Protective Effects of Sugar Cane Extracts (SCE) on *Eimeria tenella* Infection in Chickens

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ABSTRACT. The effects of oral administration of sugar cane extracts (SCE) on *Eimeria tenella* oocysts infection in chickens were studied with 2 different experiments. In Experiment 1, 3-week-old inbred chickens (MHC; H.B15) were inoculated into the crop with SCE (500 mg/kg/day) for 1 day or 3 consecutive days, and then challenged with *E. tenella* sporulated oocysts (2×10^4 cells/chicken). In Experiment 2, 1-week-old chickens were orally administered SCE at the same dose for 3 consecutive days, and then initially infected with *E. tenella* sporulated oocysts (2×10^3 cells/chicken). At 2 and 3 weeks of age, these chickens were immunized intravenously with the mixed antigens of sheep red blood cells (SRBC) and *Brucella abortus* (BA). At 4 weeks of age, chickens were challenged with *E. tenella* sporulated oocysts (1×10^5 /chicken). Challenged chickens with *E. tenella* oocysts showed markedly decreased body weight gain/day, severe hemorrhage and great number of shedding oocysts in feces and high lesion scores. Oral administration of SCE and initial infection with oocysts (2×10^3 /chicken) resulted in a remarkable improvement in body weight gain/day, hemorrhage, the number of shedding oocysts and lesion score, compare to other infected groups. In addition, SCE-inoculated chickens with the initial infection showed a significant increase in antibody responses against SRBC and BA and also improvement in decreased relative proportions of Bu-1a⁺ and CD4 cells in cecal tonsil lymphocytes of *E. tenella*-challenged chickens. Cecal tissues of chickens administered SCE and initially infected with *E. tenella* oocysts showed lower numbers of schizonts, gametocytes and oocysts than those of infected control chickens. These results suggest that SCE have immunostimulating and protective effects against *E. tenella* infection in chickens.

KEY WORDS: *Eimeria tenella*, protective effect, sugar cane extract.

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Chicken coccidiosis is caused by obligate intestinal protozoan parasites belonging to several different species of *Eimeria*. *Eimeria tenella* (*E. tenella*) primarily invades and resides in the lining of the cecum of exposed chickens [1, 14, 27–30]. Infective sporozoites enter the cecal mucosa by penetrating villus epithelial cells, resulting in extensive destruction of the cecal epithelium, hemorrhagic feces, reduction in body weight gain, and decrease in feed efficiency and eventual mortality which lead to serious economic consequences. Thus far, chemoprophylaxis and anticoccidial feed additives have controlled the disease but have been complicated by the emergence of drug resistance [4, 12, 26].

To prevent the emergence of drug resistance, new drugs have been developed and administered on a rotational basis with existing drugs. However, this has resulted in the increased cost of poultry products. Furthermore, drug or antibiotic residue in the poultry products is potentially annoyance to consumer. Therefore, the regulation of anticoccidial drugs should be strengthened gradually. It is generally agreed that protective immunity does not block sporozoites from penetrating the intestinal epithelium, but it

is not clear to what extent immune response to *E. tenella* sporozoites in the gut lumen can block penetration. Chickens orally administered attenuated vaccine or infected with a small number of *E. tenella* oocysts were shown to be resistant against challenge infection with the sublethal number of the same parasite.

Immunomodulators are a highly expanding field of studies to compete and/or control infectious diseases. Sugar cane extracts (SCE), one of native immunostimulants, have been reported to enhance immune responses, immune functions and growth in chickens [5, 6]. In immune chickens only a few sporozoites reach the cecal epithelium during reinfection and those are unable to develop further [22, 23, 27]. The purpose of the present study is to explore the feasibility of immunological protection of chickens against *E. tenella* infection by oral administration of SCE and initial infection with *E. tenella* oocysts.

MATERIALS AND METHODS

Animals: Inbred chickens (MHC; H.B15), maintained at the National Institute of Animal Health (NIAH), Tsukuba, Japan, were reared coccidia-free with free access to food and water.

Feed: Experimental feed without antibiotics and anticoccidial food additives was obtained from food manufacture

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company (Sugiji Co., Ltd., Handa, Japan).

Parasite and infection: *Eimeria tenella* (*E. tenella*), the NIAH strain, was used. The strain was originally isolated from naturally infected birds and maintained at the NIAH, Tsukuba, Japan.

SCE administration: Original materials of SCE including cane juice produced from sugar cane (*Saccharum officinarum* L.) in the raw sugar manufacturing process were subjected to the preparation of SCE (Shin Mitsui Sugar Co., Ltd., Japan) as described earlier [5, 6]. The original concentration of SCE (100 mg/ml) was prepared in phosphate buffered saline (PBS). SCE was inoculated with a pipette (Komagome pipet, Iwaki Co., Tokyo, Japan) into the crop of chickens at the dose of 500 mg/kg/day for 1 day or 3 consecutive days.

Experiment 1: This experiment was carried out to evaluate the effect of SCE on the incidence of coccidiosis in chickens. Three-week-old inbred chickens were divided into 4 groups, 1) uninfected control group (referred to as control), 2) chickens were inoculated into the crop with *E. tenella* oocysts (2×10^4 cells/chicken) (referred to as challenge), 3) chickens orally administered a single dose of SCE (500 mg/kg) and then challenged with the same dose of *E. tenella* oocysts (referred to as SCE (1) + challenge) and 4) chickens orally administered SCE for 3 consecutive days and then challenged with *E. tenella* oocysts (referred to as SCE (3) + challenge).

The body weight was measured at days 0 and 7 after challenge. Mortality was recorded and body weight gain per day was calculated. Hemorrhagic feces were observed from day 5 to day 7 post challenge. The total amount of feces was collected from all experimental groups from day 5 to day 7 after challenge for oocyst shedding determination [19]. The oocysts were diluted and counted microscopically in a plankton counter chamber. Total oocyst number was calculated as oocyst count $\times$ dilution factor $\times$ counting chamber volume $\times$ fecal sample volume. On day 7 post challenge all chickens were killed and scored for gross cecal lesions on a scale of 0 to 4 according to the method of Conway [3].

Experiment 2: This experiment was performed to evaluate the effects of SCE on immune responses and infection against *E. tenella* in chickens. First, 1-week-old chickens were divided into three groups of control, initial infection and SCE (3) + initial chickens, which were referred in Experiment 1. Each group consisted of 12 chickens. All chickens were immunized intravenously with the mixed antigens of sheep red blood cells (SRBC) and *Brucella abortus* (BA) at 2 and 3 weeks of age. Subsequently, at 4 weeks of age after evaluation of antibody responses to SRBC and BA, half chickens in the above three groups were challenged orally with *E. tenella* sporulated oocysts (1×10^5 /chicken), consisting the following six groups; 1) saline-administered uninfected control chickens (control), 2) chickens challenged with *E. tenella* sporulated oocysts (1×10^5 /chicken) (challenge), 3) chickens challenged with *E. tenella* sporulated oocysts (2×10^3 /chicken) (initial infection), 4) chickens initially infected and then challenged with

E. tenella oocysts (initial + challenge), 5) chickens administered SCE for 3 consecutive days and then initially infected with *E. tenella* oocysts (SCE (3) + initial), and 6) chickens administered SCE for 3 consecutive days, initially infected and then challenged with *E. tenella* oocysts (SCE (3) + initial + challenge). Each group consisted of six chickens. Gain in body weight/day (g/day), clinical signs, oocyst shedding and lesion scores were evaluated in all chickens after challenge at 4 weeks of age.

Immunization and determination of antibody titers: Each chicken was immunized intravenously with 0.1 ml of mixed antigens containing SRBC (5×10^8 cells) and BA (1×10^9 cells) [8] at 2 and 3 weeks of age. Agglutinin titers against SRBC and BA were evaluated in sera taken at 7 days after each immunization, as described previously [8]. The sera were also treated with 0.2 M 2-mercaptoethanol (ME) to evaluate 2-ME resistant agglutinin titers.

Isolation of intestinal leukocytes: Chicken intestinal leukocytes (IL) were prepared according to the technique of Choi *et al.* [2]. Briefly, the intestine between the duodenal loop and the region immediately prior to the Meckel's diverticulum was excised, cut longitudinally, and washed 3 times in PBS supplemented with 2% fetal bovine serum (FBS). Intestinal tissue of each chicken was treated separately, cut into small pieces, and incubated for 10 min in the same medium supplemented by 10 mM dithiothreitol to eliminate the intestinal mucous membrane. The supernatant was discarded and the small pieces of the intestine were incubated for 20 min at 37°C in PBS containing 1 mM EDTA. Cells in the supernatant were washed and gently pressed through a stainless steel mesh to remove most epithelial cells, dead cells and cellular clusters. Cells were further purified by 60% percoll density gradient centrifugation at 3,000 rpm for 20 min at 24°C to remove red cells. Cell viability was over 95% as determined by trypan blue exclusion. The cells were finally suspended to a concentration of 1×10^6 cells/ml in 2% FBS-PBS.

Relative proportions of cells positive for marker antigens: The relative proportions (RP) of cells positive for marker antigens in IL were evaluated the following procedures described by Erf *et al.* [7]. Briefly, 100 μ l of chicken leukocytes (1×10^6 cells/ml) and 100 μ l of 2% FBS-PBS were mixed in each well of a 96-well plate and centrifuged at 2,000 rpm for 1 min at 4°C. After 2 times washing, the sedimented cells were supplemented with 100 μ l of mouse-anti-chicken monoclonal antibody specific for Bu-1a, CD4, CD8, TCR1, TCR2 and TCR3 markers, generous gifts of Dr. Olli Vainio, University of Turku, Finland, and then incubated on ice for 30 min. After incubation the cells were washed 2 times with 2% FBS-PBS and supplemented with 100 μ l of FITC-conjugated anti-mouse IgG antibody (Zymed, CA, U.S.A.) diluted 1:400 in PBS. The cells were incubated on ice for 30 min, and then washed 2 times with 2% FBS-PBS. The RP of Bu-1a⁺, CD4⁺, CD8⁺, TCR1⁺, TCR2⁺ and TCR3⁺ cells in IL were evaluated using a flow cytometer (XL, Beckman Coulter Corp, U.S.A.).

Histopathology: On day 7 post challenge cecal tissue

samples were excised, fixed with neutral buffered formalin (10%), and then embedded in paraffin. Approximately 4 μ m thick cross sections were excised and stained with hematoxylin and eosin (HE) for histopathological examination [19].

Efficacy of SCE: Efficacy of SCE was evaluated on the basis of mortality, gain in body weight/day, degree of hemorrhagic feces, oocyst count/chicken, lesion scores, immune responses and RP of Bu-1a⁺ and CD4⁺ in IL. The mortality was estimated from the number of dead chickens in each infected group. The body weight gain per day was determined from challenge to 1 week post challenge. The extent of hemorrhagic fecal score was assigned corresponding to the degree of hemorrhages in the feces [29]. Oocyst shedding was investigated from day 6 to day 7 post challenge with *E. tenella* and the lesion score of each group was investigated on day 7 post challenge. The localization of schizonts, gametocytes and oocysts in the cecum was microscopically investigated.

Statistical analysis: The Student's *t* test was used for statistical significance determination. *P* values of less than 0.05 were considered to be statistically significant. All data were expressed as mean $\pm$ standard error (SE).

RESULTS

Clinical signs and lesion scores in Experiment 1: Chickens challenged with *E. tenella* (2×10^4) showed severe clinical signs such as anorexia, depression, severe and continuous hemorrhages in feces and hemorrhagic feces around the cloaca. The surviving chickens showed retarded growth with decreased body weight gain/day as shown in Table 1. Yellowish-white sausage-like structures in the feces excreted from the cecum could be found. In contrast, chickens orally administered SCE and challenged with oocysts showed no mortality, indicating a significant improvement in the body weight gain/day, milder hemorrhages and less number of oocysts shed in feces and lower lesion scores, when compared to the challenged groups (Table 1).

Immune responses: The results concerning immune responses against SRBC and BA in Experiment 2 are summarized in Table 2. SCE-administration and additional initial infection with *E. tenella* oocysts (2×10^3) resulted in an increase in antibody responses against SRBC and BA in both first and second responses, as compared with those of uninfected control group and initially infected group. In addition, the enhancing effects of SCE were shown in both

Table 1. Effect of oral administration of SCE on body gain, fecal oocyst shedding and lesion score in *E. tenella* infected chickens (Experiment 1)

Group	Number of total chickens	Number of dead chickens	Gain in body weight (g/day) ^{a)}	Hemorrhagic feces ^{b)}	Oocyst shed/chicken ($\times 10^6$) ^{c)}		Lesion score ^{d)}
					Day 6	Day 7	
Control	10	0	10.7 $\pm$ 0.1	–	0	0	0
Challenge	12	0	7.6 $\pm$ 1.2*	++	58	27	+4.0
SCE (1) + challenge	7	0	8.6 $\pm$ 1.1	+	23	16	+3.8
SCE (3) + challenge	12	0	8.9 $\pm$ 0.9**	+	16	12	+3.3

a) Mean $\pm$ SE.

b) +: Transient hemorrhage; ++: continuous hemorrhage from day 5 to day 7 post infection.

c) Values represent mean oocyst feces numbers in pooled feces of each group.

d) Values represent mean lesion scores of each group. * *P*<0.01, compared to control group, and ** *P*<0.05, compared to challenge group.

Table 2. Antibody responses to SRBC and BA in chickens orally administered SCE and initially infected with *E. tenella* oocysts (Experiment 2)

Group	SCE	Initial infection with oocysts (2×10^3)	Immune responses ^{a)}							
			SRBC				BA			
			First response		Second response		First response		Second response	
			Number of responders	Titer	Number of responders	Titer	Number of responders	Titer	Number of responders	Titer
Control	–	–	12/12	8.0 $\pm$ 0.7	12/12	10.3 $\pm$ 0.6	1/12	0.4 $\pm$ 0.4	12/12	10.8 $\pm$ 1.0
			12/12	(6.0 $\pm$ 0.3) ^{b)}	12/12	(6.7 $\pm$ 0.5)	0/12	(0)	0/12	(0)
Initial infection	–	+	12/12	7.4 $\pm$ 0.7	12/12	10.2 $\pm$ 0.9	1/12	0.2 $\pm$ 0.2	12/12	10.7 $\pm$ 0.7
			12/12	(5.8 $\pm$ 0.2)	12/12	(7.9 $\pm$ 0.4)	0/12	(0)	0/12	(0)
SCE (3) + initial	+	+	12/12	12.2 $\pm$ 1.0**	12/12	13.6 $\pm$ 0.7*	4/12	2.6 $\pm$ 1.0*	12/12	13.8 $\pm$ 1.0
			12/12	(8.3 $\pm$ 0.5)**	12/12	(11.1 $\pm$ 0.6)**	0/12	(0)	0/12	(0)

a) Mean $\pm$ SE of log₂ of the reciprocal antibody titer.

b) The parenthesis show 2-ME resistant titers.

* *P*<0.05; ** *P*<0.01, compared to control and initially infected groups, respectively.

Table 3. Mortality rate, gain in body weight, fecal oocyst shedding and lesion score in chickens orally administered SCE and infected with *E. tenella* oocysts (Experiment 2)

Group	SEC	<i>E. tenella</i>		Number of total chickens	Number of dead chickens	Gain in body weight (g/day) ^{a)}	Hemorrhagic feces ^{b)}	Oocysts shed /chicken ($\times 10^6$) ^{c)}	Lesion score ^{d)}
		Initial (2×10^3)	Challenge (1×10^5)						
Control	–	–	–	6	0	10.6 ± 0.8	–	0	0
Challenge	–	–	+	6	1	8.5 ± 1.4	++	42.0	+4.0
Initial infection	–	+	–	6	0	11.7 ± 1.7	–	0	0
Initial + challenge	–	+	+	6	0	11.0 ± 0.7	+	40.0	+3.0
SCE (3) + initial	+	+	–	6	0	$15.9 \pm 0.4^*$	–	0	0
SCE (3) + initial + challenge	+	+	+	6	0	$13.9 \pm 0.9^*$	+	3.3	+2.0

a) Mean $\pm$ standard error.

b) +: Transient hemorrhage; ++: continuous hemorrhage from day 5 to day 7 post infection.

c) Values represent mean oocyst numbers in pooled feces of each group.

d) Values represent mean lesion scores of each group.

* $P < 0.01$, compared to control, challenge and initial + challenge groups.Table 4. Relative proportions (RP) of cells positive for marker antigens in intestinal leukocytes (IL) of chickens orally administered SCE and infected with *E. tenella* oocysts (Experiment 2)

Group	SCE	<i>E. tenella</i>		RP (%) of surface marker antigen positive cells ^{a)}					
		Initial (2×10^3)	Challenge (1×10^5)	BU-1a ⁺	CD4 ⁺	CD8 ⁺	TCR1 ⁺	TCR2 ⁺	TCR3 ⁺
Control	–	–	–	11.8	10.2	34.1	12.5	16.5	12.5
Challenge	–	–	+	5.7	3.7	50.7	23.5	19.5	18.5
Initial infection	–	+	–	14.4	21.0	35.9	13.4	26.5	10.5
Initial + challenge	–	+	+	11.0	12.8	55.0	18.0	32.0	12.7
SCE (3) initial	+	+	–	24.5	10.5	35.4	28.8	15.5	9.3
SCE (3) + initial + challenge	+	+	+	15.8	7.5	49.5	36.9	19.7	18.5

a) RP was examined on day 8 after challenge. Values represent the mean PR of 3 chickens in each group.

numbers and titers of responding chickens producing antibodies to BA in the first and second responses.

Gain in body weight, oocyst shedding and lesion scores in Experiment 2: All chickens except the three groups of control, initial infection and SCE (3) + initial in Experiment 2 were challenged with *E. tenella* oocysts (1×10^5). Challenged chickens showed retarded growth as determined by sharply decreased body weight gain/day (8.5 ± 1.4 g/day) as shown in Table 3. Severe and continuous hemorrhages in feces and hemorrhagic feces around the cloaca were also observed in those chickens. In addition, challenged group and initially infected + challenged group shed a large number of oocysts in the feces/chicken on days 6 to 7 (42×10^6 and 40×10^6 , respectively) after challenge infections with typical gross lesions including mucosal edema, severe hemorrhagic intestinal inflammation, gray-white to milky yellow sausage-like structures composed of oocysts, necrotic cells, red blood cells and mucous filling the intestinal lumen and necrosis of the cecum and lower parts of small intestine (lesion score, +4 and +3, respectively). On the other hand, chickens administered SCE and initially infected with oocysts showed improved body weight gain/day (13.9 ± 0.9 g/day), milder hemorrhages and less number of oocysts (3.3×10^6) shed in the feces and mild intestinal inflammation with lower lesion score (+2) after challenge, when compared with the described two groups (Table 3).

RP of surface marker antigens: The results concerning the mean RP in IL from 3 chickens in each group are summarized in Table 4. Chickens challenged with *E. tenella* oocysts showed decreased RP of Bu-1a⁺ and CD4⁺ cells (3.7%), as compared to control (11.8% and 10.2%, respectively). On other hand, the RP of Bu-1a⁺ cells and CD4⁺ cells in SCE (3) + initial + challenge group were 15.8% and 7.8%, respectively, suggesting that oral administration of SCE just before initial infection with oocysts improved these decreased RP in IL of *E. tenella*-challenged chickens. Furthermore an apparent increase in the RP of TCR1⁺ cells was shown in chicken groups administered SCE, SCE (3) + initial and SCE (3) + initial + challenge, when compared to that of chickens not administered SCE.

Histopathology: As showed in Fig. 1, a lot of schizonts were observed in the cecal mucosa of challenged chickens (Fig. 1A) and initially infected + challenged chickens (Fig. 1B). On the other hand, very few schizonts were observed in cecal sections from SCE (3) + initial + challenge chickens (Fig. 1C).

DISCUSSION

The goal of the experiments in the present study was to determine the effects of oral administration of SCE on main variables associated with pathology caused by *E. tenella*

Fig. 1. Histology of the ceca of 5-week-old chickens challenged with *E. tenella* sporulated oocysts (1×10^5) (HE stain, $\times 100$). A) A lot of schizonts ($\rightarrow$) in the cecal mucosa of challenged chickens, B) many schizonts ($\rightarrow$) in the cecal mucosa of initially infected + challenged chickens and C) no schizonts in the cecal mucosa of SCE + initially infected + challenged chickens.

infection in chickens including mortality, body weight gain, the degree of hemorrhage in feces, oocyst shedding, lesion score and immune responses. The body weight gain/day significantly decreased after challenge with *E. tenella* oocysts. Reduced body weight gain is a major contributor to production of loss that accompanies coccidial infection in young chickens because inflammatory immune responses divert energy from growth which may affect the weight gain [13]. Oral administration of SCE significantly improved body weight gain per day in initially infected chickens and initially infected + challenged chickens. These results confirmed our previous results concerning the growth-promoting effects of SCE in chickens [5, 6]. The improving effects of SCE on body weight gain and decreasing the mortality in chickens after *E. tenella* oocyst infection may be associated with its protective effects. Youn and Noh [29] reported that administration of herb extracts improved the survival rate and body weight gain in chickens infected with *E. tenella*. However, Allen [1] reported that daily oral administration of L-arginine (500 mg/kg) did not increase the body weight gain but significantly reduced oocyst shed from *E. tenella*-infected chickens than infected control group. The body weight gain/day, hemorrhages and oocyst shedding in feces and lesion scores were investigated during 1 week after challenge with *E. tenella* oocysts. Hemorrhages in feces of almost all experimental groups, except the uninfected control group, were seen during 5–7 days after infection with *E. tenella* oocyst. But the extent of hemorrhage in feces of chickens administered SCE and initially infected with oocysts was milder than that of other infected groups. The oocyst output in SCE-administered + initially infected + challenged chickens (3.3×10^6 /chicken) was lower than that of challenged group and initially infected + challenged

group (42 and 40×10^6 /chicken, respectively). The lesion scores in the SCE + initially infected + challenged group (+2) improved better than those of challenged group and initially infected + challenged group (+4 and +3, respectively). These results indicated a protective effect of SCE on *E. tenella* infection.

The antibody responses against SRBC and BA significantly increased in both first and second responses in chickens orally administered SCE and initially infected with oocysts, when compared with those of uninfected control and initially infected chickens. These results also confirmed our previous findings that oral administration of SCE significantly increased the antibody responses against SRBC, BA and *Salmonella enteritidis* (SE) and also maintained higher antibody titers than control chickens [5], suggesting the stimulatory effects of SCE on antibody production. The enhancing effects of SCE on humoral immune responses may affect local mucosal immune responses which may correspond with the onset of specific immunity to *E. tenella* infection. These results are in agreement with those of Li *et al.* [15, 16] reported the activation of classical complement pathway by a polysaccharide from sugar cane extracts and its interaction with immunoglobulins. Parmentier *et al.* [20] reported immune responses and resistance to *Eimeria acervulina* of chickens divergently selected for antibody response to SRBC.

Flow cytometric analysis of cecal tonsil lymphocytes showed decreased relative proportions of Bu-1a⁺ and CD4⁺ cells in IL of challenged chickens. Oral administration of SCE with oocysts of *E. tenella* improved these decreased proportions in IL of challenged chickens with *E. tenella* oocysts, indicating the stimulatory effects of SCE on the local mucosal immunity. It is generally believed that CD4⁺

cells are important to develop help during antibody responses, but CD4⁺ cells also provide help during induction of cytotoxic responses [17, 25, 28]. Using the murine model system for protozoan infection, the importance of CD4⁺ cells has been reported in the control of infection with *E. tenella* [24, 25] and *Toxoplasma gondii* [10, 11]. Stimulation of antibody responses to SRBC and BA in chickens orally administered SCE and initially infected with oocysts and improved relative proportions of Bu-1a⁺ and CD4⁺ cells in IL suggested that administration of SCE with a low dose of oocysts played a role in the development of protective immunity against oocyst-reinfection by interacting with lymphocytes. The importance of lymphocytes in immune responses to coccidia has been reported in chickens [23]. The spleen cells and peripheral blood lymphocytes from immune chickens are capable of transferring the resistance against the infection to naive recipients. Furthermore, the treatment of chickens with immunosuppressive agents enhanced the severity of coccidiosis. Isobe and Lillehoj [9] reported that dexamethasone-treated chickens showed reduced T-cell proliferation, reduced interferon production and increased susceptibility to *Eimeria* infection. It was reported that cane sugar factors induced *in vivo* protective responses against *Pseudomonas aeruginosa* and *Proteus mirabilis* [21]. Youn and Noh [29] reported anticoccidial effects of herbal extracts on *E. tenella* infection. In the present study oral administration of SCE with *E. tenella* oocysts was found to show more effective improvement after challenge than challenged group and initially infected + challenged group on the basis of survival rate, gain in body weight, hemorrhages and oocyst shedding in feces and lesion scores.

Histopathological examination revealed that chickens received SCE with initial infection showed lower numbers of schizonts, gametocytes and oocysts in the cecum after challenge than any other infected groups (even if initially infected with *E. tenella* oocysts). The lack of parasite development in cecal tissues, enhanced immune responses and improved relative proportions of Bu-1a⁺ and CD4⁺ cells in IL of chickens orally administered SCE and initially infected with oocysts indicated that these birds had mounted protective immune responses which may prevent invasion and development of sporozoites in the cecal tissue after challenge. It has been reported that avian humoral immunity in the intestinal tract is mediated via secretion of antibody by plasma cells located within the gut lamina propria into the intestinal lumen [1, 18]. Vervelde *et al.* [28] reported the major role of intestinal leukocytes in protective immunity following *E. tenella* infection. The mechanism by which oral administration of SCE results in reduction of pathological lesions in *E. tenella*-infected chickens remains open. Further basic studies including the interaction between SCE and intestinal immune cells are needed.

Taken together, these results suggest that inoculation of SCE with *E. tenella* oocysts into the crop induces protective immunity against *E. tenella* infection in chickens. The protective effects of SCE-administration on local mucosal as

well as systemic immune responses may inhibit the invasion and/or natural development of the parasites.

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