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Effects of dietary selenium on host response to necrotic enteritis in young broilers

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ABSTRACT

The effects of dietary supplementation of young broiler chickens with an organic selenium (Se) formulation, B-Traxim Se, on experimental necrotic enteritis (NE) were studied. Chickens treated with three Se doses (0.25, 0.50, 1.00 mg/kg) from hatch were orally challenged with Eimeria maxima at 14 days of age followed by Clostridium perfringens to induce NE. Chickens fed with 0.50 mg/kg Se showed significantly increased body weights and antibody levels against NetB, and significantly reduced gut lesions compared with non-supplemented chickens. However, there were no significant differences in Eimeria oocyst shedding between the Se-treated and non-supplemented groups. Levels of IL-1β, IL-6, IL-8, iNOS, LITAF, TNFSF15, AvBD6, AvBD8, and AvBD13 transcripts were increased in the gut and spleen of at least one of the three Se-treated groups compared with the non-treated group. These results suggest that dietary supplementation of young broilers with Se might be beneficial to reduce the negative consequence of NE.

1. Introduction

Necrotic enteritis (NE) is a devastating enteric disease of poultry that results in more than $2 billion economic losses through growth depression, and high morbidity and mortality (Park et al., 2008; Timbermont et al., 2011). NE is caused by the anaerobic bacterium *Clostridium perfringens*, but it is difficult to experimentally reproduce the disease with *C. perfringens* alone because of various risk factors involved in the development of NE. Co-infection with *Eimeria maxima* has been commonly associated with NE (Lee et al., 2011b). With the emergence of antibiotic-resistant pathogens and increasing concerns about chemical residues in poultry meat, many antibiotics which have been traditionally used to prevent and control NE have been restricted or banned. Therefore, there is an urgent need to develop antibiotic alternative methods for reducing economic losses due to NE. Several alternative approaches to antibiotics have been proposed to reduce the negative consequences of NE in broilers, including prebiotics, probiotics, functional feed additives (yeast products, organic acids, essential oils, and phytonutrients), and vaccines against *C. perfringens* toxins (Fernando et al., 2011; Geier et al., 2010; Jerzsele et al., 2012; Lee et al., 2013; Lillehoj and Lee, 2012; Mot et al., 2013).

Selenium (Se) is an essential trace element that plays important roles in immune function, health, and animal productivity (Yoon et al., 2007). Sodium selenite and sodium selenate are the most common inorganic Se sources used in livestock feeds (Yuan et al., 2012). In prior studies, inorganic Se showed an anti-cryptosporidial effect (Huang and Yang, 2002) and promoted protective immunity against *Eimeria tenella* (Colnago et al., 1984). Recently, however, there has been interest in organic Se sources, such as Se-enriched yeast and selenomethionine. Organic Se generally shows higher efficacy and bioavailability and less toxicity compared with inorganic Se (Briens et al., 2013). A new organic Se formulation, B-Traxim Se (Pancosma SA, Geneva, Switzerland), is formed by the incorporation of inorganic Se into soybean protein, which is subsequently hydrolyzed to form a commercial product specifically designed for animal feeding (Pavlata et al., 2012). A previous study reported that breeder hens fed B-Traxim Se showed high levels of Se in eggs and lower levels of glutathione peroxidase in liver than those fed sodium selenite (Leeson et al., 2008). The objective of the present study was to evaluate the effects of different levels of dietary B-Traxim Se in broilers on host protective response against experimental NE using an *E. maxima*/*C. perfringens* co-infection model system (Park et al., 2008).

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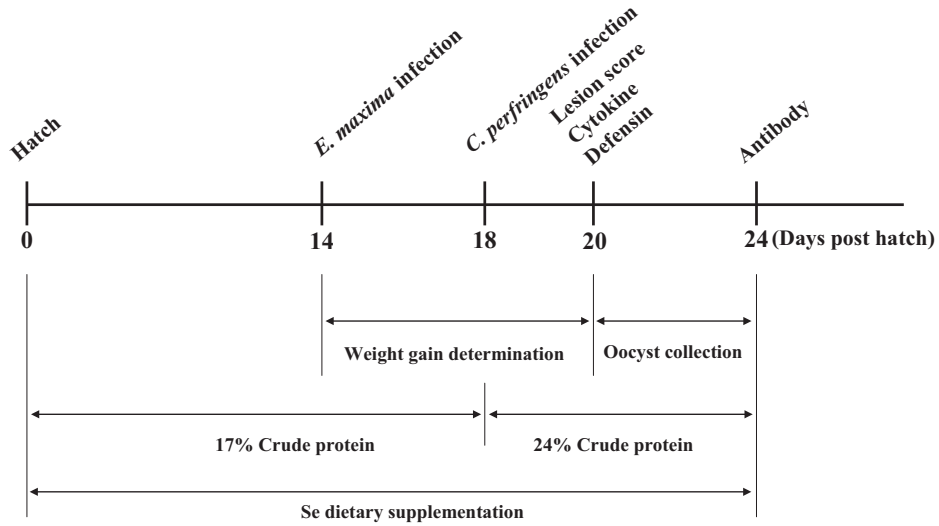

Fig. 1. Schematic outline of the experimental design.

2. Materials and methods

2.1. Experimental design

A schematic diagram of the experimental protocol is shown in Fig. 1. One-day-old Ross broiler chickens (Longenecker's Hatchery, Elizabethtown, PA) were housed in Petersime starter brooder units and provided with an antibiotic-free starter diet (Table 1) between days 1 and 18 and a grower diet (Table 1) after day 18 until the end of trial. Both basal diets include 0.10 mg Se/kg diet. Feed and water were given *ad libitum*. Chickens were randomly divided into five groups (15 chickens/group): uninfected control, infected and non-supplemented control, and infected and supplemented groups with 0.25, 0.50, or 1.00 mg/kg of B-Traxim Se. All experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of the Beltsville Agricultural Research Center.

2.2. Experimental NE model

Chickens were reared in brooder pens in an *Eimeria*-free facility for 14 days post-hatch and then transferred into large hanging cages (3 birds/cage) at a separate location where they were infected and kept until the end of the experiment. At 14 days of age, chickens were orally infected with *E. maxima* Beltsville strain 41A (1.0×10^4 sporulated oocysts/bird), followed by *C. perfringens* strain Del-1 (1.0×10^9 CFU/bird) at 18 days of age. To facilitate the development of NE, the diet formulation was changed from the starter diet to the grower diet after day 18.

Table 1
Ingredient and nutrition of the basal diets.[a]

Ingredients[b] (g/kg)	Starter diet (days 0–18)	Grower diet (days 18–24)
Crude protein	170.0	240.0
Carbohydrate	610.0	540.0
Selenium-free mineral and vitamin mixture	150.0	150.0
Fat	47.0	47.0
Fiber	24.0	24.0
Selenium[c] (mg/kg)	0.10	0.10

[a] Based on the producer's declaration that B-Traxim Se contains 1.1% (wt/wt) Se metal content, the basal diets were added to the B-Traxim Se (Pancosma S.A., Geneva) to prepare the 0.25, 0.50, and 1.00 mg/kg Se supplemented diets by calculation.
[b] Data were from USDA/FeedMill, Beltsville, MD.
[c] The Se concentration was calculated.

2.3. Evaluation of body weight gain, lesion score, and oocyst shedding

Body weight gains were measured between 0 and 6 days post-infection with *E. maxima*. Intestinal lesion scores were evaluated at 6 days post-infection with *E. maxima* on a scale of 0 (none) to 4 (high) in a blinded fashion by three independent observers as described earlier (Prescott, 1979). Fecal oocysts were individually enumerated between 6 and 10 days post-infection with *E. maxima* using a McMaster chamber as described (Jang et al., 2010).

2.4. Determination of serum antibody levels against C. perfringens *toxins*

Blood samples (4 birds/group) were collected by cardiac puncture at 6 days post-infection with *C. perfringens* following euthanasia. Sera were prepared by low speed centrifugation and serum antibodies against α-toxin and NetB toxin were measured by enzyme-linked immunosorbent assay (ELISA) as described (Lee et al., 2011c) using recombinant *C. perfringens* α-toxin and NetB toxin expressed in *Escherichia coli* as described (Lee et al., 2011a). Briefly, 96-well microtiter plates were coated overnight with 1.0 μg/well of purified recombinant toxin proteins, washed with PBS containing 0.05% Tween 20 (PBS-T), and blocked with PBS containing 1% BSA for 1 h at room temperature. Diluted serum samples (1:20, 100 μl/well) were added and incubated with gentle shaking for 2 h at room temperature. The plates were washed with PBS-T and bound antibodies were detected with peroxidase-conjugated rabbit anti-chicken IgG and peroxidase-substrate (Sigma, St. Louis, MO). The optical density (OD) at 450 nm was determined with an automated microtiter reader (Bio-Rad, Richmond, CA). All samples were analyzed in quadruplicate.

2.5. Quantification of pro-inflammatory cytokines and AvBD transcript levels

The levels of transcripts for pro-inflammatory cytokines and AvBD were measured as described (Hong et al., 2006; Kim et al., 2008) by quantitative RT-PCR. At 2 days post-infection with *C. perfringens*, the spleen and jejunum located proximal to the Meckel's diverticulum