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Short communication

Anticoccidial effect of green tea-based diets against *Eimeria maxima*

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Abstract

Anticoccidial effects of green tea (GT)-based diets were evaluated in chickens following oral infection with *Eimeria maxima* an ubiquitous intestinal parasite of poultry that impairs the growth and feed efficiency of infected birds. Five-week-old chickens were assigned to four groups (GT 0.5%, GT 2.0%, untreated/infected and non-infected control) and each group consisted of 15 chickens. Chickens were fed a standard diet supplemented with ground green tea for 2 weeks prior to infection with *E. maxima* (10,000 sporulated oocysts per bird). The effects of green tea on *E. maxima* infection were assessed by two parameters, fecal oocyst shedding and body weight gain. The green tea-fed chickens produced significantly reduced fecal oocysts ($P < 0.05$) when compared to the *E. maxima*-infected group fed standard diet. The green tea-based diet, however, did not improve body weight loss caused by *E. maxima* infection. This study is the first to demonstrate anticoccidial effect of green tea on *Eimeria* parasites.

Keywords: Green tea; *Eimeria*; Chickens; Immunity

1. Introduction

Seven species of *Eimeria*, an intracellular protozoa belonging to the phylum Apicomplexa, cause avian coccidiosis, an economically important disease in the poultry industry. *Eimeria* infection causes the extensive destruction of the intestinal epithelium which results in reduced feed efficiency, body weight gain, and a temporary reduction in egg production (Dalloul and Lillehoj, 2005; Min et al., 2004). Although coccidiosis is mainly controlled by the use of chemotherapeutic agents, novel approaches are urgently needed due to the increasing emergence of drug-resistant parasite strains in commercial poultry production settings (Allen and Fetterer, 2002; Chapman, 1997; Williams, 2002). Feeding natural dietary supplements or probiotics to animals to enhance their innate defense mechanisms could effectively reduce or prevent the need for therapy of these enteric infections.

Green tea which is a popular beverage comes from a nonoxidized and unfermented product of leaves from the evergreen plant *Camellia sinensis*. Green tea contains a number of polyphenolic compounds, collectively termed

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catechins such as epicatechin, epicatechin gallate, epigallocatechin, and epigallocatechin gallate (EGCG). EGCG accounts for approximately 50% of the total amount of catechins (Crespy and Williamson, 2004; Fujiki, 2005). These components are known to have anti-tumorigenic, anti-inflammatory, antioxidative, antiproliferative, antibacterial, antiviral, and anti-parasitic properties (Crespy and Williamson, 2004; Fujiki, 2005; Isogai et al., 2001; Molan et al., 2003, 2004; Ryu, 1982; Weber et al., 2003). Although a variety types of natural products have been investigated in search for alternative controls of coccidiosis in chickens (Allen and Fetterer, 2002; Dalloul and Lillehoj, 2005), the effects of green tea on *Eimeria* infection has not been studied. This present work investigated the effects of green tea-based diets on *E. maxima* infection.

2. Materials and methods

2.1. Experimental chickens and treatments

Fertilized eggs were produced from the mating of female Rhode Island Red chickens and male Korean native chickens and hatched at the National Livestock Research Institute (Daejeon, Korea). Five-week-old male chickens ($n = 60$) were randomly assigned to four groups (GT 0.5%, GT 2.0%, untreated/infected and non-infected control) and each group was consisted of 15 chickens. Two groups were fed with a standard diet supplemented with 0.5% and 2.0% powdered green tea. The third group was composed by untreated and infected birds, and the fourth group was composed by untreated and uninfected control. Green tea powder (Boseong, Korea) was prepared by air drying at room temperature and grinding evergreen plant leaves of *C. sinensis*. The chickens were given unlimited access to feed and water and constant light was provided for the duration of the experiment.

2.2. Eimeria infections and assessment of fecal oocyst production and body weight changes

E. maxima were cleaned by flotation on 5.25% sodium hypochlorite and washed three times with phosphate buffered saline. Chickens were orally infected with 1×10^4 sporulated oocysts of *E. maxima* at 7 weeks of age and transferred to wire-floored grower cages (three birds/cage). Fecal materials were collected from 6 to 10 days post-infection, and the number of oocysts were assessed using a McMaster counting chamber. Total oocyst numbers were calculated using the following formula: [total number oocysts = oocyst count × dilution factor × (fecal sample volume/counting chamber volume)/number of birds per cage]. Body weights were individually measured during 2 weeks before infection and for the 10 days post-infection.

2.3. Statistical analysis

Differences in mean oocyst production and in mean weight gain between the four groups were tested by a one-way ANOVA (GraphPad InStat, GraphPad Software Inc., San Diego, CA) and considered significant at $P < 0.05$.

3. Results

Fecal oocyst shedding and body weight loss were the most reliable disease parameters that have been used to measure the effects of coccidiosis (Chapman et al., 2005; Lillehoj and Trout, 1996). As shown in Fig. 1, the chickens fed diets supplemented with 0.5% and 2% green tea exhibited significantly reduced oocyst shedding when compared to the untreated and infected birds ($P < 0.05$). The group fed diet supplemented with 0.5% green tea (GT 0.5%) showed a reduction of 38.5% in fecal oocyst output and the group supplemented with 2% green tea (GT 2.0%) showed a reduction of 51.5% in fecal oocyst output following *E. maxima* infection. No statistical differences were found in body weight between the control and green tea-fed chickens before infection ($P > 0.05$). There was no significant difference in body weight between the untreated/infected and green tea-fed and infected groups (Fig. 2).

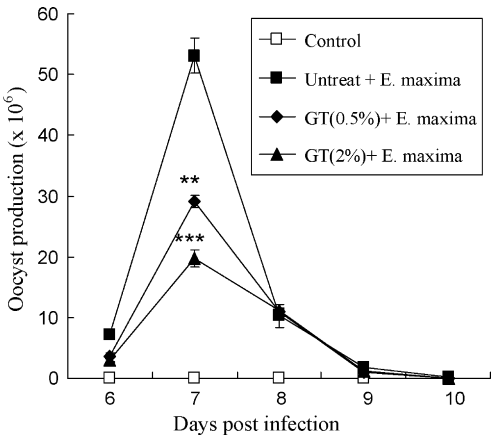

Fig. 1. Effect of green tea-based diets on the fecal oocyst output. Bars represent the means ± standard deviation of 15 chickens. The asterisks indicate significantly reduced oocyst shedding compared to the untreated and infected group ($^{**}P < 0.01$, $^{***}P < 0.001$).

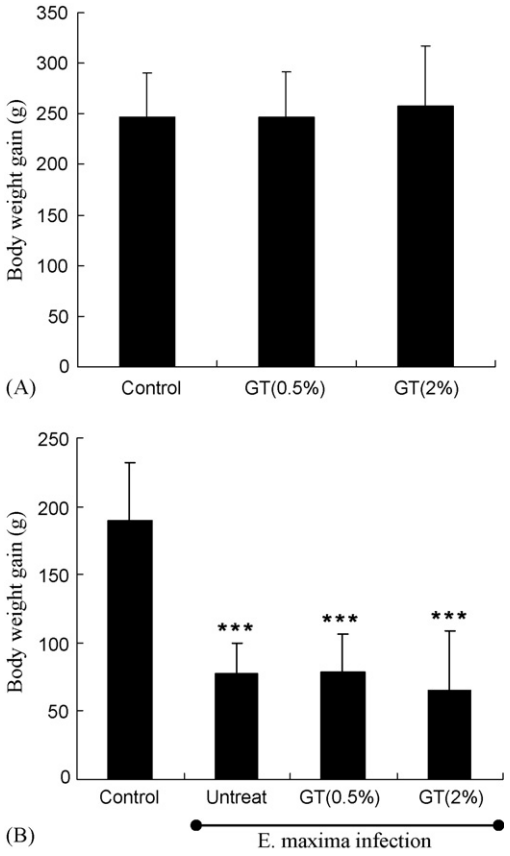

Fig. 2. Comparsion of body weight gain following green tea-based diets and *E. maxima* infection. Green tea-based diets provided for 2 weeks prior to infection and kept during the entire experimental period. Body weight gains for 2 weeks before infection (A) and for the 10 days post-infection (B). Bars represent the means ± standard deviation of 15 chickens. The asterisks indicate significantly different body weight compared to the control group ($^{***}P < 0.001$).

4. Discussion

Prophylactic medication has been used to control and prevent coccidiosis in commercially grown chickens. However, *Eimeria* develops drug resistance rapidly and increasing consumers' concerns over the use of drugs for coccidiosis control has prompted to develop alternative control strategies against avian coccidiosis. The new approaches include the use of natural products, probiotics, improved farm management practics, and modulation of the chicken immune system (Allen and Fetterer, 2002; Dalloul and Lillehoj, 2005). This study investigated the effect of green tea on chicken coccidiosis since previous studies have shown the anti-parasitic activity against *Trichostrongylus colubriformis* and *Toxoplasma gondii* (Molan et al., 2003, 2004; Ryu, 1982).

Assessment of host disease susceptibility to avian coccidiosis has been evaluated by enumerating fecal oocysts and body weight changes following challenge infection with live coccidia parasites (Chapman et al., 2005; Min et al., 2002). The anticoccidial effect of green tea was assessed using fecal oocyst shedding and body weight gains. Supplementation of chickens with green tea extract significantly reduced fecal oocyst output in chickens infected with *E. maxima*, although the same treatment did not effect body weight loss caused by coccidiosis. In general, the higher concentration of green tea supplementation showed more protective effect and reduced fecal oocyst shedding. In this regard, it is interesting to note that green tea components have shown anti-parasite activities *in vitro* and exerted the inhibition of egg hatching and larval development, and inactivated the infective larvae (Molan et al., 2003, 2004; Ryu, 1982). Considering that hosts are infected by contaminated litter in the chicken houses, significant reduction of fecal oocyst shedding by green tea diet will lead to less environmental contamination of coccidia parasites.

The underlying immunological mechanism responsible for green tea-mediated protection against coccidiosis is not known. However, many cytokines are known to mediate protective cell-mediated immune response against intracellular pathogens including coccidia. Increased levels of IFN-γ, in particular, has been associated with protective immunity against avian coccidiosis (Choi et al., 1999; Yun et al., 2000). In this study, we found no significant difference in the level of IFN-γ transcripts in spleen samples at 1 and 3 days post-infection between untreated and green tea-treated chickens. In conclusion, the anticoccidial effect of green tea-based diets in chickens infected with *E. maxima* was limited to a reduction in the numbers of oocysts shed. This result needs to be further investigated in the other species of coccidia that infect chickens.

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