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# Effects of dietary tannic acid and vaccination on the course of coccidiosis in experimentally challenged broiler chicken

#### Behzad Mansoori\*, Mehrdad Modirsanei

Department of Animal and Poultry Health and Nutrition, Faculty of Veterinary Medicine, University of Tehran, PO Box 14155-6453, Tehran, Iran

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#### ABSTRACT

An experiment was carried out to assess the influence of tannic acid (TA) on integrity of the intestine in broiler chicks vaccinated against coccidiosis and challenged with the disease. In a  $2 \times 2$  factorial design, the trial had five groups of 10 chickens each, including positive (group 2) and negative (group 1) controls. The chickens were kept on wood shavings and fed a commercial maize and sovbean-based starter-grower diet. From day 1. groups 3 and 5 received TA (10 g kg<sup>-1</sup>) in their diet. On day 4, birds of groups 4 and 5 were vaccinated orally against coccidiosis with anti-coccidial vaccine, Livacox T<sup>TM</sup>. Each dose of the vaccine contained 300-500 sporulated oocysts of each of Eimeria acervulina, Eimeria maxima and *Eimeria tenella*. On day 18, all experimental groups except for the negative (group 1) were challenged with 10-fold dose of Livacox T<sup>TM</sup> to produce a mild coccidiosis infection. Faecal samples of individual birds were collected on day 23, and the number of faecal oocysts was determined. D-Xylose absorption test was also carried out on all birds on day 23. Immediately after D-xylose absorption test, all birds were killed humanely and the intestinal tract was removed, weighed and examined for gross lesions. Results showed that negative (group 1) and positive controls (group 2) had the highest and lowest levels of plasma D-xylose postingestion of the substrate, respectively. Vaccination and/or feeding TA raised the level of plasma D-xylose in infected birds, although this was not significant for TA-fed birds. Vaccination reduced but TA increased the total number of oocysts per gram of faeces. Birds of groups 2-5 had distinct intestinal lesions when compared with group 1. However, vaccination prevented intestinal lesions. Relative weights of intestinal parts were the lowest in group 1 and the highest in group 2. Vaccination but not TA reduced the relative weights of intestinal parts in infected birds. It was concluded that dietary tannins may reduce the efficacy of anticoccidial vaccines and alter the proper development of immunity against the disease.

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Coccidiosis is the most important parasitic disease of chicken in many parts of the world. The parasite, *Eimeria*, invades the cells of the intestine producing enteritis and diarrhoea, resulting in a disability to absorb dietary nutrients through the disruption of the integrity of the intestinal mucosa (Adams et al., 1996; Greif, 2000; Persia et al., 2006; Zhao et al., 2006). The disease is passed from bird to bird via droppings, which means that the problem is greatest in intensive husbandry. Coccidiosis is controlled mainly by coccidiostats or live attenuated vaccines supplemented in the feed or water (Lillehoj et al., 2000; Badran and Lukesova, 2006).

It is suggested that, in poultry, diets containing plantbased bioactive compounds, such as green tea extract or grape seed proanthocyanidins, lower the small intestinal lesion scores, oocyst output and mortality as well as improve weight gains and feed conversion ratio during

<sup>\*</sup> Corresponding author. Tel.: +98 21 61117105.

*E-mail addresses*: bmansoori@ut.ac.ir, mansoori61@yahoo.co.uk (B. Mansoori).

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Eimeria infections (Allen and Fetterer, 2002; Jang et al., 2007; Naidoo et al., 2008; Wang et al., 2008; Wallace et al., 2010). These compounds may exert their anticoccidial activity by protecting infected intestinal tissues from oxidative damage, thereby reducing the severity of coccidiosis. However, the effect of these kinds of antioxidants seems to be restricted to certain *Eimeria* species only, especially E. acervulina and E. maxima (Allen and Fetterer, 2002). Water-soluble extracts from green tea or pine bark containing condensed tannins have anti-coccidial activity as evidenced by their ability to decrease significantly the sporulation of the oocysts of Eimeria tenella, E. maxima and E. acervulina under laboratory conditions, to a great extent (Molan and Thomas, 2007; Molan et al., 2009). Moreover, tannins possess the ability of inhibiting a wide range of fungi, yeasts and bacteria as these metabolites form complexes with microbial enzymes or metal ions, thus inhibiting their activity, or disturb the electron transport system of the microorganism membrane (Scalbert, 1991; Chung et al., 1998).

This study aimed to evaluate the influence of tannic acid (TA), a model hydrolysable tannin, on the integrity of the intestine and the efficacy of live attenuated anti-coccidial vaccine in broiler chickens challenged with experimental coccidiosis. Intestinal integrity of the experimental birds was assessed by estimating oocyst excretion, xylose absorption, lesion scores and relative intestinal weight.

#### 1. Materials and methods

Based on a  $2 \times 2$  factorial design, the experiment was arranged to have four experimental groups plus one group as negative control. Fifty newly hatched male broiler chicks (Ross 308), with a mean body weight (BW) of  $42 \pm 2$  g, were individually labelled by feet rings and randomly divided into five groups of 10 birds. Chicks were kept on wood shavings at a room temperature of 31 °C in the first 3 days followed by a reduction of 1 °C for every 2-3 days until the temperature reached 24°C. Lighting was continuous and the relative humidity was set at 60%. A commercial maize and soybean-based starter-grower diet, without any growth-promoting additives, was fed according to the feeding standards of the breed. It contained 12.1 MJ kg<sup>-1</sup> metabolisable energy,  $200 \text{ g kg}^{-1}$  crude protein,  $4.6 \text{ g kg}^{-1}$ total methionine, 12.3 g kg<sup>-1</sup> total lysine, 9.8 g kg<sup>-1</sup> total calcium, 4.2 g kg<sup>-1</sup> available phosphorus and other nutrients were provided with the feed in at least sufficient quantity (http://www.aviagen.com). Feed and water were provided ad libitum. From day 1, groups 3 and 5 received TA (10 g kg<sup>-1</sup> of diet, Sigma–Aldrich Chemie GmbH, Deisenhofen, Germany) in their diet. On day 4, birds of groups 4 and 5 were vaccinated orally against coccidiosis with Livacox T<sup>TM</sup> (Biopharm Co., Prague, Czech Republic). Each dose of vaccine contained 300-500 sporulated oocysts of each of E. acervulina, E. maxima and E. tenella in 0.5 ml distilled water. At day 18, all experimental groups except the negative control (group 1) were challenged with 10 doses of the same vaccine  $(3-5 \times 10^3$  sporulated oocysts of each *Eimeria* in 1 ml distilled water) orally, to produce a mild coccidiosis infection. Birds of group 1 were sham-inoculated with 1 ml of distilled water. Excreta samples of individual birds were

collected on day 23 (5 days after challenge infection), and the number of faecal oocysts was determined according to the method described by Molan et al. (2009).

### 1.1. D-Xylose administration and measurement in plasma

D-Xylose absorption test was carried out on all birds of each experimental group on day 23. Briefly, D-xylose solution was prepared by dissolving D-xylose powder (50 mg ml<sup>-1</sup>, Sigma Chemical Co., St. Louis, MO, USA) in distilled water. Food and water were removed from each group of the bird 12 h prior to the first collection of blood. All birds were weighed individually and given D-xylose solution at a dose of  $500 \text{ mg kg}^{-1}$  BW via oral gavage. One blood sample prior-to and the other sample 80 min post-ingestion of the test material were collected by ulnar vein puncture, using heparinised micro-haematocrit capillary tubes (Code - No. 9100260, Hirschmann Laborgeräte Techcolor, Germany). Tubes were centrifuged, plasma was separated and the concentration of D-xylose in plasma was measured according to the method described by Goodwin et al. (1985).

By the termination of the experiment and immediately after D-xylose absorption test, all birds were killed humanely by cervical dislocation and the intestinal tract was removed, weighed and carefully examined externally and internally for coccidial lesions. The coccidial lesion scores were assigned from 1 (no gross lesion) to 4 (most severe gross lesion) in the appropriate regions according to the method described by Johnson and Reid (1970). Weights of intestinal parts were recorded in percent as relative to live BW of each bird.

#### 1.2. Statistical analysis

Analysis of data was carried out using one-way analysis of variance (ANOVA; Minitab 13.2 statistical package, Minitab Inc. State College, 2000). Fisher's least significant difference (LSD) method was used to find confidence intervals for all pairwise differences between means. General linear model was performed to measure the main effects and interaction of factors (vaccine and TA) on each measured variable of coccidiosis-infected birds. All statements of significance were based on a probability of P < 0.05.

#### 2. Results

Uninfected birds (group 1) and infected birds neither vaccinated nor having TA in their diet (group 2) had the highest and lowest levels of D-xylose in their plasma at 80 min post-ingestion of the substrate, respectively (Table 1). Vaccination and/or feeding TA raised the level of plasma D-xylose in infected birds, although it was not significant for TA fed birds (Table 2). The number of oocysts present in per gram of faeces was high in groups 2 and 3 (Table 1), and vaccination resulted in a reduction in total oocyst output of infected birds (group 4). Although group 5 had a lower oocyst output, the difference was only numerical when compared with groups 2 and 3. In general, vaccination reduced but TA increased the number of

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