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Oxidative stress linked to changes of cholinesterase and adenosine deaminase activities in experimentally infected chicken chicks with *Eimeria* spp

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ABSTRACT

Both oxidative stress and alterations in adenosinergic and cholinergic systems participate in initiation and progression of parasitic infectious diseases. Nevertheless, the involvement of these pathways during eimeriosis remains poorly understood. Therefore, the aim of this study was to evaluate the involvement of adenosinergic and cholinergic systems in regulation of inflammatory response and oxidative stress in chicken chicks experimentally infected with *Eimeria* spp. Two groups were formed for comparison at 3 time points (days 5, 10 and 15) of infection (PI): uninfected (control) and infected. Erythrocyte counts, hematocrit and hemoglobin levels were lower in infected chicks on day 15 post-infection (PI). Total leukocytes, heterophil and lymphocyte counts were higher in infected chicks on days 5 and 10 PI, while eosinophil counts were higher only on day 10 PI. Serum levels of total protein and globulins were higher in infected chicks on days 10 and 15 PI, while triglycerides and cholesterol levels were lower on day 15 PI. Acetylcholinesterase activity in total blood and butyrylcholinesterase activity in serum were higher in infected chicks on day 15 PI, while adenosine deaminase activity was higher on day 10 PI and lower on day 15 PI compared with the respective control. Finally, serum levels of reactive oxygen species and catalase activity in total blood were higher in infected chicks on day 15 PI, while superoxide dismutase activity in total blood was lower at the same time of infection. These data suggest that cholinergic and adenosinergic systems display a pro-inflammatory profile that contributes to impairment of immune and inflammatory responses in a mixed *Eimeria* infection. Furthermore, oxidative stress may contribute to clinical signs of disease as well as to pathogenesis. In summary, the impairment of immune response and alterations in blood antioxidant/oxidant status contributes to disease pathophysiology.

1. Introduction

Coccidiosis, commonly caused by parasite belonging to genus *Eimeria,* is an important problem in poultry production that is associated with economic losses of 3 billion dollars in the poultry production chain [1]. According to Ritzi et al. [2], the reproduction of parasites in the small and large intestines leads to intestinal cell damage, causing impairment of zootechnical indices, such as weight loss and increased food conversion. Intestinal villi and crypts are affected by infection, giving rise to hemorrhage and necrosis, and consequently biochemical and metabolic disturbances that affect chicks performance and overall health [3].

Oxidative stress is a disturbance between antioxidant/oxidant status in favor of excessive generation, or slower removal of free radicals, such as reactive oxygen species (ROS) [4]. Excessive ROS content leads to damage of proteins, lipids and nucleic acids, with consequent loss of their biological functions and subsequent tissue injury [4]. Oxidative stress has been linked to initiation and progression of several infectious diseases [5], including eimeriosis [6]. According to Galli et al. [7], eimeiriosis causes an augmentation of production of antioxidant

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enzymes, including catalase (CAT), in an attempt to neutralize the excessive production of free radicals and/or to reduce the oxidative damage provoked by infection. Chicks infected with *Eimeria* spp. suffer intestinal lesions provoked by direct parasite action, eliciting an exacerbated inflammatory response that may contribute to oxidative stress [8]. This stressor also negatively impacts animal performance as a result of tissue lesions, in the form of reduction in nutrient absorption, decreased intestinal villi and augmentation of intestinal crypts [9,10]. Furthermore, the inflammatory response increases the production of intestinal mucus, contributing to impairment of nutrient absorption [11]. In this sense, study conducted by Georgieva et al. [12] revealed that *E. acervulina* infection elicits a disturbance on antioxidant/oxidant status of chickens via augmentationon on plasma lipid damage and inhibition on superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities, which contribute to disease pathophysiology. Also, Koinarski et al. [13] also demonstrated a significant decrease on plasma endogenous antioxidants, as carotene, vitamin A and vitamic C, in chicks infected by *E. acervulina*. In addition, these authors observed a significant increase on lipid damage and inhibition in SOD activity in total blood, which contributed to impairment on chicks growth and increase of mortality

According to the literature, when a pathogen breaches the first line of defense (often a mucosal barrier), that organism's molecular signature is recognized by resident macrophages, and these cells respond by releasing a cocktail of pro-inflammatory cytokines (including interleukin-1 and -6) that signal the brain via several pathways (humoral as well as neural) of the peripheral innate immune response. These may include changes in animal behavior [14]. We hypothesized that adenosinergic and cholinergic systems have a role in the pathogenesis of coccidiosis, especially in the modulation of the immune response, as well as behavior. Adenosine deaminase (ADA) is an enzyme of the adenosinergic system that catalyzes the deamination of deoxyadenosine to inosine, participating in immune and inflammatory responses via regulation of adenosine, a molecule with anti-inflammatory properties. Adenosine may protect host tissue from damage as well as participating in lymphocyte proliferation [15,16]. Moreover, the cholinergic system has two important enzymes linked to regulation of acetylcholine (ACh), a molecule with anti-inflammatory properties [17,18], acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) [19]. Therefore, the aim of this study was to evaluate the involvement of adenosinergic and cholinergic systems in regulation of inflammatory responses and oxidative stresses in chicks experimentally infected with *Eimeria* spp.

2. Materials and methods

2.1. Chicks, accommodation and feed

Forty chicks (Cobb 500 lineage) were acquired from a commercial hatchery located in Chapecó (SC, Brazil). The chicks were randomly allocated to two pens containing wood shavings that were heated with incandescent bulbs. The diets were based on corn and soybean meal, following the recommendations of the Brazilian Tables for Poultry and Pigs [20]. Water and fed were provided ad libitum for all chicks.

2.2. Experimental design

The chicks were randomly divided in two groups ($n = 20$ each group) identified as: control (uninfected) and infected, and each chick an experimental unit, exposed to the same effect (treatment). On day 2 of age, the experimentally infected chicks received an oral solution containing 35,000 oocysts of *Eimeria* spp. (*E. mitis* = 10,000 oocysts; *E. acervulina* = 8000 oocysts, *E. praecox* = 8000 oocysts; *E. tenella* = 5000 oocysts; and *E. maxima* = 4000 oocystis). The inocula (non-attenuated strains) used in this study were made available by Biovet (SP, Brazil). The chicks, individually, were weighed at the beginning and end of the experiment (day 1 and 15 of life), using digital scale.

2.3. Sample collection

On days 5 ($n = 7$ per group), 10 (n = 7 per group) and 15 ($n = 6$ per group) post-infection (PI), chicks were anesthetized in a chamber containing halothane. Each bird was an experimental unit and therefore, being an individually analyzed sample. Total blood was collected by cardiac puncture followed by euthanasia via cervical dislocation. Total blood was allocated to tubes containing sodium citrate as anticoagulant, and in tubes without anticoagulant to obtain serum after centrifugation at 3500 rpm over 10 min. The sera were stored and frozen at −20 °C until use. For histopathological analysis, intestinal fragments (duodenum, jejunum and cecum) were collected and stored in buffered formalin solution (10%). fecal samples were collected to perform the fecal examination during the same days of infection (days 5, 10 and 15 PI).

Protein levels in serum and blood samples were determined by the Coomassie blue method according to Bradford [21], using bovine sera albumin as the standard.

2.4. Fecal examination

The numbers of *Eimeria* ssp. oocysts per gram of feces were evaluated using the centrifugal-flotation technique, as described by Monteiro [11]. The feces (1 g) were dissolved in 15 mL of sucrose solution and centrifuged for 5 min at 2000 rpm. The fecal exam was performed by light microscopy (100 x).

2.5. Hematology analysis

Erythrocytes and leukocytes counts were performed in a Neubauer chamber as described by Natt and Herrick [22], while hemoglobin content was determined using commercial kits following the manufacturer's recommendations. At blood sampling, blood smears were performed and stained with a commercial dye (*Panótipo Rapido)* to perform the differential count of leukocytes using a light microscope at 1000 x magnification [23].

2.6. Serum biochemistry

Serum levels of total protein, albumin, uric acid, triglycerides and cholesterol were evaluated using the semi-automated analyzer BioPlus (Bio-2000) using commercial kits (Analisa®). Serum globulin levels were calculated using the formula: total protein – albumin.

2.7. Cholinesterase activities

Blood samples with anticoagulant were diluted 1:50 (v/v) in lysis solution (0.1 mmol/L potassium/sodium phosphate buffer containing 0.03% Triton X-100) to determine AChE activity in blood samples, and frozen at −20 °C for 7 days. The AChE (EC 3.1.1.7; AChE) enzymatic assay was performed in total blood by the method of Ellman et al. [24], as previously described by Worek et al. [25]. The activity was obtained using acetylcholine (ACh) as substrate, and the enzymatic activity was expressed as μmol AcSCh/h/mg of protein.

Serum BChE (EC 3.1.1.8; BUChE) activity was determined as described by Ellman et al. [24]. The BChE activity was assayed in a medium containing sodium phosphate buffer 0.1 mM, pH 7.4, DTNB 0.30 mM and 15 μL of serum. After 3 min of pre-incubation at 37 °C, the reaction was started with 1 mM of butyrylthiocoline (BuSCh), and the reading was performed for 2 min at intervals of 20 s in a spectrophotometer at 412 nm. The specific activity was expressed in μmol BuSCh/h/mg protein.

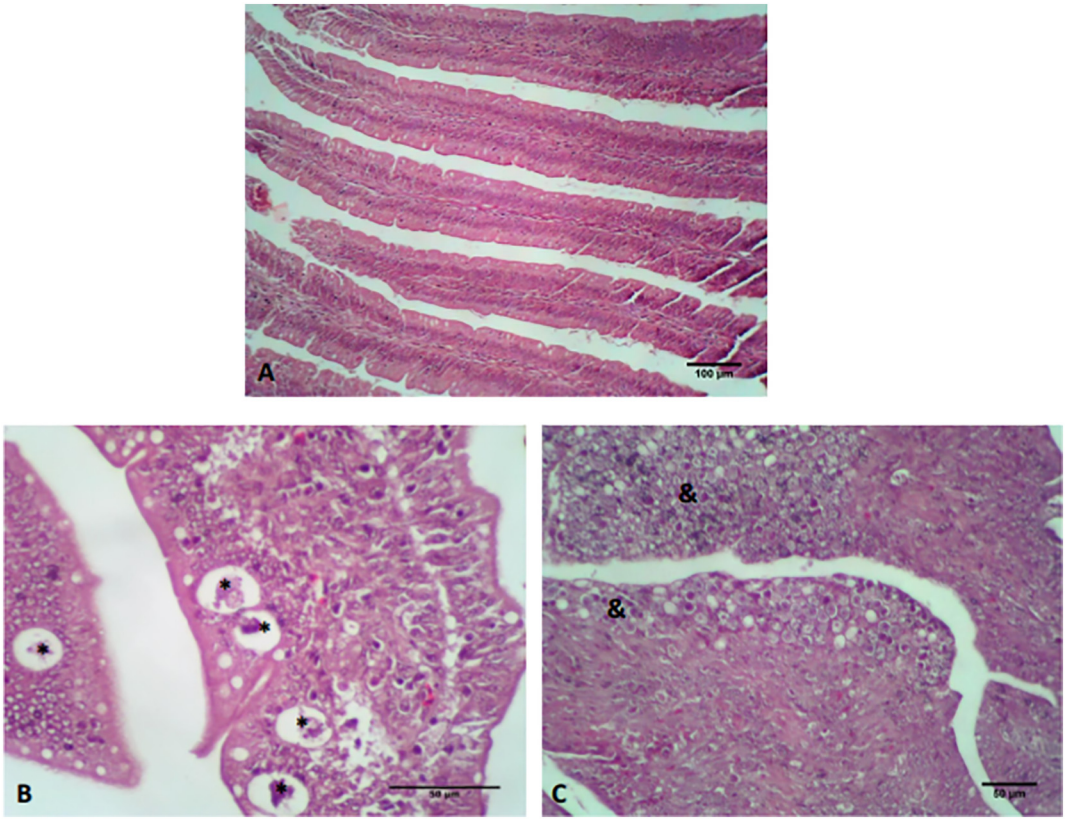

Fig. 1. Intestinal histopathology of chicks experimentally infected with *Eimeria* spp. No intestinal lesions were observed in the control group (days 5, 10 and 15 post-infection), and in the infected group (day 5 post-infection) [A]. However, was observed moderate-to-severe quantity of degenerate gametes with occasional micro- and macrogametocytes in the infected groups on days 10 and 15 post-infection [B, C].

2.8. Serum ADA activity

Seric ADA activity was measured spectrophotometrically by the method of Giusti and Gakis [26]. The reaction started by the addition of the substrate (adenosine) to a final concentration of 21 mmol/L, and incubation was carried out for 1 h at 37 °C. The reaction was stopped by the addition of 106 mmol/L/0.16 mmol/L of phenol-nitroprusside/mL solution. The reaction mixture was immediately mixed with 125 mmol/L/11 mmol/L of alkaline-hypochlorite (sodium hypochlorite) and vortexed. Ammonium sulfate (75 µmol/L) was used as ammonium standard. The concentration of ammonia was directly proportional to the absorption of indophenol at 650 nm. Serum ADA activity was expressed as U Ado/mg of protein.

2.9. Serum ROS levels

Serum ROS levels were determined as an index of peroxide production by cellular components. This experimental method of analysis was based on the deacetylation of the probe DCFH-DA, and its subsequent oxidation by reactive species to DCFH, a highly-fluorescent compound [27]. The serum was added to a medium containing Tris–HCl buffer (10 mM; pH 7.4) and DCFH-DA (1 mM). After DCFH-DA addition, the medium was incubated in the dark for 1 h until the start of the fluorescence measurement procedure (excitation at 488 nm and emission at 525 nm, and both slit widths used were 1.5 nm). Serum ROS levels were expressed as U DCF/mg of protein.

2.10. Antioxidant enzymes in total blood

Catalase (EC 1.11.1.6; CAT) activity was determined in total blood by the decomposition of H_2O_2 at 240 nm according to the method described by Nelson and Kiesow [28], and modified by Aebi [29]. Superoxide dismutase (EC 1.15.1.1, SOD) activity was spectrophotometrically quantified in total blood according to Misra and Fridovich [30] by determining the inhibition of auto-oxidation of epinephrine to adrenochrome at an alkaline pH at 480 nm. Results were expressed as nmol H_2O_2/mL of serum and U SOD/mL of serum, respectively.

2.11. Histopathology

Intestinal samples (duodenum, jejunum and cecum) were collected on days 5,10 and 15 PI, stored in buffered formalin solution (10%), stained with hematoxylin and eosin (HE) and analyzed using a light microscope.

2.12. Statistical analysis

The vast majority of the data did not present normal distribution (Shapiro-wilk test). Therefore, was used a nonparametric test for two independent groups (Mann-Whitney test), considering also each chick an independent variable, that is, experimental unit. Results were considered significant when $P < .05$. Results were presented as mean ± standard deviation.

3. Results

3.1. Body weight, parasitic burden, clinical signs and histopathology

The body weight of the infected chicks (305 ± 19 g) was lower ($P < .05$) compared to the non-infected chicks (485 ± 32 g). No significant difference was observed between groups regarding the number of *Eimeria* spp. oocysts on day 5 PI. No oocysts of *Eimeria* spp. were observed in the control group on days 10 and 15 PI, while 800 ± 421

Table 1
Hematological analysis of chicks experimentally infected by *Eimeria* spp. compared to control (uninfected).

Variable	Day	Control	Infected	*P* value
Erythrocytes ($\times 10^6$ μL)	5	2.13 (0.35)	2.19 (0.30)	0.902
	10	3.02 (0.39)	3.17 (1.0)	0.741
	15	2.8 (0.9)	1.5 (0.23)	0.001*
Hematocrit (%)	5	24 (3.9)	29.7 (3.4)	0.082
	10	25.5 (4.2)	26.7 (3.3)	0.749
	15	26.4 (2.3)	20.5 (3.0)	0.038*
Hemoglobin (g/dL)	5	10.4 (1.56)	9.22 (3.09)	0.804
	10	9.25 (1.20)	10.0 (1.44)	0.882
	15	8.88 (2.15)	6.3 (0.2)	0.050*
Total leukocytes (μL)	5	3537.5 (715)	6267.5 (1219.9)	0.001*
	10	4325 (900.4)	7600 (1190.9)	0.001*
	15	4140 (1135)	3200 (433.9)	0.129
Heterophil (μL)	5	578.6 (167.5)	1473.7 (1170.8)	0.001*
	10	787 (291.6)	2213.5 (987.9)	0.001*
	15	1478.8 (916.4)	1409.5 (753.5)	0.903
	5	2681 (393.7)	4265.2 (1254.9)	0.001*
Lymphocytes (μL)	10	3324 (714.8)	4975.5 (470.7)	0.001*
	15	2443.8 (153.5)	1689.2 (1002)	0.099
	5	213.7 (171.6)	368.2 (307.5)	0.340
Monocytes (μL)	10	195.7 (124.3)	282.8 (160.0)	0.249
	15	148.5 (139.3)	68.8 (43.5)	0.421
	5	46.7 (76.5)	48.1 (58.3)	0.640
Eosinophils (μL)	10	0.0 (0.0)	88.8 (62.4)	0.013*
	15	26.8 (26.5)	24.1 (16.6)	0.536
	5	17.3 (21.0)	112.2 (119.0)	0.320
Basophils (μL)	10	18.2 (21.0)	39.2 (46.3)	0.530
	15	42.1 (38.8)	8.25 (16.5)	0.189

* $P < .05$ indicates statistical difference between groups.

and 1736 ± 838 oocysts per gram of feces were found for the infected group on days 10 and 15 PI, respectively.

No clinical signs were found in either group on days 5 and 10 PI. However, the infected chicks presented apathy, tremors and ruffled feathers compared to the control group on day 15 PI.

No intestinal lesions or intestinal alterations were observed in either group on day 5 PI. However, infected chicks presented mild-to-moderate (day 10 PI) and moderate-to-severe (day 15 PI) quantities of undifferentiated gametes with occasional microgametocytes, macrogametocytes and oocysts with morphology consistent with *Eimeria* spp. (Fig. 1).

3.2. Hematological analysis

Erythrocytes, hematocrits and hemoglobin levels were lower in infected chicks than in control chicks on day 15 PI. Total leukocytes, heterophils and lymphocytes counts were higher in infected chicks than in control on days 5 and 10 PI, while eosinophil counts were higher only on day 10 PI. No significant difference was observed between groups regarding basophil and monocyte counts (Table 1).

3.3. Serum biochemistry

Serum levels of total protein and globulin were higher in infected chicks than in control on days 10 and 15 PI, while cholesterol and triglycerides levels were lower on day 15 PI. No significant differences were observed between groups regarding albumin and uric acid levels (Table 2).

3.4. Cholinesterase and ADA activities

AChE activity in total blood and BChE activity in serum were higher in infected chicks than in uninfected on day 15 PI. Furthermore, ADA activity in serum was higher in infected chicks than in control on day 10 PI, but was lower on day 15 PI (Fig. 2).

Table 2
Serum biochemistry of chicks experimentally infected by *Eimeria* spp.

Variable	Day	Control	Infected	P value
Total protein (g/dL)	5	3.18 (0.54)	2.9 (0.54)	0.740
	10	3.53 (0.5)	4.22 (0.4)	0.020*
	15	3.6 (0.31)	4.28 (0.6)	0.043*
Albumin (g/dL)	5	1.34 (0.27)	1.14 (0.34)	0.730
	10	1.70 (0.43)	1.72 (0.5)	0.849
	15	1.52 (0.17)	1.34 (0.25)	0.631
Globulin (g/dL)	5	1.84 (0.23)	1.75 (0.70)	0.774
	10	1.83 (0.60)	2.5 (0.48)	0.046*
	15	2.07 (0.22)	2.94 (0.79)	0.023*
Uric acid (mg/dL)	5	7.78 (3.18)	8.27 (4.0)	0.729
	10	9.98 (3.66)	8.65 (4.46)	0.540
	15	7.65 (1.81)	5.80 (1.91)	0.062
Cholesterol (mg/dL)	5	55.5 (17.2)	57.0 (13.7)	0.860
	10	79.4 (11.9)	63.1 (7.66)	0.270
	15	108.2 (9.5)	82.2 (20.2)	0.047*
Triglycerides (mg/dL)	5	53.2 (22.6)	60.7 (42.1)	0.530
	10	65.4 (16.2)	65.8 (28.5)	0.823
	15	53.5 (9.8)	28.4 (11.7)	0.001*

* $P < .05$ indicate statistical difference between groups.

3.5. Antioxidant/oxidant status

Serum ROS levels were lower in infected chicks than in uninfected on day 10 PI, but were higher on day 15 PI. Catalase activity in total blood was higher in infected chicks on day 15 PI, while SOD activity in total blood was lower in infected chicks on days 5 and 15 PI (Fig. 3).

4. Discussion

In this present study, was observed that eimeriosis altered hematological and biochemical profiles of chicks experimentally infected with five species of *Eimeria*. The most important finding was that adenosinergic and cholinergic systems were involved in impairment of immune response, as well as in exacerbation of oxidative stress.

In the current study, was observed a significant reduction in erythrocyte counts and hematocrits, as well as in hemoglobin contents, in accordance to observations by Akhtar et al. [31] in broilers experimentally infected with *E. tenella, E. maxima, E. acervulina* and *E. necatrix*. According to these authors, the reduced levels of erythrocytes and hemoglobin might correlate with hemorrhage in the ceca and intestine, as well as tissue injury. This accords with our observation of undifferentiated gametes with occasional micro- and macrogametocytes as well as oocysts consistent with *Eimeria* spp. Confirming the occurrence of inflammatory response, was observed a significant augmentation of total leukocytes, heterophil and lymphocyte counts in chicks infected by *Eimeria* spp., in agreement with observations by Akhtar et al. [31] in broilers experimentally infected with *E. tenella, E. maxima, E. acervulina* and *E. necatrix*. According to Campbell and Ellis [32], the increased lymphocyte and heterophil counts might be attributable to the induction of an immune response in the infected animals, since these cells participate in the first line of defense against several parasitic infections. Furthermore, a study conducted by Rothwell et al. [33] demonstrated that increased leukocyte levels play an important regulatory role during the immune response to *E. tenella* infection mediated by cytokines, including interferon-gamma (IFN- γ), a interleukin associated with inhibition of pathogen replication and stimulation of immune cells [34]. Similarly, augmentation of lymphocyte counts may be an attempt to stimulate the release of IFN- γ, as observed by Lillehoj et al. [35] in chicks experimentally infected *E. acervulina* and *E. tenella*. Finally, was observed a significant increase in eosinophil counts in infected chicks on day 10 PI, in disagreement with several studies using chicks [31], and rats [36] as experimental models of eimeriosis. Is important emphasize that eosinophils are innate immune cells that exert important cytotoxic functions, causing damage to parasite

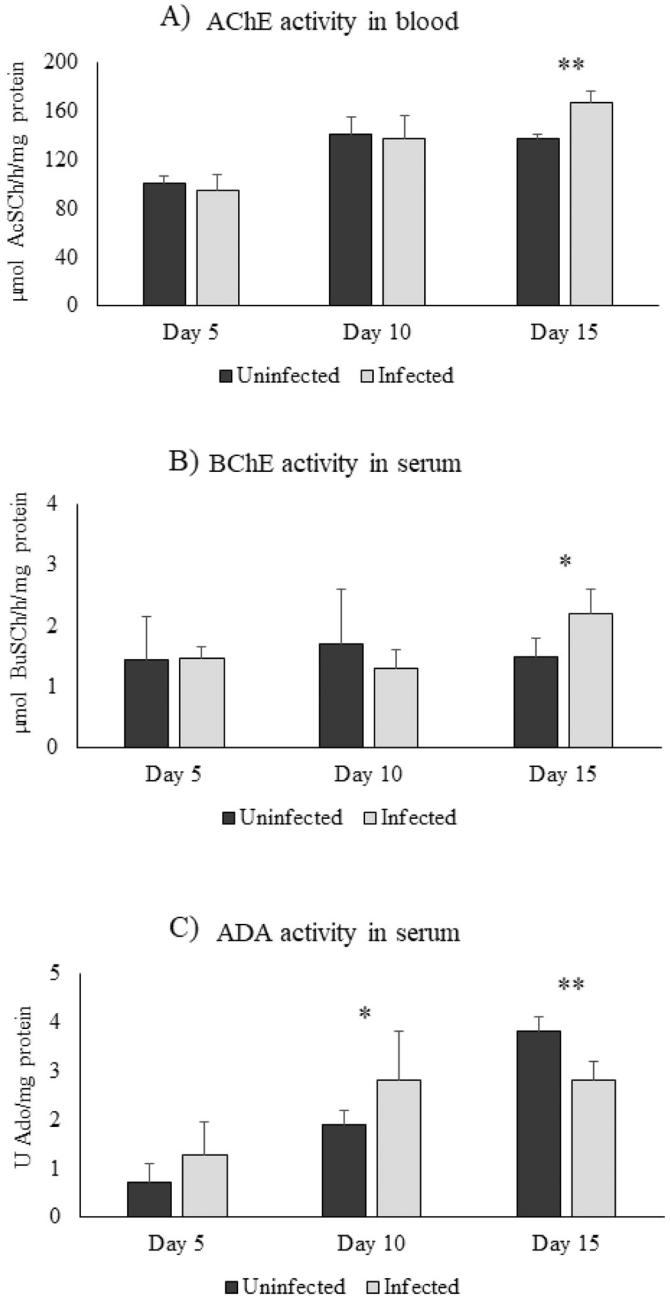

Fig. 2. Acetylcholinesterase (AChE) activity in total blood [A], and butyrylcholinesterase (BChE) [B] and adenosine deaminase (ADA) activities [C] in serum of chicks experimentally infected with *Eimeria* spp. on days 5, 10 and 15 post-infection. *$P < .05$; **$P < .01$.

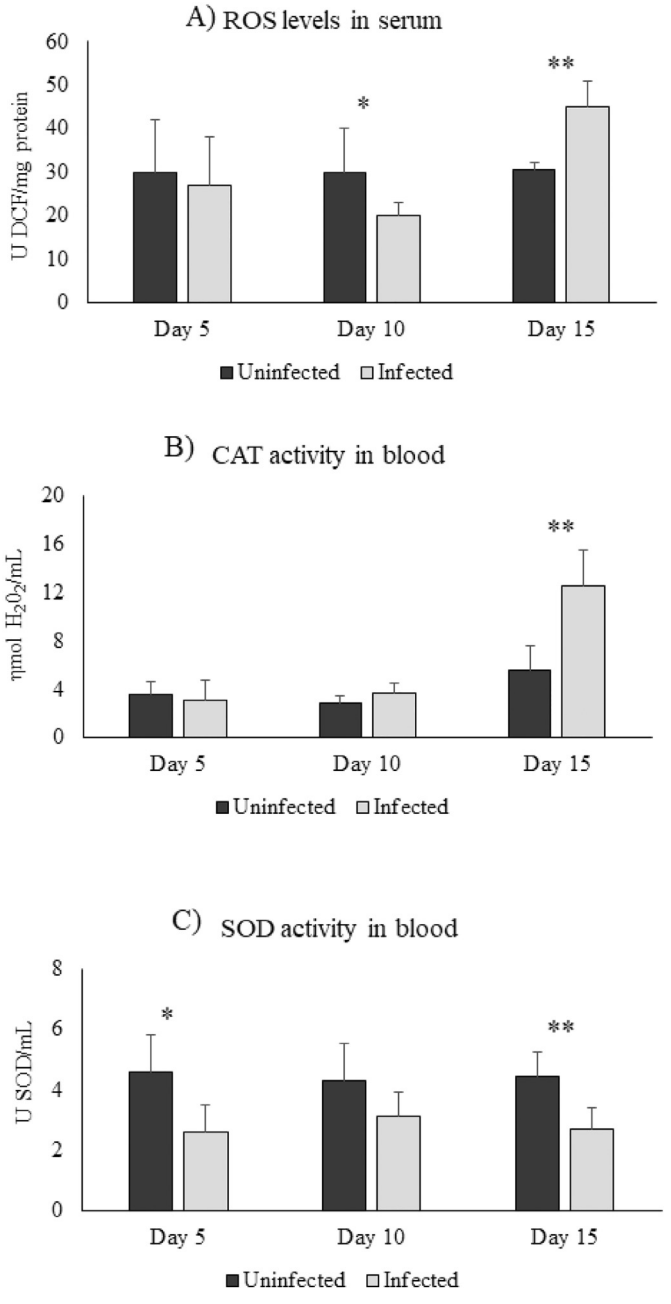

Fig. 3. Reactive oxygen species (ROS) levels in serum [A], and catalase (CAT) [B] and superoxide dismutase (SOD) [C] activities in total blood of chicks experimentally infected with *Eimeria* spp. on days 5, 10 and 15 post-infection. *P < .05; **$P < .01$.

pathogens. Furthermore, there is evidence to suggest the involvement of eosinophils in the maintenance of the intestinal epithelial barrier function, as well as on the store of preformed cytokines and chemokines for immediate release [37]. Therefore, The eosinophil counts increased may be an attempt to maintain the integrity of the intestinal barrier, as well as an attempt to improve the immune response in order to eliminate the parasite.

Serum proteins are largely composed of albumin and globulins, responsible for hormone transport, vitamins and lipids [38], among other activities. These proteins play an interesting role during infectious diseases [39]. In this study, was observed that infected chicks showed augmentation of serum total protein and globulin levels, in accordance to observations by Gomez-Bautista et al. [40] in rabbits infected with *E. stiedai* and in rats infected with *E. niestulzi* [41]. According to these authors, hyperproteinemia was mainly due to increases in gamma-globulins, alpha-globulins and beta-globulins, explaining the augmentation on serum globulin levels observed in the present study. Is important to highlight that globulins exerts an interesting role in the immune response, constituting the basis of the humoral immune response, developing an effective immune response against several microorganisms, including parasites [38]. In this study, was observed a significant reduction in serum levels of cholesterol and triglycerides, possibly due to failure of their synthesis in the intestine [42], as observed in *Gallus gallus* experimentally infected with *E. acervulina* [43]. In this sense, the hypothesis arises that intestinal damage provoked by mixed *Eimeria* spp. infection contributes to impairmet of cholesterol synthesis.

The significant increase in AChE activity in total blood can be

interpreted as a pro-inflammatory effect due to the reduction in AChE, a cholinergic molecule with anti-inflammatory and immunomodulatory properties [44]. This is similar to the results observed by Baldissera et al. [45] in spleen of silver catfish (*Rhamdia quelen*) naturally infected by protozoa *Ichthyophthirius multifiliis*. Similarly, serum BChE activity was elevated in infected chicks compared to that of the control group, in accordance to observations by Schwertz et al. [46] in the sera of cattle naturally infected by *Eurytrema coelomaticum*. According to these authors, augmentation of serum BChE activity also can be interpreted as a pro-inflammatory response caused by rapid degradation of ACh content, possibly contributing to impairment of immune and inflammatory responses. Furthermore, serum ADA activity showed varying profiles according to the disease evolution. On day 10 post-infection, significant augmentation of serum ADA activity may be considered a pro-inflammatory response mediated by rapid deamination of adenosine into inosine, because adenosine is a molecule with anti-inflammatory and immonumodulatory properties [17,47]. This phenomenon was observed by Tadayon et al. [48] in serum of goats experimentally infected with *E. ninakohlyakimovae, E. caprina* and *E. arloingi* on day 3 and 7 PI. Nevertheless, serum ADA activity was lower in infected chicks on day 15 PI, a period in which the animals showed clinical signs of the disease. At this moment, ADA had an anti-inflammatory profile due to reduction in adenosine degradation and consequent accumulation in the extracellular medium, as observed by Baldissera et al. [45] in spleens of silver catfish naturally infected by protozoa *I. multifiliis*. Therefore, the cholinergic system contributes to impairment of the immune response and consequent inflammatory damage, while the adenosinergic system exerts distinct profiles depending on disease evolution.

On day 15 PI, was observed a significant increase in serum ROS levels in chicks experimentally infected with *Eimeria* spp., in accordance with observations by Del Cacho et al. [49] in serum of chickens infected by *E. tenella*. According to Winterbourn [4], the increase on ROS content may lead to biomolecular damage of lipids, proteins and nucleic acids, causing loss of their biological functions and tissue injury. This may explain the various intestinal lesions on day 15 PI. In order to respond to oxidative stress, biological systems protect themselves with endogenous antioxidants, including hepatic CAT and SOD enzymes [50]. CAT activity in total blood was significantly higher in chicks infected with *Eimeria* spp. on day 15 PI, while SOD activity reduced at the same time of infection, in accordance with observations by Koinarski et al. [51] and Georgieva et al. [12] in plasma of chicks experimentally infected by *E. acervulina*. CAT is an enzyme responsible for catalyzing decomposition of H_2O_2 into water and oxygen. According to Lushchak [52], increased CAT activity may be considered an attempt to remove excessive H_2O_2, a molecule linked to tissue injury and inflammation [53]. In summary, stimulation of CAT activity indicates an increased capacity to scavenge H_2O_2 that is produced in response to oxidative stress caused by eimeriosis, contributing to reduction of oxidative damage. Like our observations, Georgieva et al. [12] observed a significant increase on plasma lipid peroxidation with concomitant inhibition on SOD and GPx activities, which contributed to disease pathophsiolgy of chickens infected by *E. acervulina*. However, SOD activity in total blood was lower in infected chicks, indicating a minor capacity to scavenge superoxide anion into oxygen and H_2O_2, because superoxide anion is a molecule linked to damage to constituents of cell membranes, including lipids and proteins [54]. Thus, inhibition of SOD activity indicates a reduced capacity to scavenge superoxide anion, thereby contributing to cell damage and pathogenesis of eimeriosis. Also, study conducted by Koinarki et al. [13] revealed a significant increase on total blood lipid damage and CAT activity, while the total blood SOD activity and the endogenous antioxidants (carotene, vitamin A and vitamin C) were inhibited in chicks infected by *E. acervulina*, contributing to animal mortality. Other study also reported that the experimental infection with *E. acervulina* in chicks brought about a depletion in the concentration of ascorbic acid (vitamin C) in blood plasma, duodenum, jejunum, ileum, liver and the adrenal glands [55].

5. Conclusion

Based on these data, concluded that the cholinergic and adenosinergic systems display a pro-inflammatory profile contributing to impairment of immune and inflammatory responses in mixed *Eimeria* infection. Furthermore, oxidative stress may contribute to clinical signs of disease and pathogenesis. In summary, the impairment of the immune response and alterations in blood antioxidant/oxidant status contribute to disease pathophysiology in experimental infection.

Ethics committee

This study was approved by the Comissão de Ética no Uso de Animais (CEUA) of the Universidade do Estado de Santa Catarina (UDESC), number 3096260917.

Conflict of interest

The authors have declared no conflict of interest.

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