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Short communication

Effects of feed-borne *Fusarium* mycotoxins and an organic mycotoxin adsorbent on immune cell dynamics in the jejunum of chickens infected with *Eimeria maxima*

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ABSTRACT

An experiment was conducted to explore the effects of *Fusarium* mycotoxins, common animal feed contaminants, on intestinal immune responses to coccidia (*Eimeria*) in chickens. Effects of feed-borne *Fusarium* mycotoxins and a polymeric glucomannan mycotoxin adsorbent (GMA) on immune cell populations were studied in the jejunum of broiler breeder pullets using an *Eimeria maxima* infection model. Birds were fed a control diet, a diet naturally contaminated with *Fusarium* mycotoxins, contaminated diet plus 0.2% GMA, or control diet plus 0.2% GMA. Contaminated diets contained up to 6.5 μg/g deoxynivalenol (DON), 0.47 μg/g 15-acetyl-DON and 0.73 μg/g zearalenone. Birds received a primary oral inoculation (1000 oocysts/bird) with *E. maxima* USDA strain 68 at 2 weeks of age and a secondary oral inoculation (30,000 oocysts/bird) with the same strain at 4 weeks of age. Diet-related differences in $CD4^+$ cell, $CD8^+$ cell and macrophage recruitment pattern into the jejunum were observed following both the primary and secondary infections. It was concluded that feed-borne *Fusarium* mycotoxins and GMA have the potential to modulate immune response to coccidial infections.

1. Introduction

Eimeria maxima is an apicomplexan coccidian parasite which infects the jejunum of chickens after ingestion of sporulated oocysts during feeding (Rothwell et al., 1995). Local immune responses to *Eimeria* have been found to vary with the age and genetic makeup of experimental birds, and the dose and strain of the *Eimeria* species inducing infection (Bessay et al., 1996; Yun et al., 2000). Cell-mediated immune responses, nevertheless, are known to be predominant in the intestine of *Eimeria*-infected animals (Lillehoj, 1998). $CD4^+$ cells, macrophages and intraepithelial lymphocytes are the main cells responsible for controlling primary infections, while $CD8^+$ cells play the major role in immunity to secondary infections (Lillehoj, 1998).

Information is lacking on the effects of feed-borne contaminants on intestinal immune responses to *Eimeria*. *Fusarium* mycotoxins, for example, are common contaminants of animal feed. Among these mycotoxins, the trichothecene deoxynivalenol (DON) and acetylated DON derivatives are a major concern due to their high incidence and stability (Pestka and Smolinski, 2005). Repetitive exposure to trichothecenes may result in injury to actively dividing cells such as those present in bone marrow and intestinal mucosa, mainly due to inhibition of protein synthesis (Rocha et al., 2005). The intestinal epithelium could

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be exposed to high concentrations of mycotoxins following ingestion of contaminated feed (Bouhet and Oswald, 2005).

In previous studies on *Fusarium* mycotoxicoses under field-relevant conditions, it was observed that feed-borne *Fusarium* mycotoxins at concentrations that did not affect performance could modulate the relative proportions of lymphocyte subsets in the blood of broiler breeder pullets infected with multiple *Eimeria* species (Girgis et al., 2008). Feed-borne *Fusarium* mycotoxins also altered the morphology of villus–crypt units in the intestine (Girgis et al., 2010). The current study is an investigation of the dynamics of immune cell populations in the jejunum (multiplication site of *E. maxima*) of broiler breeder pullets fed diets containing grains naturally contaminated with *Fusarium* mycotoxins and supplemented with a polymeric glucomannan mycotoxin adsorbent (GMA).

2. Materials and methods

2.1. Experimental birds

Two hundred and sixty four 1-day-old Ross 308 broiler breeder female chicks (Aviagen Inc., Blairsville, GA) were raised in the Animal Facility Isolation Unit of the Ontario Veterinary College. Birds were managed as prescribed by the Canadian Council on Animal Care with the Animal Utilization Protocols approved by the Animal Care Committee of the University of Guelph.

2.2. Experimental diets

Corn-, wheat- and soybean meal-based control diets were formulated to meet the standard nutritional specifications for starter and grower broiler breeder chickens. The mycotoxin-contaminated diets were formulated to the nutrient specifications of the control diets by replacing control corn and wheat with corn and wheat naturally contaminated with *Fusarium* mycotoxins. GMA-supplemented diets contained 0.2% GMA (Mycosorb®, Alltech Inc., Nicholasville, KY). No coccidiostat was included in any of the experimental diets.

Dietary contents of DON, 3-acetyl-DON, 15-acetyl-DON, nivalenol, T-2 toxin, HT-2 toxin, diacetoxyscirpenol and neosolaniol were analyzed by gas chromatography-mass spectrometry as described by Raymond et al. (2003). Fumonisin concentrations were determined by immunochemistry, whereas zearalenone, ochratoxin A and the aflatoxins G_1, G_2, B_1 and B_2 were analyzed by high performance liquid chromatography following extraction using immunoaffinity columns (Wang et al., 2008).

2.3. Experimental design

One-day-old chicks were randomly divided into four groups of 66 birds each. Each group was fed one of the four experimental diets. Birds were orally inoculated with *E. maxima* (USDA strain 68; 1000 sporulated oocysts/bird) at 2 weeks of age. At 4 weeks of age, birds were orally inoculated again with a challenging dose of the same *E. maxima* strain (30,000 sporulated oocysts/bird). At day 0, 3 and 6 post-primary infection and day 0, 1, 2, 3 and 6 post-secondary infection, six birds from each group ($n = 24$) were randomly selected, killed by cervical dislocation and immediately sampled as described below.

Birds were fed starter diets *ad libitum* from day 1 to 3 weeks of age and grower diets on skip-a-day feed restriction program from 3 weeks to 6 weeks of age. Birds were reared on the floor for the entire experimental period. On the fifth day after each oral inoculation, four birds from each group ($n = 16$) were individually housed in cages with wire mesh floor in order to prevent fecal–oral re-infection and collect droppings for determination of oocyst output. Droppings were collected from day 6 to day 14 post-infection (PI). Ten birds remaining in each group after the last sampling point ($n = 40$) were raised for one more week in order to measure body weight gain.

2.4. Body weight gain and Eimeria oocyst count

Birds were weighed individually on a weekly basis throughout the experimental period, and weekly body weight gains were calculated. *Eimeria* oocyst counts were calculated using McMaster method as described by Girgis et al. (2010).

2.5. Sample collection and immunohistochemistry staining

Intestinal segments were collected from the jejunum of freshly killed birds 5 cm proximal to Meckel's diverticulum. Tissues were flushed with saline, embedded in OCT (Tissue-Tek®, Sakura, Finetek Inc., Torrance, CA) and immediately frozen in liquid nitrogen. Frozen tissues were sectioned at 5 μm thickness, placed on Superfrost Plus glass slides (Fisher Scientific, Ottawa, ON) and stored at −20 °C until used.

Tissue sections were fixed with ice-cold acetone for 10 min. Endogenous peroxidase activity was inhibited by incubating the tissues for 10 min with 3% H_2O_2 prepared in phosphate buffered saline (PBS) containing 0.3% normal goat serum. Blocking of nonspecific sites was done by incubating the tissues for 30 min with 5% normal goat serum in PBS. Sections were then incubated with the appropriate primary antibodies (Southern Biotech, Birmingham, AL) for 30 min. Mouse monoclonal anti-chicken CD4 (clone CT-4), anti-chicken CD8a (clone CT-8), anti-chicken IgM (clone M-1), and anti-chicken monocyte/macrophage (clone KUL01) were used as the primary antibodies at dilutions of 1:200, 1:200, 1:150 and 1:400 in blocking buffer, respectively. Biotinylated goat anti-mouse IgG (H + L) (Vector Laboratories, Burlingame, CA) was used as the secondary antibody at a dilution of 1:250 in blocking buffer. Sections were incubated with the secondary antibody for 30 min. Avidin–biotin–peroxidase system (Vectastain® ABC kit, Vector Laboratories, Burlingame, CA) was used for immunoperoxidase staining according to the manufacturer's protocol. Enzyme-linked antibodies were then visualized by reaction with 3,3-diaminobenzidine–H_2O_2 solution (DAB substrate kit for peroxidase, Vector Laboratories, Burlingame, CA). Tissue sections were counterstained with hematoxylin and mounted with Cytoseal-60 (Richard-Allan-Scientific, Kalamazoo, MI).