

# Evaluation of nitro compounds as feed additives in diets of *Eimeria*-challenged broilers in vitro and in vivo

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**ABSTRACT** Coccidiosis is a disease caused by *Eimeria* spp., resulting in approximately 3 billion US dollar loss in the poultry industry annually. The present study evaluated the effects of potential feed additives, 2-Nitro-1-propanol (NP) and nitroethanol (NE), on control of coccidiosis. An in vitro experiment indicated that both NP and NE inhibited the development of sporozoites in Madin-Darby bovine kidney cells (MDBK). The in vivo study was further conducted to evaluate the effects of NP and NE on growth performance, nitrogen-corrected apparent metabolizable energy (AMEn), and intestinal lesion scores of broilers challenged with *Eimeria* spp. Six treatments were tested in the study, including the nonchallenged control, challenged control, 100 ppm NP, 200 ppm NP, 100 ppm NE, and 200 ppm NE. Broilers were fed the treatment diets from day 12 until the end of the trial.

All birds except the unchallenged control were challenged with *Eimeria maxima*, *Eimeria tenella*, and *Eimeria acervulina* on day 14. The growth performance was calculated, and the intestinal lesion was scored on day 20. The results showed that *Eimeria* challenge significantly reduced growth performance, increased intestinal lesion scores, and decreased AMEn compared with the nonchallenged control group. Birds fed with 200 ppm of NP had reduced growth performance compared with the nonchallenged control and challenged control. However, the supplementation of NP significantly improved AMEn and reduced cecal damage. Overall, NP and NE reduced sporozoites numbers in the MDBK cells. NP improved dietary digestibility of energy and reduces lesion scores in the ceca but could not maintain growth performance in broiler chickens infected with *Eimeria* spp.

**Key words:** nitro compounds, broiler, coccidiosis, *Eimeria*, AMEn

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## INTRODUCTION

Coccidiosis is a disease caused by *Eimeria* spp, resulting in 3 billion US dollar loss in the poultry industry annually (Dalloul and Lillehoj, 2006). Application of anticoccidial drugs and vaccines has been the conventional means for preventing coccidiosis. Monensin is one of the ionophores used as effective coccidiostats for decades (Chapman et al., 2010). Owing to the unique mode of action which could disrupt ion gradient crossing cell membrane, it was believed that to develop resistance against *Eimeria* spp. was difficult using monensin. (Chapman, 2014). However, the monensin-resistant *Eimeria* was found 10 y after introducing monensin in the United States in 1971 (Shivaramaiah et al., 2014). Moreover, the high percentage of *Eimeria maxima*,

*Eimeria tenella*, and *Eimeria acervulina* have developed resistance to synthetic drugs such as nicarbazin and narasin (Shivaramaiah et al., 2014). Alternatively, vaccination could trigger protective immune responses by giving day-old birds a low dosage of *Eimeria* oocysts (Yun et al., 2000). Introducing vaccine strains that are sensitive to anticoccidial drugs in poultry house could also replace some drug-resistant strains, subsequently slowing down the development of resistant *Eimeria* (Chapman et al., 2002). To eliminate the development of drug resistance, the rotation program that uses different drugs and vaccination has been practiced in the poultry industry. However, even though the resistant strains are diluted by vaccines, they are not completely eliminated in poultry farms. Thus, it is necessary to develop potential anticoccidial feed additives to replace the drugs used in the rotation programs to control the outbreak of drug-resistant coccidiosis.

Nitro compounds are organic compounds that contain a nitro functional group on the carbon of alcohol (Olender et al., 2018). 2-Nitro-1-propanol (NP, C<sub>3</sub>H<sub>7</sub>NO<sub>3</sub>) and nitroethanol (NE, C<sub>2</sub>H<sub>5</sub>NO<sub>3</sub>) have the similar ability on inhibiting microorganism (Dimitrijevic et al., 2006; Kim

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et al., 2006). NE and NP are derivatives of alcohol groups and belong to the same chemical category. Boiling point of NE is higher than that of NP because of the additional carbon structure. Limited research has compared nitro alcohols, ethanol, and propanol, but a previous study indicated that NP and NE exhibit stronger microbial inhibitory effects than their acid and alcohol counterparts (Kim et al., 2006). It has been reported that nitro compounds inhibited *Salmonella*, *Enterococcus*, *Campylobacter*, and *Eimeria coli in vitro* (Jung et al., 2004a; Dimitrijevic et al., 2006; Horrocks et al., 2007). Both nitro compounds were first introduced in the ruminant diets to replace nitrate because nitrate could be toxic to animals when it was metabolized to nitrite in the rumen (Anderson et al., 2003). The first study with broilers reported that oral administration of NP reduced the amount of *Salmonella typhimurium* in the intestine of broilers (Jung et al., 2004b). In addition, both NP and NE reduced intestinal *Salmonella* colonization and upregulated immune responses in laying hens as well (Adhikari et al., 2017). Besides, nitro compounds decreased 94% methane production during 24 h ruminal fluid incubation (Anderson et al., 2003). It is interesting that monensin, a common coccidiostat in the poultry farm, has also been used as a methane inhibitor in ruminant production (Anderson et al., 2003). As monensin reduces both methanogenesis and *Eimeria* spp. infection in the intestine of animals, it is speculated that NP and NE may have similar effects to monensin on pathogens, methane production, and *Eimeria* spp. Thus, we hypothesized that nitro compounds as feed additives could inhibit the development of *Eimeria* spp. and reduce its invasiveness in broilers. The objective of this study was to evaluate the effects of dietary NP and NE on *E. tenella* development in the MDBK cells (in vitro) and growth performance, energy digestibility, and intestinal lesions scores in *Eimeria*-challenged broilers (in vivo).

## MATERIALS AND METHODS

### Cell Viability

The trypan blue dye exclusion test was conducted to determine cell viability. The trypan blue method was modified according to the study by Ageely et al., 2016. In brief, MDBK cells (10,000 cells/well) were cultured and grown to 100% confluency in a 24-well plate. At confluency, the media were removed and replaced by different levels of NP and NE (0, 0.125, 0.25, 0.5, 1, and 2 mM). MDBK cells were incubated at 41°C for 72 h. After incubation, 0.5 ml of floating and adhering cells were mixed with 0.5 ml of 0.4% trypan blue for 2 min at room temperature. Live cells and dead cells were counted by using a hemocytometer (Hausser Scientific Company, Horsham, PA) under a microscope (Olympus IX71, Olympus America Inc., Melville, NY). The cell viability (%) was calculated by using the following formula:

$$\text{Cell Viability}(\%) = \frac{\text{Live cells}}{\text{Total of dead cells and Live cells}} \times 100\%$$

### Preparation of *E. tenella* Sporozoite

Sporozoite preparation was modified according to the method listed by Hessenberger et al., (2016). Briefly, sporulated *E. tenella* oocysts were washed once with bleach and 3 times with water. The oocyst walls were cracked by 0.5-mm sterilized plastic beads to release sporocysts. The sporocysts were incubated in phosphate-buffered saline containing 0.25% trypsin and 0.75% taurodeoxycholic acid at 41°C for 3 h to excyst sporozoites. After incubation, the sporozoites were washed with PBS and separated from oocyst walls and debris by using a filter (Whatman paper, grade 589/2). Sporozoites were counted by using a hemocytometer under a microscope.

### In Vitro Cell Culture and Sporozoite Incubation

The in vitro experiment was modified from the method described by Fuller and McDougald (1990). MDBK cells were cultured and grown to 100% confluency in a 24-well plate which was divided into 6 treatments, including 4 different levels of NP or NE (1, 0.5, 0.25, and 0.125 mM), the negative control, and the positive control (0.5 µg/ml monensin). Each treatment had 4 replicate wells in the in vitro test. Hundred thousand of excysted *E. tenella* sporozoites were coincubated with MDBK cells at 41°C for 72 h. After incubation, monolayers were fixed with iced methanol and incubated with mouse antisporozoite primary antibody (UGA 1A3) for 6 h (Fuller and McDougald, 2001). After washed with PBS, monolayers were incubated with fluorescein isothiocyanate-conjugated goat antimouse IgG (NETA Scientific Inc., Hainesport, NJ; MILL-AP127 F) (Fuller and McDougald, 1990). The intracellular sporozoites were counted in 20 random fields in each well under a microscope (Olympus IX71, Olympus America Inc., Melville, NY).

### Experimental Birds and Housing

The study was approved by the Institutional Animal Care and Use Committee at the University of Georgia, Athens, GA. A total of 144 twelve-day-old male broilers (males from the Cobb 500 female line) were randomly allocated to 6 treatments with 4 replicates of 6 birds each. Birds were raised in the battery cages from day 12 to day 20. There were 6 treatments in the study, including the nonchallenged control (NC), the challenged control (CC), 100 ppm NP (100 NP), 200 ppm NP (200 NP), 100 ppm NE (100 NE), and 200 ppm NE (200 NE). The nonchallenged and challenged control groups were fed with corn and soybean meal basal diet, and for the nitro compound treatment diets, the basal diet was added with 100 ppm of NP, 200 ppm of NP, 100 ppm of NE, or 200 ppm of NE. Feed and water were *ad libitum* provided to birds during the experiment. All test diets contained 0.3% chromium dioxide as an indigestible marker for measuring nitrogen-corrected apparent metabolizable energy (AMEn). The environmental

**Table 1.** Effects of different levels of NP and NE on cell viability of MDBK cells.

Items	0 mM	0.125 mM	0.25 mM	0.5 mM	1 mM	2 mM	SEM	P value
NP	87% <sup>a</sup>	87% <sup>a</sup>	86% <sup>a</sup>	76% <sup>b</sup>	72% <sup>b</sup>	59% <sup>c</sup>	0.022	<0.0001
NE	84% <sup>a</sup>	82% <sup>a</sup>	79% <sup>a</sup>	83% <sup>a</sup>	52% <sup>b</sup>	3% <sup>c</sup>	0.072	<0.0001

<sup>a,b,c</sup>Means within the same row with different superscript letters are significantly different ( $P < 0.05$ );  $n = 4$ .

Abbreviations: MDBK, Madin-Darby bovine kidney; NE, nitroethanol; NP, 2-Nitro-1-propanol; SEM, standard error of mean.

The MDBK cells were incubated in different levels of NP and NE at 41°C for 72 h. The cell viability was determined by the trypan blue dye exclusion test.

temperature program was followed by the recommendation of Cobb Broiler Management Guide (Cobb 2013). All treatments, except the unchallenged control group, were challenged with *E. maxima* (100,000 oocysts per bird), *E. tenella* (100,000 oocysts per bird), and *E. acervulina* (250,000 oocysts per bird) on day 14.

### Growth Performance, Lesion Scores, and Feces Collection

Body weight of chickens per cage and feed intake (FI) were recorded and calculated for body weight gain (BWG) and feed conversion ratio (FCR) on day 12 and day 20. The intestinal lesion of each bird was scored according to the four-score scale (Johnson and Reid,

according to Dansky and Hill (1952). Briefly, 0.5 g of feed or feces sample was ashed in a nickel crucible at 600°C overnight to burn out organic materials. The additional 5.8 g of fusion mixture (190 g KNO<sub>3</sub>–100 g Na<sub>2</sub>CO<sub>3</sub>) and 5.6 g of NaOH were added in the nickel crucible and burned at 600°C for additional 2 h. The ashed sample was dissolved in water, and chromite in the sample was oxidized to chromate by H<sub>2</sub>O<sub>2</sub>. The concentration of chromate was determined at 400 nm by using a spectrophotometer (Spectramax M5; Molecular Devices, San Jose, CA), and several levels of chromic oxide were used as a standard curve. AMEn was calculated according to the method listed by Lammers et al. (2008).

$$AMEn = GE_{Diet} - (GE_{Excreta} * Cr_2O_{3Diet}) / (Cr_2O_{3Excreta}) - 8.22 * (N_{Diet} - NE_{Excreta} * Cr_2O_{3Diet} / Cr_2O_{3Excreta})$$

1970), and feces were collected from each cage on day 20 and dried in the oven for further analyses.

### Nitrogen-Corrected AMEn

Oven-dried feed and feces were grounded to measure energy content by using a calorimeter (IKA Calorimeter C1; IKA Works Inc., Wilmington, NC). Crude protein levels of feed and feces were measured by the Experiment Station Chemical Laboratories, University of Missouri. The analysis of chromic oxide was performed

### Statistical Analyses

Data were analyzed as the PROC GLM in SAS (SAS Institute Inc., Cary, NC). The Duncan's multiple-range test was used to separate means with significance levels at  $P \leq 0.05$ . Lesion scores were analyzed as the Proc Mixed in SAS, and the  $t$  value was further used for wise comparisons among treatments (Price et al., 2014). Moreover, both the average of lesion scores and the percentage of scores in each treatment were calculated and showed in the results.

**Table 2.** Effects of different levels of NP and NE on development of *E. tenella* sporozoites in the MDBK cells.

Items <sup>1</sup>	NC	PC	1 mM	0.5 mM	0.25 mM	0.125 mM	SEM	P value
NP	7.88 <sup>a</sup>	0.69 <sup>d</sup>	4.61 <sup>c</sup>	5.54 <sup>b,c</sup>	5.41 <sup>b,c</sup>	6.65 <sup>a,b</sup>	0.50	<0.0001
NE	7.36 <sup>a</sup>	0.61 <sup>d</sup>	ND <sup>2</sup>	2.06 <sup>b</sup>	2.68 <sup>c</sup>	3.80 <sup>b</sup>	0.55	<0.0001

<sup>a-c</sup>Means in the same row with different superscript letters are significantly different ( $P < 0.05$ );  $n = 4$ .

Abbreviations: MDBK, Madin-Darby bovine kidney; ND, nondetectable; NE, nitroethanol; NP, 2-Nitro-1-propanol; SEM, standard error of mean.

MDBK cells and *E. tenella* sporozoites were coincubated in different levels of NP or NE in the 24-well plates at 41°C for 72 h. Mouse antisporozoite primary antibody (UGA 1A3) and fluorescein isothiocyanate-conjugated goat antimouse IgG were used to target sporozoites in the cells. The number of sporozoites was counted in 20 random fields in each replicate well.

<sup>1</sup>NC = negative control (no chemical was added in the cell medium); PC = positive control (0.5 µg/ml of monensin).

<sup>2</sup>MDBK cells were dead and detached from the plate in the 1-mM NE treatment.

**Table 3.** Effects of NP and NE on growth performance of mixed *Eimeria*-challenged broilers (day 12–day 20).

Items	NC	CC	NP 100	NP 200	NE 100	NE 200	SEM	<i>P</i> value
BW (g)	804 <sup>a</sup>	600 <sup>b</sup>	582 <sup>b</sup>	512 <sup>c</sup>	602 <sup>b</sup>	590 <sup>b</sup>	11.91	<0.0001
BWG (g)	476 <sup>a</sup>	272 <sup>b</sup>	254 <sup>b</sup>	183 <sup>c</sup>	273 <sup>b</sup>	262 <sup>b</sup>	19.05	<0.0001
FI (g)	608 <sup>a</sup>	513 <sup>b</sup>	485 <sup>c</sup>	421 <sup>d</sup>	506 <sup>b,c</sup>	493 <sup>b,c</sup>	19.1	<0.0001
FCR (g/g)	1.28 <sup>c</sup>	1.89 <sup>b</sup>	1.92 <sup>b</sup>	2.32 <sup>a</sup>	1.86 <sup>b</sup>	1.89 <sup>b</sup>	0.07	<0.0001

<sup>a-c</sup>Means within the same row with different superscript letters are significantly different ( $P < 0.05$ );  $n = 4$ .

Abbreviations: BW, body weight; BWG, body weight gain; CC, challenge control; FCR, feed conversion ratio; FI, feed intake; NC, nonchallenge control; NE 100, 100 ppm of nitroethanol; NE 200, 200 ppm of nitroethanol; NP 100, 100 ppm of 2-Nitro-1-propanol; NP 200, 200 ppm of 2-Nitro-1-propanol; SEM, standard error of mean.

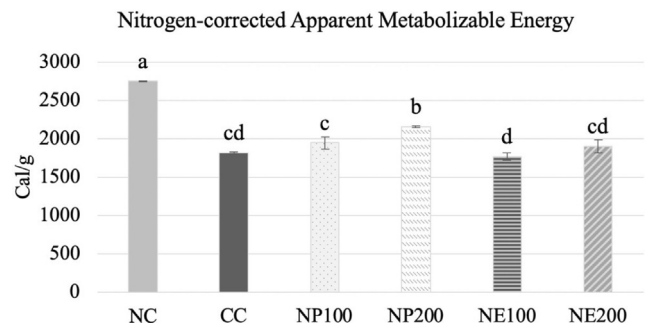
Birds were fed with treatment diets on day 12 and challenged with *E. maxima* (100,000 oocysts per bird), *E. tenella* (100,000 oocysts per bird), and *E. acervulina* (250,000 oocysts per bird) on day 14.

## RESULTS AND DISCUSSION

The in vitro test evaluated the effects of NP and NE on inhibiting cell invasion and the development of *E. tenella* sporozoites in the MDBK cells. The MDBK cells have been used as a host of *E. tenella* in previous studies to screen potential feed additives in vitro (Hessenberger et al., 2016; Marugan-Hernandez et al., 2017) because they are more stable than primary chicken epithelial cells. To find out appreciate levels of NP and NE in the in vitro test, cell viability was determined by the trypan blue method. The results showed that MDBK cells which were incubated in 0.125 and 0.25 mM of NP did not reduce cell viability compared with the control group (0 mM of NP) (Table 1). On the other hand, there was no significant difference among the control group birds, 0.125, 0.25, and 0.5 mM of NE. However, the cell viability was reduced from 87 to 76% and 72% in the 1-mM NP and 0.5-mM NP treatments, respectively. Furthermore, more than 60% of cells were dead in the 2-mM NP, 1-mM NE, and 2-mM NE groups. Similar to our results, a previous study indicated that 1-mM NP and 3-mM NE could kill the bovine primary cells after 48 h (Paik et al., 2008). Furthermore, it was reported that human skin fibroblasts did not survive in the treatments above 1-mM NE and 0.8-mM NP (Kim et al., 2014). Based on the previous and present results, 4 levels of NP and NE, including 1 mM, 0.5 mM, 0.25 mM, and 0.125 mM, were selected and tested in the present study. The in vitro results demonstrated that NP and NE reduced sporozoites invasion into MDBK cells (Table 2). The positive control, 0.25-, 0.5-, and 1-mM NP, and 0.125-, 0.25-, 0.5-mM NE significantly reduced numbers of sporozoites compared with the negative control treatment (NC). However, both nitro compounds were not as effective as 0.5  $\mu\text{g}/\text{ml}$  monensin, which inhibited the development of sporozoites dramatically (from 7.88–0.69 in the 24-well plate of NP; from 7.36–0.61 in the plate of NE).

Although the actual mode of action of nitro compounds on inhibiting sporozoites remains unclear and needed to be elucidated in the future, we speculated that there are 2 possible mechanisms that might be involved in the inhibition of *Eimeria* invasion. First, NP and NE might directly kill parasites before the

sporozoites invade the host cells. Second, nitro compounds could trigger the egress of sporozoites from host cells and reduce their productivity. Previous studies reported that ethanol, isopropanol, and acetaldehyde could stimulate the egress of *E. tenella* in vitro (Yan et al., 2015, 2016). Acetaldehyde could enhance  $\text{Ca}^{2+}$  levels in intracellular cells of parasites, leading to an increase of microneme discharge (Yan et al., 2016). Once micronemes are released, reinvasion ability and oocyst production of egressed sporozoites will be reduced because microneme is a protein structure related to sporozoite's mobility and plays a crucial role in the migration of sporozoites across the host cell membrane (Yan et al., 2016). There was no clear evidence that NP and NE could act as ethanol and propanol, regulating  $\text{Ca}^{2+}$  levels in the intracellular cells of parasites, but a previous study reported that NP and NE were more capable of inhibiting microorganisms than propanol and ethanol (Kim et al., 2006). Further research is needed to understand the actions of mechanism of how NP and NE inhibit parasite invasion in the MDBK cells. Moreover, it should be noticed that the relationship between *E. tenella* and MDBK might be different from the situation of other *Eimeria* spp. and intestinal epithelial cells of



**Figure 1.** Effects of 2-Nitro-1-propanol and nitroethanol on nitrogen-corrected apparent metabolizable energy (AMEn) of mixed *Eimeria*-challenged broilers. Birds were fed with different treatment diets on day 12 and challenged with *E. maxima* (100,000 oocysts per bird), *E. tenella* (100,000 oocysts per bird), and *E. acervulina* (250,000 oocysts per bird) on day 14. Six treatments were tested in the study, including nonchallenge control (NC); challenge control (CC); 100 ppm of 2-Nitro-1-propanol (NP100); 200 ppm of 2-Nitro-1-propanol (NP200); 100 ppm of nitroethanol (NE100); 200 ppm of nitroethanol (NE 200);  $n = 4$ . Means with different letters are significantly different ( $P < 0.05$ ).



chicken, even though the in vitro results showed that NP and NE inhibited sporozoites development in the present study.

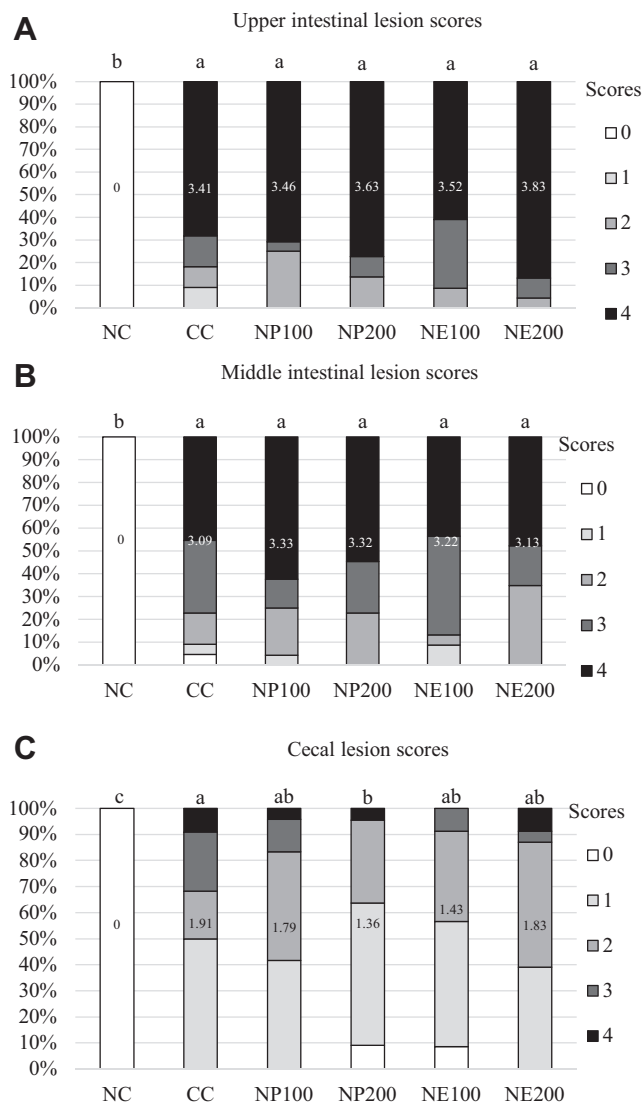
In addition to the in vitro test, an in vivo study was further conducted to test NP and NE on control of coccidiosis in broilers. During *Eimeria* infection, parasites invade into the intestinal epithelial cells, causing severe damage in the gut of broilers and reducing nutrient digestibility and growth performance (Dalloul and Lillehoj, 2005; 2006; Adedokun et al., 2016). The growth performance results in the present study are shown in Table 3. BWG and FI were significantly reduced by *Eimeria* challenge, whereas FCR was increased in the challenged birds compared with that in the nonchallenged control birds ( $P < 0.0001$ ). Similarly, a previous study demonstrated that *Eimeria* challenge reduced growth performance, amino acid, and energy digestibility in broilers (Adedokun et al., 2016). Furthermore, higher *Eimeria acervulina*-challenged doses could cause a linear decrease in growth performance of broilers (Rochell et al., 2016). In the present study, there was no significant difference in growth performance among the challenged control, NP100, NE100, and NE200. However, NP 200 treatment had the highest FCR and the lowest body weight, BWG, and FI ( $P < 0.0001$ ). The toxicity of NP has not been studied in broiler chicken. There are only 2 studies evaluating the effects of NP in chicken, reporting that broilers gavaged with 13 mg of NP did not reduce growth performance (Jung et al., 2004b), and no unfavored growth performance was observed in laying hens fed with 200 ppm of NP (Adhikari et al., 2017). In the present study, 100-ppm NP did not affect growth performance, but 200-ppm NP reduced BWG and FI and increased FCR in broilers.

Besides growth performance, nutrient digestibility could be another parameter to monitor the gut health of broilers during *Eimeria* infection. As we expected, the results of AMEn in the present study showed a significant AMEn reduction in challenge groups compared with nonchallenge control group ( $P < 0.01$ , Figure 1); birds challenged with *Eimeria* spp. decreased AMEn from 2,753 to 1,819 Cal/g. Moreover, it is interesting that birds in the 200 NP group had higher AMEn than the challenge control group, suggesting that the digestion and absorption of nutrient were improved in the 200 NP treatment.

The lesion score results are present in Figure 2. The nonchallenged group had the least intestinal lesion compared with the other treatments in the upper intestine, middle intestine, and ceca. Interestingly, NP 200 significantly decreased lesion scores in the ceca, reducing the combination of scores 3 and 4 from 32 to 5%. The reduction of lesion scores, the decrease of in vitro sporozoites numbers, and the improvement of AMEn suggest that NP could inhibit *Eimeria* spp. infection, especially the *E. tenella*, in broiler chicken. However, NP reduced growth performance, indicating that the dose of NP should be addressed in the future study.

In summary, in vitro and in vivo studies were conducted to evaluate the effects of NP and NE on inhibition of

*Eimeria* infection. The in vitro results indicated that both NP and NE significantly reduced sporozoites numbers in the MDBK cells. Supplementation of 200 NP in broiler diets improved AMEn of broilers challenged with mixed *Eimeria* spp. However, growth performance and intestinal lesion scores were not significantly improved by the NP and NE treatments, except NP 200 that showed significantly lower cecal lesion scores than the challenged control. In addition, NP at 200 ppm was too high to maintain efficient growth performance during the *Eimeria* infection period. Future studies are needed to find out the mechanisms of nitro compounds on inhibiting sporozoites invasion, as well as an appropriate dosage of NP which will not affect growth



**Figure 2.** Effects of 2-Nitro-1-propanol and nitroethanol on intestinal lesion scores of mixed *Eimeria*-challenged broilers. Birds were fed with different treatment diets on day 12 and challenged with *E. maxima* (100,000 oocysts per bird), *E. tenella* (100,000 oocysts per bird), and *E. acervulina* (250,000 oocysts per bird) on day 14. Six treatments were tested in the study, including nonchallenge control (NC); challenge control (CC); 100 ppm of 2-Nitro-1-propanol (NP100); 200 ppm of 2-Nitro-1-propanol (NP200); 100 ppm of nitroethanol (NE100); 200 ppm of nitroethanol (NE 200). Intestinal lesion was scored at 6 days after infection. The average and percentage of lesion scores were both present in the figures (A, upper intestine; B, middle intestine; C, Ceca.)  $n = 24$ . Means with different letters are significantly different ( $P < 0.05$ ).

performance but improve gut health and inhibit *Eimeria* development in the intestine of broilers.

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## REFERENCES

- Adedokun, S. A., A. Helmbrecht, and T. J. Applegate. 2016. Investigation of the effect of coccidial vaccine challenge on apparent and standardized ileal amino acid digestibility in grower and finisher broilers and its evaluation in 21-day-old broilers. *Poult. Sci.* 95:1825–1835.
- Adhikari, P., D. E. Cosby, N. A. Cox, and W. K. Kim. 2017. Effect of dietary supplementation of nitrocompounds on *Salmonella* colonization and ileal immune gene expression in laying hens challenged with *Salmonella* Enteritidis. *Poult. Sci.* 96:4280–4286.
- Ageely, H. M., A. E. Agag, S. Mohan, and A. Shehata. 2016. Catha edulis (khat) induces apoptosis in Madin-Darby bovine kidney cell line. *Pharmacogn. Mag.* 12(Suppl):454–459.
- Anderson, R. C., T. R. Callaway, J. A. Van Kessel, Y. S. Jung, T. S. Edrington, and D. J. Nisbet. 2003. Effect of select nitrocompounds on ruminal fermentation; an initial look at their potential to reduce economic and environmental costs associated with ruminal methanogenesis. *Bioresour. Technol.* 90:59–63.
- Chapman, H. D. 2014. Milestones in avian coccidiosis research: a review. *Poult. Sci.* 93:501–511.
- Chapman, H. D., T. E. Cherry, H. D. Danforth, G. Richards, M. W. Shirley, and R. B. Williams. 2002. Sustainable coccidiosis control in poultry production: the role of live vaccines. *Int. J. Parasitol.* 32:617–629.
- Chapman, H. D., T. K. Jeffers, and R. B. Williams. 2010. Forty years of monensin for the control of coccidiosis in poultry. *Poult. Sci.* 89:1788–1801.
- Cobb Vantress. 2013. Broiler management guide. Accessed July 2017. COBB Broiler Management Guide [www.tt-trade.cz/docs/cobb-broiler-en.pdf](http://www.tt-trade.cz/docs/cobb-broiler-en.pdf)
- Dalloul, R. A., and H. S. Lillehoj. 2006. Poultry coccidiosis: recent advancements in control measures and vaccine development. *Expert Rev. Vaccines.* 5:143–163.
- Dansky, L. M., and F. W. Hill. 1952. Application of the chromic oxide indicator method to balance studies with growing chickens. *J. Nutr.* 47:449–459.
- Dimitrijevic, M., R. C. Anderson, T. R. Callaway, Y. S. Jung, R. B. Harvey, S. C. Ricke, and D. J. Nisbet. 2006. Inhibitory effect of select nitrocompounds on growth and survivability of *Listeria monocytogenes* *in vitro*. *J. Food Prot.* 69:1061–1065.
- Fuller, A. L., and L. R. McDougald. 1990. Reduction in cell entry of *Eimeria tenella* (Coccidia) sporozoites by protease inhibitors, and partial characterization of proteolytic activity associated with intact sporozoites and merozoites. *J. Parasitol.* 76:464–467.
- Fuller, A. L., and L. R. McDougald. 2001. Cell membrane labeling of *Eimeria tenella* sporozoites with the fluorescent dye PKH-67 GL for tracking parasite-host interactions. *Parasitol. Res.* 87:521–525.
- Hessenberger, S., G. Schatzmayr, and K. Teichmann. 2016. *In vitro* inhibition of *Eimeria tenella* sporozoite invasion into host cells by probiotics. *Vet. Parasitol.* 229:93–98.
- Horrocks, S. M., Y. S. Jung, J. K. Huwe, R. B. Harvey, S. C. Ricke, G. E. Carstens, T. R. Callaway, R. C. Anderson, N. Ramlachan, and D. J. Nisbet. 2007. Effects of short-chain nitrocompounds against *Campylobacter jejuni* and *Campylobacter coli* *in vitro*. *J. Food Sci.* 72:50–55.
- Johnson, J., and W. M. Reid. 1970. Anticoccidial drugs: lesion scoring techniques in battery and floor-pen experiments with chickens. *Exp. Parasitol.* 28:30–36.
- Jung, Y. S., R. C. Anderson, T. R. Callaway, T. S. Edrington, K. J. Genovese, R. B. Harvey, T. L. Poole, and D. J. Nisbet. 2004a. Inhibitory activity of 2-nitropropanol against select food-borne pathogens *in vitro*. *Lett. Appl. Microbiol.* 39:471–476.
- Jung, Y. S., R. C. Anderson, T. S. Edrington, K. J. Genovese, J. A. Byrd, T. R. Callaway, and D. J. Nisbet. 2004b. Experimental use of 2-nitropropanol for reduction of *Salmonella typhimurium* in the Ceca of broiler chicks. *J. Food Prot.* 67:1945–1947.
- Kim, M., A. Takaoka, Q. V. Hoang, S. L. Trokel, and D. C. Paik. 2014. Pharmacologic alternatives to riboflavin photochemical corneal cross-linking: a comparison study of cell toxicity thresholds. *Invest. Ophthalmol. Vis. Sci.* 55:3247–3257.
- Kim, W. K., R. C. Anderson, A. L. Ratliff, D. J. Nisbet, and S. C. Ricke. 2006. Growth inhibition by nitrocompounds of selected uric acid-utilizing microorganisms isolated from poultry manure. *J. Environ. Sci. Health B.* 41(1):97–107.
- Lammers, P. J., B. J. Kerr, M. S. Honeyman, K. Stalder, W. A. Dozier, 3rd, T. E. Weber, M. T. Kidd, and K. Bregendahl. 2008. Nitrogen-corrected apparent metabolizable energy value of crude glycerol for laying hens. *Poult. Sci.* 87:104–107.
- Marugan-Hernandez, V., R. Fiddy, J. Nurse-Francis, O. Smith, L. Pritchard, and F. M. Tomley. 2017. Characterization of novel microneme adhesive repeats (MAR) in *Eimeria tenella*. *Parasit. Vectors* 10:491.
- Olender, D., J. Zwiak, and L. Zaprutko. 2018. Multidirectional efficacy of biologically active nitro compounds included in medicines. *Pharmaceuticals* 11.
- Paik, D. C., Q. Wen, R. E. Braunstein, and S. L. Trokel. 2008. Short chain aliphatic beta-nitro alcohols for corneal cross-linking: corneal endothelial toxicity studies. *J. Refract. Surg.* 24(Suppl):741–747.
- Price, K. R., M. T. Guerin, and J. R. Barta. 2014. Success and failure: the role of relative humidity levels and environmental management in live *Eimeria* vaccination of cage-reared replacement layer pullets. *J. Appl. Poult. Res.* 23:523–535.
- Rochell, S. J., C. M. Parsons, and R. N. Dilger. 2016. Effects of *Eimeria* acervulina infection severity on growth performance, apparent ileal amino acid digestibility, and plasma concentrations of amino acids, carotenoids, and alpha-1-acid glycoprotein in broilers. *Poult. Sci.* 95:1573–1581.
- Shivaramaiah, C., J. R. Barta, X. Hernandez-Velasco, G. Tellez, and B. M. Hargis. 2014. Coccidiosis: recent advancements in the immunobiology of *Eimeria* species, preventive measures, and the importance of vaccination as a control tool against these Apicomplexan parasites. *Vet. Med. Res. Rep.* 5:23–34.
- Yan, X., X. Liu, Y. Ji, G. Tao, and X. Suo. 2015. Ethanol and isopropanol trigger rapid egress of intracellular *Eimeria tenella* sporozoites. *Parasitol. Res.* 114:625–630.
- Yan, X., G. Tao, X. Liu, Y. Ji, and X. Suo. 2016. Calcium-dependent microneme protein discharge and *in vitro* egress of *Eimeria tenella* sporozoites. *Exp. Parasitol.* 170:193–197.
- Yun, C. H., H. S. Lillehoj, and E. P. Lillehoj. 2000. Intestinal immune responses to coccidiosis. *Dev. Comp. Immunol.* 24:303–324.