TÜBİTAK

Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Research Article

Turk J Vet Anim Sci
(2019) 43: 76-81
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doi:10.3906/vet-1704-68

Assessment of low doses of *Eimeria tenella* sporulated oocysts on the biochemical parameters and intestinal microflora of chickens

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Received: 22.04.2017 • **Accepted/Published Online:** 06.12.2018 • **Final Version:** 12.02.2019

Abstract: This study was carried out to evaluate the biochemical parameters and intestinal microflora in broilers with low doses of *Eimeria tenella*. Birds from the experimental groups were orally infected with 500 (GI) or 1000 (GII) sporulated oocysts of *E. tenella* and then examined for weight gain, biochemical indicators, and microbiota on day 9 and 16 after infection. Experimental infection of *E. tenella* on day 9 post infection (pi) increased concentrations of uric acid, cholesterol, triacylglycerols, and glucose; however, these measurements did not differ from those of the control group on day 16 pi. On day 16 pi, bifidobacteria counts were lower in both infected groups, whereas *E. coli* counts were higher in GII than in GI and the control group. Statistical differences were not observed when average weight gains between the infected and noninfected groups were compared.

Key words: *Eimeria tenella*, subclinical coccidiosis, broilers, experimental infection, biochemical parameters

1. Introduction

Coccidiosis is an intestinal disease caused by coccidia, common protozoal parasites that affect both domestic and wild animals. Coccidia are commonly found in small poultry farms (often in subclinical forms); however, some species found in large production farms are highly pathogenic and can cause enormous losses (1).

Eimeria tenella is one of the most economically significant species of *Eimeria*. Infections from this parasite can lead to bird mortality through hemorrhaging, which is accompanied by the emergence of second stage schizonts from the cecal subepithelium (2). There are many studies that research the influence of these pathogenic coccidia at oocyst doses of 1×10^4 and above (e.g., 3–5). Subclinical *Eimeria* infections have not been studied at great length. However, recently we see a growing number of small farms where poultry are kept under different types of alternative farming systems (organic or bio regime), and subclinical coccidia infection occurs in almost 100% of cases in these small farms (1).

Subclinical infections are often reported in very general terms. Studies commonly find that these infections negatively affect weight gain and feed conversion rate (6). However, experiments with predetermined low doses of oocysts are rarely reported. Marshall et al. (7) found no significant effects on weight gain in broilers (compared with the uninfected control group) that received 350, 1250, and 5000 oocysts ($P = 0.64$). Similarly, repeated doses of 100 oocysts did not affect mean body weight in experimental chickens (8). Three thousand *E. tenella* oocysts affected weight gain in infected broilers; however, differences between weight gain in the infected group and that of the control groups were statistically significant only in one of two broiler breeds (9). Clinical signs observed in all infected birds were very mild and included decreased activity, reduced feed intake, and mild diarrhea; aside from

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these symptoms, the birds appeared normal (9). Marshall et al. (7) observed, at 6 days post infection (pi), edema and cellular infiltration of the cecal mucosa in birds infected with 350–5000 oocysts of *E. tenella*. Cecal lesion scores ranged from none to 3.

Biochemical parameters and changes in the microflora have been primarily monitored in chickens infected with *E. tenella* doses of 1×10^4 oocysts and more. In such type of infections, severe intestinal damage occurs, causing detrimental effects on the absorption of nutrients. The most commonly reported effects were decreases in protein, sodium, and chloride concentrations, and, conversely, increases in glucose and uric acids (10–12). Changes in the microflora were especially reflected in increased quantities of coliforms and *Clostridium perfrigens*; conversely, lactic acid bacteria and bifidobacteria counts were lower in the infected groups compared with those of the control groups (13).

The objective of this study was to evaluate the effects of low doses of *Eimeria tenella* sporulated oocysts in broilers used in the poultry industry. More specifically, this study focused on changes in weight gain, biochemical indicators, and microflora in the cecum of the chicken.

2. Materials and methods

2.1. Birds

All bird care and handling procedures were approved by Czech University of Life Sciences Prague and the Institutional Ethical Committee and Central Committee for Animal Welfare, an organ of the Ministry of Agriculture. One-day-old broiler chickens (Ross 308) were used in the experiments. They were raised until they reached 2 weeks of age, at which time they were transferred to the experimental rooms. Thirty-six birds were divided into three groups of 12 (randomly allocated in separate pens): group I (GI), group II (GII), and a control group (GC). GI and GII were the experimental groups, whereas GC served as the control group. To avoid contamination, the control group was housed in a separate room. Birds were kept under a lighting program that provided 23 h of light and 1 h of darkness for the first 7 days, and 16 h of light and 8 h of darkness from day 8 until the end of the experiment. Feed and water were supplied ad libitum and the feed was not supplemented with anticoccidial compounds. During the experiment, chickens were given three feed mixtures. A starter diet was provided until 10 days of age and contained 23.8% crude protein (CP) and 12.6 MJ of metabolizable energy (ME). Chickens between 11 and 21 days old received a grower diet that comprised 21.3% CP and 13.0 MJ ME. For the remainder of the experiment, chickens were provided with a finisher diet that comprised 19.5% CP and 13.4 MJ ME.

2.2. Experimental design

All broilers were individually weighed before being transferred to the experimental rooms and subsequently weighed once every 7 days. At 16 days of age, chickens from GI (n = 12) and GII (n = 12) were infected with 5×10^2 and 1×10^3 *Eimeria tenella* sporulated oocysts, respectively; GC (n = 12) served as the uninfected control group. Chickens were infected by administering various doses of *E. tenella* oocysts in 1 mL of aqueous suspensions using a gavage. A laboratory strain of *E. tenella* was originally obtained from the Biofarm Research Institute of Biopharmacy and Veterinary Drugs, Jílové u Prahy. Oocyst counts were determined from fecal samples taken daily from each group from day 16 to the end of the experiment. Samples were examined through the use of a McMaster chamber and presented as the number of oocysts per gram of fresh feces (14).

Six chickens from each group were euthanatized 9 days pi, which is the period of intensive parasite reproduction in the intestine. Chickens from each group were killed by cervical dislocation and samples were removed as rapidly as possible. All birds were scored for lesions (15). The same examinations were performed 16 days pi.

2.3. Blood collection and measured parameters

Blood samples were taken through cardiac puncture from all chickens after euthanasia. The blood samples were coagulated at laboratory temperature and then centrifuged at $1000 \times g$ for 15 min. The separated serum was deep-frozen (–80 °C) until analysis. The following biochemical parameters were measured from blood (serum) samples: total protein (TP), albumin, urea, glucose, triacylglycerols (TG), nonesterified fatty acids (NEFA), cholesterol, aspartate aminotransferase (AST), and uric acid (UA). All of the parameters were determined by spectrophotometry using an Erba XL 200 (Erba Lachema) automated analyzer from the laboratory of the Department of Veterinary Sciences (FAFNR, CULS Prague). Commercial reagents (Erba Lachema; Randox) were used for the analysis.

2.4. Enumeration of bacteria populations in the cecum

Fecal material from the cecum was aseptically transferred in aliquots of 1 g to tubes containing minimal media in an anaerobic environment, and microbiological analysis was performed immediately using the plate counting method. The total bacteria and bifidobacteria counts were analyzed under anaerobic conditions; lactobacilli were incubated under microaerophilic conditions, whereas enterococci, *Escherichia coli*, and coliform bacteria were analyzed under aerobic conditions. pH measurements were also done on the contents of the cecum (Table 1).

2.5. Statistical analysis

Results were evaluated using the SAS 9.4 program (SAS Institute Inc.) and ANOVA (16). Growth was assessed by one-way analysis of variance. Duncan's multiple range

Table 1. The selective media used in the experiment.

Group of bacteria tested	Medium*
Total anaerobic bacteria count	Wilkins-Chalgren anaerobe agar supplemented with Soya peptone and cysteine (W+S agar)
Bifidobacteria	W+S agar supplemented with mupirocin (1 mg/100 mL), pH adjusted with 0.1 mL/100 mL glacial acetic acid
Lactobacilli	Rogosa agar adjusted with 0.132 mL/100 mL glacial acetic acid
Enterococci	Slanetz-Bartley agar
Escherichia coli	T.B.X. agar
Storage medium	Nutrient broth supplemented with tryptone and cysteine

*All media were purchased from Oxoid.

test was used to appraise differences between the groups. Biochemical constituents and bacteria populations in ceca were evaluated by two-way analysis of variance. A t-test was used to evaluate differences between group and age interactions. All data were expressed as mean ± standard deviation values and $P < 0.05$ was considered statistically significant.

3. Results

3.1. Performance parameters

Chickens given low doses (500 and 1.000 *E. tenella* oocysts/bird) exhibited the same weight gains as those in the uninfected control group (Figure 1). No statistical differences were observed when average weight gains between the infected and noninfected groups were compared.

3.2. Biochemical constituents

Experimental infection of *E. tenella* at 9 days pi (at 25 days of age) had increased concentrations of uric acid ($P < 0.05$), cholesterol ($P < 0.05$), triacylglycerols ($P < 0.05$), and glucose ($P < 0.05$); however, these measurements did not differ from those of the control group with advancing age (32 days of age, i.e. 16 days pi). Levels of nonesterified fatty acids ($P < 0.05$) were increased only in group GI at day 9 pi (Table 2).

3.3. Enumeration of bacteria populations in ceca

The composition of the cecal microbiota of the experimental chickens is shown in Figures 2 and 3. At 9 days pi, the numbers of bacteria between different groups did not differ significantly; however, cecal pH was significantly lower ($P < 0.05$) in chickens of GII (Figure 2). Bifidobacteria counts were lower ($P < 0.05$) in both infected groups and *E. coli* counts were higher ($P < 0.05$) in GII compared to those of the control group (GC) and GI.

4. Discussion

Due to the development of effective prophylactic drugs, clinical outbreaks of coccidiosis are considered rare in modern poultry production; however, subclinical *Eimeria* infection is still considered one of the most significant problems in the industry. Accurate estimations of the effects caused by coccidial infections are difficult to achieve because different *Eimeria* species impact their hosts in different ways (1,17). We believe this work will contribute to the understanding of the complex issues associated with the impact of subclinical *Eimeria tenella* infection on a host.

Eimeria tenella is most often monitored through experimental infection with doses of 1×10^4 to 1×10^5, which are known to cause clinical signs and mortality. A dose of 10^4 oocysts is associated with a 12.5% mortality rate (18), whereas doses of $2–8 \times 10^4$ produce a 20%–70% mortality rate (7,13). According to Witlock et al. (10), four major physiological stresses are responsible for death in chickens infected with *E. tenella*: hypothermia, depletion of easily mobilized energy reserves, acute metabolic acidosis, and renal tubule cell dysfunction.

The aim of this study was to evaluate changes in the body that are caused by low doses of *E. tenella* and

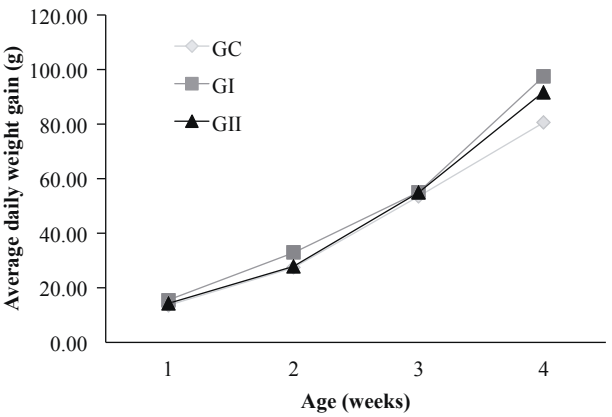

Figure 1. Effect of low doses of *E. tenella* on weight gains of broiler chicken. GC – control group, GI – group infected with 500 *E. tenella* oocysts, GII – group infected with 1000 *E. tenella* oocysts.

Table 2. Hematological and clinical biochemistry variables for control group and the groups infected with sporulated oocysts of *E. tenella*.

		Group		
	Days pi	Control	Group I	Group II
UA	9	174.8 ± 66.14	403.5 ± 100.47	443.3 ± 138.92
(μmol/L)	16	165.2 ± 71.65	146.0 ± 56.82	168.8 ± 63.67
CHOL	9	1.42 ± 0.56	3.09 ± 0.88	3.12 ± 1.41
(mmol/L)	16	1.42 ± 0.51	1.57 ± 0.3	1.57 ± 0.3
TG	9	0.175 ± 0.05	0.878 ± 0.39	0.540 ± 0.13
(mmol/L)	16	0.318 ± 0.2	0.318 ±- 0.13	0.398 ± 0.14
AST	9	1.28 ± 0.27	2.53 ± 0.73	2.65 ± 0.85
(μkat/L)	16	1.61 ± 0.69	1.73 ± 0.33	1.75 ± 0.34
UREA	9	0.160 ± 0.05	0.310 ± 0.09	0.258 ± 0.05
(mmol/L)	16	0.165 ± 0.08	0.135 ± 0.04	0.160 ± 0.08
GLU	9	6.68 ± 1.15	12.42 ± 2.19	12.07 ± 3.25
(mmol/L)	16	6.79 ± 2.37	6.55 ± 1.05	7.33 ± 1.3
TP	9	21.50 ± 2.21	24.63 ± 2.69	23.93 ± 0.97
(g/L)	16	21.13 ± 2.72	19.38 ± 2.11	19.87 ± 1.45
ALBU	9	10.78 ± 1.51	11.53 ± 0.77	11.13 ± 0.82
(g/L)	16	11.15 ± 1.37	10.20 ± 1.13	10.22 ± 0.91
NEFA	9	0.168 ± 0.09	0.555 ± 0.23	0.308 ± 0.12
(mmol/L)	16	0.158 ± 0.07	0.200 ± 0.11	0.217 ± 0.05

pi – post infection, UA – uric acid, CHOL – total cholesterol, TG – triacylglycerols, AST – aspartate aminotransferase, GLU – glucose, TP – total protein, ALBU – albumin, NEFA – nonesterified fatty acids, GC – control group, GI – group infected with 500 *E. tenella* oocysts, GII – group infected with 1000 *E. tenella* oocysts.

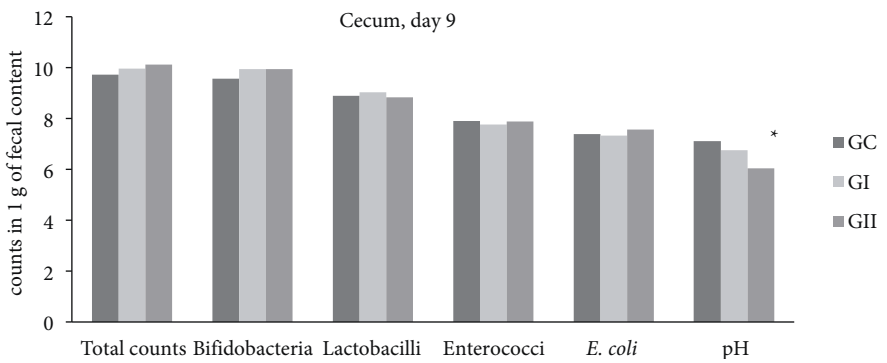

Figure 2. Effect of low doses of *E. tenella* on cecal bacteria populations of broiler chickens at 9 days post infection. GC – control group, GI – group infected with 500 *E. tenella* oocysts, GII – group infected with 1000 *E. tenella* oocysts; * – significant difference between control and infected group.

experimental studies using low doses of *E. tenella* oocysts are rare. These studies indicate, as does our study, that weight gain in slightly infected broilers (50–1250 oocysts/bird) does not significantly differ from that of uninfected control chickens. According to values from Johnson and Reid (15), intestinal lesions ranged from 0 to 2.3 (7,8,18). However, even mild coccidia infections can certainly bring about a state of stress, which is reflected in the biochemical parameters of the host. In our study, experimental infection by *E. tenella* increased levels of cholesterol,

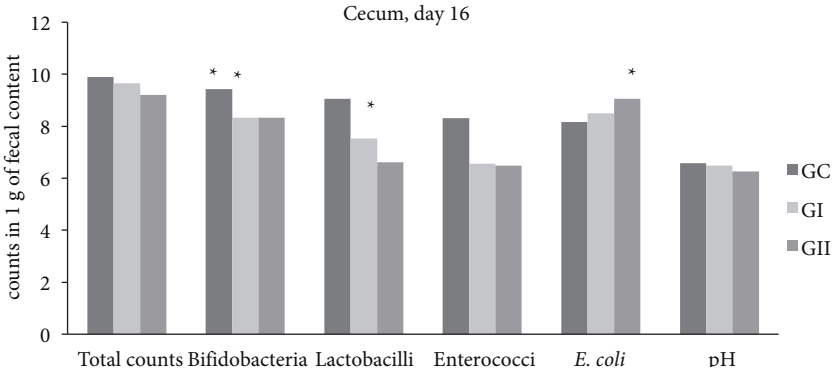

Figure 3. Effect of low doses of *E. tenella* on cecal bacteria populations of broiler chickens at 16 days post infection. GC – control group, GI – group infected with 500 *E. tenella* oocysts, GII – group infected with 1000 *E. tenella* oocysts; * – significant difference between control and infected group.

triacylglycerols, nonesterified fatty acids, glucose, and uric acid mainly at day 9 pi. These parameters decreased after 7 days, and progress of infection did not differ from that of the control group. Similar results were observed by Fukata et al. (12), who observed increasing cholesterol, triacylglycerols, glucose, and urate after inoculation with *E. tenella*. These authors observed the effects of inoculation up to 11 days after infection, after which point all levels decreased with the exception of cholesterol. An increase in lipid metabolism indicators is associated with higher lipolysis (19). Similarly, a higher glucose level in the present study at 9 days pi occurred presumably as a preventive measure against hypoglycemia in infected chickens. Similar trends were observed in uric acid, which plays a role in oxidative stress (20). Rosebrough et al. (21) found that increase in the level of plasma uric acid was associated with a decrease in the dietary energy to protein ratio. Based on our results, we can hypothesize that chickens subjected to infection had intestinal changes that brought about a decrease in lipid and energy metabolism within the first 9 days pi. With advancing age, the negative effects of coccidiosis infection decrease and metabolism indicators stabilize. This hypothesis is supported by the fact that the concentration of total protein was not affected by experimental infection, and therefore protein synthesis was not affected. This corresponds to the growth rate of chickens, which was unaffected by infection.

Maintenance of gut health is complex and relies on a delicate balance between diet, the commensal microflora, the mucosa, the digestive epithelium, and the overlying mucus layer. This balance is also frequently affected by the presence of parasites and enteric bacteria with pathogenic potential. The proliferation and metabolic activity of these pathogens may perturb digestive function and lead to diarrhea, poor growth rates, and even death (22). Intestinal microflora is essential for health and disease prevention in hosts (23), and it also plays a particularly significant role in protection against pathogen colonization. The ability of lactobacilli to inhibit pathogens has been reported in vitro (24,25). Tierney et al. (26) demonstrated that *Lactobacillus* also significantly inhibited *E. tenella* invasion. However, many previous studies confirmed the presence of synergy between normal intestinal flora and *Eimeria tenella*, resulting in clinical coccidiosis (27). Kimura et al. (28) reported that *Clostridium perfringens* proliferated 5 days pi following the decrease in lactobacilli and bifidobacteria.

Aside from lower pH values, our study indicated no changes in microflora 9 days pi. However, a week later (at day 16 pi), microbial examinations resulted in an increase in *E. coli* and a decrease in bifidobacteria counts in the infected groups. These changes were similar to those of studies that administered doses of 10^4 to 10^5 oocysts (13).

In conclusion, chickens given low doses (500 and 1000 oocysts/bird) exhibited the same weight gains as those in the uninfected control group. All infected birds showed significantly higher levels of uric acid, cholesterol, triglycerides, and glucose 9 days pi; a week later, values were comparable to those of the control group. In this study there were no changes in microflora 9 days pi. At day 16 pi, microbial examinations revealed an increase in *Escherichia coli* and a decrease in bifidobacteria counts in the infected groups.

Acknowledgments

This study was funded by the Ministry of Agriculture of the Czech Republic (Project NAAR No. QJ1510038) and the Grant of National Agency for Agricultural Research of the Czech Republic, v.v.i. CIGA No. 20152021. We would also like to thank Brian Kavalir for his proofreading services.

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