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Dietary oregano essential oil alleviates experimentally induced coccidiosis in broilers

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ABSTRACT

An experiment was conducted to determine the effects of oregano essential oil on growth performance and coccidiosis prevention in mild challenged broilers. A total of 250 1-d-old chicks were used in a completely randomized design with 5 treatments and 5 replicates with 10 birds in each replication. Experimental treatments included: (1) negative control (NC; unchallenged), (2) positive control (PC; challenged with sporulated oocysts of *Eimeria*), (3) PC fed 200 ppm Diclazuril in diet, (4) PC fed 300 ppm oregano oil in diet, and (5) PC fed 500 ppm oregano oil in diet. At 22 d of age, all the experimental groups except for NC were challenged with 50-fold dose of Livacox T as a trivalent live attenuated coccidiosis vaccine. On d 28, two birds were slaughtered and intestinal coccidiosis lesions were scored 0–4. Moreover, dropping was scored in the scale of 0–3, and oocysts per gram feces (OPG) were measured. Oregano oil at either supplementation rate increased body weight gain ($P = 0.039$) and improved feed conversion ratio ($P = 0.010$) from d 22 to 28, when compared with PC group. Using 500 ppm oregano oil in challenged broilers diet increased European efficiency factor than PC group ($P = 0.020$). Moreover, challenged broilers fed 500 ppm oregano oil or Diclazuril in diets displayed lower coccidiosis lesions scores in upper ($P = 0.003$) and middle ($P = 0.018$) regions of intestine than PC group, with the effect being similar to unchallenged birds. In general, challenged birds fed 500 ppm oregano oil or Diclazuril in diets had lower OPG ($P = 0.001$), dropping scores ($P = 0.001$), litter scores ($P = 0.001$), and pH of litter ($P = 0.001$) than PC group. It could be concluded that supplementation of oregano oil at the dose of 500 ppm in diet may have beneficial effect on prevention of coccidiosis in broilers.

1. Introduction

Coccidiosis is an important parasitic disease of the intestinal tract of chickens caused by coccidian protozoa of the genus *Eimeria*. The disease especially affecting young chicks and spreads from one chick to another by contact with infected dropping. Parasite proliferation in the epithelium cells causes tissue destruction that increased incidence of diarrhea, intestinal hemorrhage and may overset digestive processes, leading to decreased body weight gain and feed consumption, and impaired feed conversion ratio (Williams, 2005; Hafez, 2008). Furthermore, it is responsible for significant economic loss in the poultry industry in many parts of the worlds (Chapman et al., 2010). Therefore, coccidiosis is known as the most costly transmissible disease in commercial poultry flocks.

Applying coccidiostat drugs and attenuated vaccines, are the common approaches to prevent and control this disease. The frequent use of anticoccidial medications leading to the egress of drug-resistant *Eimeria* strains, and the

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vaccines are expensive to produce (Peek and Landman, 2003; Abbas et al., 2011). Therefore, developing safe and inexpensive methods to control coccidiosis in poultry are needed. The use of herbals and their derived compounds could be a potential alternative to control poultry coccidiosis (Abbas et al., 2012).

The essential oil of *Origanum vulgare* is famous for its antimicrobial, antifungal, and antiprotozoal activity (Milhau et al., 1997; Adam et al., 1998; Botsoglou et al., 2002). It contains majorly carvacrol and thymol that constitute almost 78–82% of the total oil (Lee et al., 2004), which has shown antiprotozoal effect *in vitro* (Milhau et al., 1997), and *in vivo* (Giannenas et al., 2003, 2013; Tsinas et al., 2011), though there is some available information about the effect of oregano essential oil on avian coccidiosis, more investigations are needed to detect efficacious dose of administration for alleviating harmful effects of *Eimeria* spp. (Tsinas et al., 2011). The objective of this study was to test the efficacy of oregano essential oil in comparison with an anticoccidial drug (Diclazuril) on growth performance and coccidiosis prevention in challenged broiler chickens.

2. Materials and methods

2.1. Birds, diets and management

The trial was conducted at the agricultural experiment station of University of Guilan from January to February 2014. Two hundred and fifty straight-run 1-d-old Ross 308 broiler chicks, with average body weight of 46.8 ± 0.8 g were used in this study. The chicks were feather sexed and distributed into 25 homogenous groups of floor pens (1 m × 1 m) according to their sex and initial weight. Pens were randomly assigned to five treatments with each treatment having five replicates of 10 birds. Chicks were kept on wood shavings at 32 °C during first 7 d of age, and then temperature was stepped down to 23 °C by 21 d of age. There was continuous light regimen during the first 2 d, then 23 h lighting was applied up to 42 d of age. Light intensity was 20 lux at the first 2 d then was decreased to 5 lux for the remaining period (3–42 d). All the chicks were vaccinated based on a routine program. Diets were provided in mash form and formulated according to Ross 308 recommendations without including any antimicrobial product. Feed and fresh water offered *ad libitum* throughout the experiment. Diets were prepared at pilot feed mill at University of Guilan. Table 1 describes the diet ingredients and nutrient contents of the basal diets. The two control treatments (NC; unchallenged, and PC; challenged group) included no measure for coccidiosis prevention, however, other groups challenged with coccidiosis received a dietary treatment. As a reference treatment, Diclazuril (Clinacox® 0.5%; Huverpharma, Inc.) at 200 ppm was administered. Oregano essential oil was tested at 2 rates of 300 and 500 ppm. Oregano oil was in the form of a powder called Orego-Stim® (Meriden Animal Health Ltd., Luton, UK) that contains 5% essential oil of *O. vulgare* subsp. Hirtum plants and 95% natural feed grade inert carrier (Giannenas et al., 2003). All the dietary treatments were fed continuously for 42 d from 1 d old.

2.2. Inoculation

At d 22, all the experimental groups except NC, were challenged with 50 doses of the Livacox T (Biopharm Co., Prague, Czech Republic) orally, to produce a mild coccidiosis infection as described by Mansoori and Modirsanei (2012). Each dose of vaccine contained 300–500 sporulated oocysts of each of *Eimeria acervulina*, *Eimeria maxima*, and *Eimeria tenella* in 0.01 ml distilled water. Birds of NC were sham-inoculated with 0.5 ml of distilled water.

2.3. Collection of samples and measurements

Body weight of the birds and feed consumption were recorded weekly by replicate, and mortality was recorded and weighed as produced. From these data, body weight gain (BWG), average daily feed intake (ADFI), and feed conversion ratio (FCR) were calculated by week and for the entire experimental period. At the end of experiment (d 42), the European efficiency factor (EEF) was calculated using the following formula: BW (kg) × %liveability × 100/FCR × trial duration (d) (Huff et al., 2013).

Coccidial lesion scoring was carried out 6 d after challenge using the method described by Johnson and Reid (1970). At d 28, two birds per pen were randomly selected, weighed, and slaughtered humanely by knife. Three regions (upper duodenum, middle jejunum, and cecum) of intestinal tract were examined for coccidial lesions. Generally, these regions correspond grossly to natural predilection sites for *E. acervulina*, *E. maxima*, and *E. tenella*, respectively. Based upon severity of the lesions, a score of 0 (no lesions), 1 (mild lesions), 2 (moderate lesions), 3 (severe lesions) or 4 (extremely severe lesions) was recorded for each chicken. A cumulative lesion score was used to judge overall multiple species anticoccidial efficacy. This cumulative lesion score was calculated by adding the lesion scores of each of the three coccidial lesion scored regions.

Excreta samples of each pen were collected on d 28 (6 d after challenge infection), and the oocysts per gram feces (OPG) counting performed by means of a modification of the McMaster counting chamber technique of Hodgson (1970) and Peek and Landman (2003). Droppings and litter scores was scored visually on d 28 by 3 observers blind to the treatments, as described by Morehouse and Baron (1970) and Benabdeljelil and Ayachi (1996), respectively. The means of the 3 values were used for statistical analysis. Droppings scored 0–3, in which 0 was assigned for normal dropping, 1 for few droppings were purplish or brownish in color, 2 for more reddish droppings, some dropping mixed with flakes of blood, and 3 for bloody droppings, absence of normal fecal content. The scale used for litter varied from 1 to 5, with a score of 1 indicating no caking and extremely dry litter and a score of 5 indicating heavy caking and extremely wet litter.

Litter samples for measuring pH were collected from five locations within each pen (4 peripheral samples and 1 central one) and thoroughly mixed to obtain material representative of the entire pen. The pH of each litter type was measured using a pH meter after litter samples of nearly