226	282	4.5	802	212	3.8
NCNP200	200	None	2,311	315	7.9	1,311	293	4.7	831	239	3.8
CC	0	Yes	1,572	384	4.4	880	279	3.3	654	286	2.3
CNP150	150	Yes	1,690	435	3.9	957	285	3.6	692	255	2.8
CNP200	200	Yes	1,836	424	4.4	954	342	2.9	678	256	2.7
	0		1,976	324	6.8	1,096	294	3.9	791	248	3.5
	150		2,029	356	6.4	1,092	284	4.1	747	233	3.3
	200		2,074	370	6.2	1,132	318	3.8	754	247	3.2
		None	2,374	270	9.0	1,283	295	4.6	854	220	4.0
		Yes	1,858	378	5.4	930	302	3.3	674	265	2.6
P value											
Source of variation											
Dosage effect			0.450	0.174	0.504	0.798	0.499	0.782	0.567	0.582	0.772
	Linear		0.213	0.091	0.257	0.580	0.412	0.878	0.415	0.983	0.491
	Quadratic		0.949	0.670	0.802	0.698	0.398	0.495	0.495	0.301	0.851
Challenge effect			<0.001	<0.001	<0.001	<0.001	0.763	0.000	<0.001	0.001	<0.001
Challenge*Dosage			0.106	0.629	0.335	0.470	0.404	0.461	0.165	0.182	0.070
	Challenge*Linear dosage		0.099	0.986	0.459	0.843	0.391	0.724	0.431	0.182	0.164
	Challenge*Quadratic dosage		0.963	0.640	0.741	0.568	0.984	0.596	0.401	0.984	0.428

N = 8.

Birds were fed with different treatment diets on day 13 and challenged with *Eimeria maxima* (50,000 oocysts per bird), *Eimeria tenella* (50,000 oocysts per bird), and *Eimeria acervulina* (250,000 oocysts per bird) on day 15. Intestinal morphology was measured on 6 D after infection.

Abbreviations: CC, challenge control; CD, crypt depth; CNP150, *Eimeria* challenge and 150-ppm NP supplementation; CNP200, *Eimeria* challenge and 200-ppm NP supplementation; NCC, nonchallenge control; NCNP150, nonchallenge and 150-ppm NP supplementation; NCNP200, nonchallenge and 200-ppm NP supplementation; VH, villi height; VH:CD, ratio of villi height to crypt depth.

the lumen of guts. Our previous study found that the reduction of AID of sodium and potassium was linear in response to the graded *Eimeria* challenge doses (Teng et al., 2020b). Furthermore, oxidative stress caused by *Eimeria* could inhibit mitochondrial creatine kinase, causing reduction of ATP and leading to the

Table 4. Effects of 2-nitro-1-propanol (NP) on gut permeability of *Eimeria*-challenged broilers (day 20).

Items			FITC-d (μg/mL)
Treatment	Dosage (ppm)	Challenge	
NCC	0	None	0.1694
NCNP150	150	None	0.1185
NCNP200	200	None	0.1454
CC	0	Yes	0.6806
CNP150	150	Yes	0.7247
CNP200	200	Yes	0.7037
	0		0.4250
	150		0.4216
	200		0.4245
		None	0.1445
		Yes	0.7030
P value			
Source of variation			
Dosage effect			0.998
	Linear		0.993
	Quadratic		0.950
Challenge effect			<0.001
Dosage*Challenge			0.713
	Challenge*Linear dosage		0.922
	Challenge*Quadratic dosage		0.771

N = 8.

Birds were fed with different treatment diets on day 13 and challenged with *Eimeria maxima* (50,000 oocysts per bird), *Eimeria tenella* (50,000 oocysts per bird), and *Eimeria acervulina* (250,000 oocysts per bird) on day 15. Gut permeability was measured on 5 D after infection.

Abbreviations: CC, challenge control; CNP150, *Eimeria* challenge and 150-ppm NP supplementation; CNP200, *Eimeria* challenge and 200-ppm NP supplementation; FITC-d, fluorescein isothiocyanate dextran; NCC, nonchallenge control; NCNP150, nonchallenge and 150-ppm NP supplementation; NCNP200, nonchallenge and 200-ppm NP supplementation.

Table 5. Effects of 2-nitro-1-propanol (NP) on oocyst shedding of *Eimeria*-challenged broilers.

Items	CC	CNP150	CNP200	SEM	Linear	Quadratic
Oocysts per gram (Log_{10})						
Eimeria acervuline	6.178	6.065	6.003	0.033	0.033	0.704
Eimeria maxima	4.007	3.794	4.028	0.051	0.865	0.041
Eimeria tenella	4.042	3.908	3.872	0.045	0.139	0.614
Total oocysts	6.184	6.072	6.011	0.033	0.031	0.687

N = 8.

Birds were fed with different treatment diets on day 13 and challenged with *Eimeria maxima* (50,000 oocysts per bird), *Eimeria tenella* (50,000 oocysts per bird), and *Eimeria acervulina* (250,000 oocysts per bird) on day 15. Oocysts shedding was measured on 6 D after infection.

Abbreviations: CC, challenge control; CNP150, Eimeria challenge and 150-ppm NP supplementation; CNP200, Eimeria challenge and 200-ppm NP supplementation.

failure of sodium movement across cell membrane, which generates electrical potential difference to absorb nutrients through cotransporters (Goff, 2015; Adedokun et al., 2016). Moreover, NP increased AID of sodium from −38 (CC) to 8% (CNP200), which was numerically as high as the NCC (8%). These results suggest that NP may improve electrolyte balance in the intestine and regulate nutrient transporters, further enhancing nutrient absorption in the *Eimeria*-challenged birds.

Intestinal morphology is one of the gut health parameters associated with the AID and growth performance of the *Eimeria*-challenged broilers. The shorter villi height, higher crypt depth, and lower ratio of villi height to crypt depth were observed in the duodenum, jejunum, and ileum of the birds challenged with higher doses of *Eimeria* spp. (Teng et al., 2020b). With shorter villi height, infected birds are not able to secrete as much endogenous enzymes as health birds do to degrade protein and carbohydrates complex efficiently. The reduced villi height and surface area of the intestine are also related to the poor digestibility during *Eimeria* infection (Di Genova and Tonelli, 2016). Furthermore, *Eimeria* spp. activate epithelial cells turnover in the intestine and induce host immune response (Yun et al., 2000; Sun et al., 2016); thus, the infected birds have to waste additional energy and nutrients on accelerating cell turnover rate and generating immune responses to inhibit parasites. In the present study, NP improved intestinal morphology of birds infected with *Eimeria* spp. A trend of linear doses effect was interacted with *Eimeria* challenge. The NP increased villi height from 1,572 to 1,836 nm in the duodenum of the *Eimeria*-challenged birds, but it did not show same patterns in the nonchallenge groups. In general, digestion and absorption of dietary nutrients and intestinal morphology are remarkably reduced during *Eimeria* infection. However, NP supplementation improved nutrient digestibility as well as numerically enhanced villi height in the small

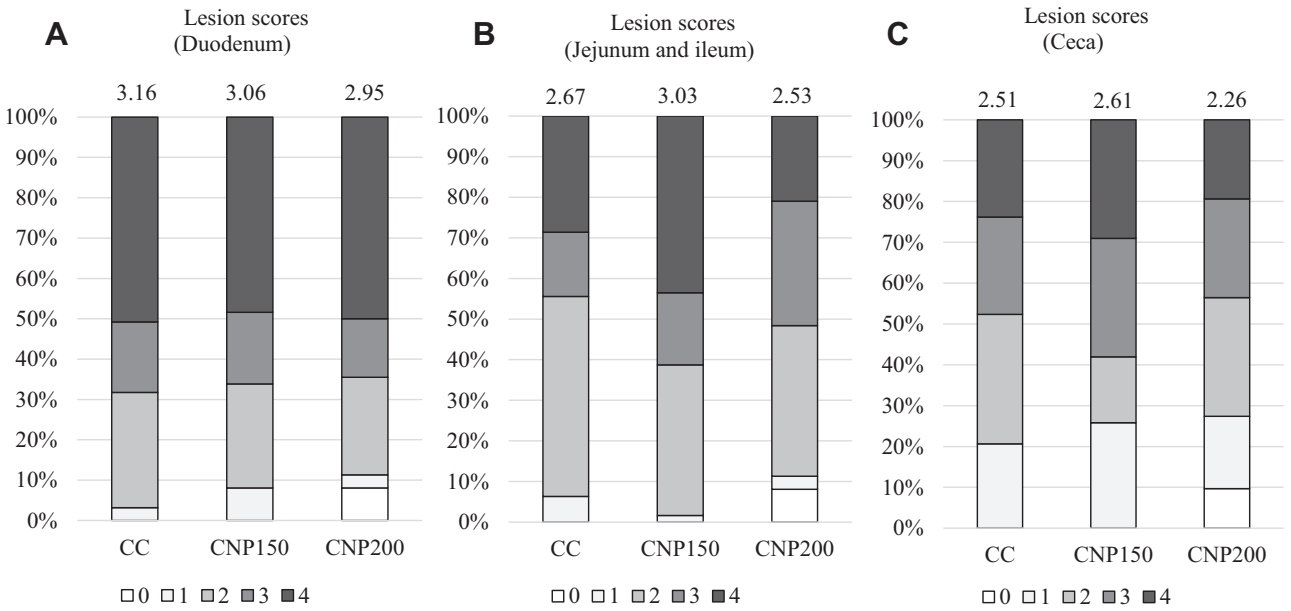

Figure 1. Effects of 2-nitro-1-propanol on intestinal lesion scores of mixed *Eimeria*-challenged broilers (6 D after infection). Birds were fed with different treatment diets on day 13 and challenged with *Eimeria maxima* (50,000 oocysts per bird), *Eimeria tenella* (50,000 oocysts per bird), and *Eimeria acervulina* (250,000 oocysts per bird) on day 15. Six treatments were tested in the study, including the nonchallenge control (NCC, no NP), 2 groups of NP supplementation (150 ppm or 200 ppm) without Eimeria challenge (NCNP150; NCNP200), challenge control (CC, no NP), and 2 groups of NP supplementation (150 ppm or 200 ppm) with Eimeria challenge (CNP150; CNP200). Intestinal lesion was scored at 6 D after infection. The average of lesion scores was present on the top of the bars in the figure (A: duodenum; B: jejunum; and ileum; C: ceca.). n = 64.

intestine. The results suggest that increasing NP supplementation may ameliorate intestinal damage from coccidiosis without improving growth performance.

The NP did not improve intestinal lesion scores in the present study; however, linear reduction of oocysts shedding of *E. acervulina* and total oocysts shedding was observed in response to the graded NP supplementation, whereas there was no difference in oocysts shedding of *E. maxima* among treatments. The mechanism of action that NP reduced oocysts shedding is not clear yet. It is speculated that NP could regulate ion levels in the gut lumen, interfering with membrane penetration of sporozoites. The previous in vitro study found that NP could inhibit development of *E. tenella* (Teng et al., 2020a). The amount of sporozoites or schizonts in the Madin-Darby bovine kidney cells was reduced by 1-mM NP in the medium. Similarly, previous studies reported that isopropanol, ethanol, and acetaldehyde caused the egress of sporozoites of *E. tenella* from the host cells (Yan et al., 2015, 2016). Furthermore, egressed sporozoites had less reinvading ability and productivity than the fresh sporozoites (Yan et al., 2015, 2016). The egressed sporozoites triggered by chemicals might be one of the reasons that 200-ppm NP reduced 30% of the oocysts production in the present study.

FITC-d has been used to evaluate gut leakage in *Eimeria* or necrotic enteritis–challenged models (Zhang et al., 2016; Baxter et al., 2017; Latorre et al., 2018; Bortoluzzi et al., 2019). FITC-d, which is normally not absorbed by enterocytes, can move across through epithelial cells and enter in blood circulation when *Eimeria* spp. damage the intestinal tight junction structure. Thus, intestinal permeability could be measured by gavaging birds with FITC-d and evaluating levels of FITC-d in the serum. In the present study, the challenged birds had higher serum levels of FITC-d than the nonchallenged groups (703 vs. 145 ng), but NP did not improve gut permeability regardless of challenge. Furthermore, there was no significant linear effect, suggesting that 150- or 200-ppm NP did not affect gut integrity of broilers.

In conclusion, *Eimeria* infection significantly increased gut permeability, but NP did not improve gut leakage of the birds. On the other hand, the increase of NP in diets linearly enhanced IDE and AID of nitrogen and linearly reduced oocysts shedding in challenge groups. Moreover, graded NP numerically improved villi height in the intestine. Although NP was not able to maintain growth performance during the infection period, it improved nutrient digestibility and reduced total oocysts shedding in broiler chickens challenged with mixed *Eimeria* spp.

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