Antioxidant status of broiler chickens, infected with *Eimeria acervulina*

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**SUMMARY**

The antioxidant status of broiler chickens (Cobb 500 hybrids) experimentally infected with *E. acervulina* was monitored via determination of blood plasma malondialdehyde (MDA) reactive products, the activities of the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT), as well as blood carotene, vitamin A and vitamin C concentrations. The results of the experiment showed a statistically significant increase of MDA concentrations (a marker of radical-induced damage) in *E. acervulina*-infected birds compared to healthy chickens (p<0.05). A decreased SOD activity was also observed in infected birds (p<0.001), whereas significant increase of CAT activities were obtained (p<0.001). Carotene, vitamin A and vitamin C concentrations were dramatically reduced in infected chickens (p<0.001, p<0.01 and p<0.05 respectively). The observed deviations in studied enzymes and non-enzymatic parameters evidence the occurrence of oxidative stress following the infection and impaired antioxidant status of broiler chickens infected with *E. acervulina*. The observed changes in small intestine, the oocyst production and the economical parameters (weight gain and feed conversion ratio) were indicative for a severe infection, in which the oxidative stress was also involved during pathogenesis.

**Key-words:** *E. acervulina* - oxidative stress - MDA - SOD - Catalase - Vitamin A - Vitamin C.

**RÉSUMÉ**

Capacités antioxydantes des poulets de chair infestés par *Eimeria acervulina*. Par V. KOINARSKI, N. GEORGIEVA, V. GADJEVA et P. PETKOV.

Le statut antioxydant des poulets de chair (hybrides Cobb 500) infestés expérimentalement par *E. acervulina* a été évalué en mesurant les concentrations plasmatiques de malonédiadehyde (MDA), les activités intra-érythrocytaires de 2 enzymes anti-oxydantes (Superoxyde Dismutase (SOD) et Catalase (CAT)) ainsi que les concentrations sanguines de carotènes, vitamine A et vitamine C. Une augmentation statistiquement significative des concentrations de MDA (marqueur des lésions induites par des réactions radicales) a été observée chez les sujets infestés par *E. acervulina* par rapport aux contrôles (p<0.05). Une activité diminuée de la SOD a aussi été constatée chez les animaux infestés (p<0.001) alors que l’activité de la CAT a significativement augmenté (p<0.001). Les concentrations en carotènes (p<0.001), en vitamine A (p<0.01) et en vitamine C (p<0.05) ont été considérablement réduites lors d’infestation par *E. acervulina*. Les modifications ainsi obtenues des activités enzymatiques et des concentrations des anti-oxydants de nature non enzymatique mettent en évidence l’apparition d’un stress oxydatif consécutif à l’infestation et l’induction d’un déficit des capacités anti-oxydantes chez les poulets infestés. Les lésions observées de l’intestin grêle, la forte production d’œocystes et la diminution des paramètres économiques (gains de poids et ratios de conversion alimentaire) traduisent une infection sévère, dont la pathogénie implique fort probablement la survenue d’un stress oxydatif.

**Mots-clés:** *E. acervulina* - stress oxydatif - MDA - SOD - Catalase - Vitamine A - Vitamine C.

**Introduction**

Under usual conditions, the production of reactive free radicals and their elimination are in a dynamic equilibrium. This balance could be disturbed when the generation of free radicals becomes higher than the protection capacity of systemic antioxidant defence. The impaired equilibrium in favour of oxidants is named oxidative stress and it is involved in the pathogenesis of numerous diseases, including parasitic infections [8, 15].

Avian coccidiosis is a common parasitic disease, causing extensive economic losses [37]. *Eimeria acervulina* is among the commonest *Eimeria* organisms in chickens, responsible for considerably increased costs in modern poultry industry [38]. In some regions, the infection rates with *E. acervulina* are even higher than those, provoked by *E. tenella* [18, 26]. The high levels of infection with this *Eimeria* species result in significant lower live body weights of chickens [15] and a more severe aflatoxicosis in the infected birds [31]. The invasion is reported to induce serious histopathological alterations in avian intestines and parenchymal organs [20].

There are several reports evidencing the presence of antioxidant desequilibrium in birds with parasitic diseases. In chickens with eimeriosis, hypovitaminosis C was observed [5]. According to other investigators [3, 12, 30] the blood levels of vitamin A and xanthophylls concentrations in chickens, infected with *E. acervulina*, were very low. GABRASHANSKA *et al.* [14] reported a deficiency of antioxidant vitamins C and E in chickens infected with *Ascaridia galli*.

The equilibrium between the activity and the intracellular amounts of antioxidant enzymes is vital for the survival and the health of aerobic organisms. The number of facts evidencing the existence of a changed expression of the principal antioxidant enzymes in various diseases is increasing, but the reports are rather conflicting [2, 6, 9, 23].

In our previous studies, we reported that the incidence of eimeriosis was about 20-50% of the poultry population in...
Bulgaria and that the prevalence of *E. acervulina* infection rate in studied birds was 18.3% [19].

The aim of the present study was to determine the blood antioxidant status of broiler chickens infected with *Eimeria acervulina*, via the measurement of blood lipid peroxidation products (malondialdehyde MDA), vitamin A, vitamin C and carotene concentrations and the activities of superoxide dismutase (SOD) and catalase (CAT), as primary enzyme and non-enzyme antioxidants.

**Material and methods**

1. **ANIMALS**

The study was performed on 50 clinically healthy 11-day old broiler chickens, Cobb 500 hybrids, weighing 288.0-411.0 g. Up to the age of 10 days, they were housed in cages on slat floors under conditions excluding an additional *Eimeria* infection and received a standard diet without antibiotics or coccidiostatics. At the age of 11 days, 2 groups of 25 birds each were formed. The first experimental group was untreated and non-infected (negative controls). The second experimental group was infected three times with $3 \times 10^5$ sporulated *E. acervulina* oocysts, at 2-day intervals (at 12th, 14th, 16th days) using an ingluvial tube [15].

In order to determine some parasitological parameters (lesion scores per bird [17] and the oocyst index [7]), 8 chickens from each group were sacrificed by cervical dislocation 8 days after the infection.

At the beginning and at the end of the experiment (8 days after the infection) determinations of the live body weight and the amount of forage expenditure were done in order to establish the weight gain and the feed conversion ratio (FCR).

2. **INFECTION MATERIAL**

*E. acervulina* oocysts were obtained from naturally infected chickens, passed through 2-week-old broiler chickens and stored in 2.5% potassium bichromate solution.

3. **BIOCHEMICAL STUDIES**

**Peripheral blood processing**

Blood for biochemical analyses (5 mL) was sampled by post infection day 8 from *v. subcutanea ulnaris* or *v. braehialis* for MDA, SOD and CAT assays. Ethylenediamine-tetraacetic acid (EDTA) was used as anticoagulant.

Collected blood was centrifuged at 2000 - 3000 g for 15 min and plasma was separated. Then, the plasma was deproteinized with 25% trichloracetic acid by continuous mixing for 5 min and centrifugation at 2000 g for 15 min. The deproteinized plasma was used for lipid peroxidation products determination. The total amount of lipid peroxidation products in plasma was assayed using the thiobarbituric acid (TBA) method, measuring spectrophotometrically malondialdehyde (MDA) reactive products at 532 nm [28].

**Erythrocyte processing**

The erythrocyte pellet was washed three times with saline and lysed. The hemoglobin was separated by precipitation with ethanol/chloroform mixture. The mixture was continuously shaken for 5 min and centrifuged at 2500 g for 20 min. The obtained supernatants were used for determination of enzyme activity.

- **Determination of superoxide dismutase (SOD) activities**
  - Erythrocyte lysates were assayed for SOD activities using the xanthine/xanthine oxidase system for superoxide anion ($O_2^\cdot$) generation. This anion reduced nitroblue tetrazolium (NBT) to formazan, which was monitored at 560 nm [34].

- **Determination of catalase (CAT) activities**
  - CAT activity was assessed in the erythrocyte lysates by the method described by BEERS and SIZER [4]. Hydrogen peroxide (30 mM) was used as a substrate and the decrease in H$_2$O$_2$ concentration at 22°C in phosphate buffer (50 mM, pH 7.0) was followed spectrophotometrically at 240 nm for 1 min. Results are presented as units per g hemoglobin (U/g Hb). One unit of CAT activity is defined as the amount of enzyme that degrades 1µM H$_2$O$_2$ per minute. Hemoglobin concentrations of lysates were determined spectrophotometrically at 546 nm by the cyanmethaemoglobin method of MAHONEY et al. [22].

- **Determination of plasma carotene, vitamin A and vitamin C**
  - Carotene (µmol/L) and vitamin A (UI/100mL) were assayed via the Carr-Price reaction spectrophotoscopically at 430 nm for carotene and 610 nm for vitamin A [16]. Plasma vitamin C concentrations (µmol/L) was assayed using the 2,2-nitrophenylhydrazine method, measuring spectrophotometrically ozazon products at 515 nm after 10 min [27].

**Statistical analysis**

The data were statistically processed one way analysis of variance (ANOVA). All results are presented as mean ± SEM. The differences were considered as significant when P values were less than 0.05.

**Results**

The number of lesions in the duodenum of infected birds [17] and the oocyst index [7] are shown in Table I. As early as the 5th day after the infection, *E. acervulina* caused considerable injuries to intestines and although at a lesser extent, they persisted until the 8th day.

Table II presents the live body weights, the weight gains and the feed conversion ratios (FCR) in chickens infected with *E. acervulina* and in healthy chickens. The results evidenced that at the beginning of the experiment, the body weight of both groups was equal but at the end, it was significantly lower in infected birds ($p<0.01$) and the weight gain by chicken was markedly depressed ($p<0.01$). This decrease was obviously due to the relatively low weight gain (52.2%) in these chickens compared to healthy controls. At the same time, FCR in infected birds was higher ($2.33 ± 0.02$ g/kg of

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food) than in healthy controls (1.35 ± 0.1 g/kg of food) (p<0.05).

Blood MDA concentrations and the activities of antioxidant enzymes SOD and CAT in studied birds are presented in Table III. The data showed a statistically significant increase of MDA concentrations (a marker of radical-induced damage) in *E. acervulina* - infected chickens vs. the healthy birds (p<0.05, Table III). SOD activities were significantly lower in infected chickens than in negative controls (p<0.001), but a significant increase of CAT activity was observed in infected birds compared to controls (p<0.001).

The changes in blood carotene (µmol/L) and vitamin A (UI/100mL) are shown in Table IV. Those parameters were lower in infected chickens compared to healthy controls. Carotene concentrations were more than twice lower (p<0.001) and those of vitamin A were considerably reduced by a factor 2 (p<0.01). Vitamin C concentrations (µmol/L) in healthy birds were 101.06 µmol/L (Table IV), whereas in infected ones - 69.26 µmol/L, i.e. a statistically significant decrease by 68.5 % was noticed (p<0.05).

### Discussion

Bibliographic reports demonstrate the presence of oxidative stress in parasitic diseases [10, 11]. The oxidative stress is manifested primarily via alterations of antioxidant enzyme activities and the reductions of some non-enzymatic antioxidants such as the vitamins A, C and E [13]. The superoxide dismutase is involved in the antioxidant defence system in a first attempt to control and eliminate the toxic reactive oxygen species (ROS) [24]. According AMSTAD et al. [1] the decrease of the activities of antioxidant enzymes could have a negative impact on cellular resistance against the oxidant-induced damage of cell genome and cell killing.

On the other hand, SPERANZA et al [33], POPOVA and POPOV [29] reported that the antioxidant enzyme catalase was important for adaptation of cells to oxidative stress and preserved cells via degradation of the reactive hydrogen peroxide. In the present study, the increases of plasma MDA concentrations and the marked reduction of the blood SOD activity in *E. acervulina* infected birds evidenced the occur-

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**Table I.** — Lesion score and oocyst index in *E. acervulina* infected broiler chickens (n = 8), and in uninfected chickens (n = 8). Results are expressed as Mean ± SEM.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Lesion score</th>
<th>Oocyst index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected with <em>E. acervulina</em></td>
<td>3.1 ± 0.1</td>
<td>32.3 ± 1.5</td>
</tr>
<tr>
<td>Healthy (negative controls)</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

**Table II.** — Changes in the live body weights, the weight gains and the feed conversion ratio (FCR g/kg of food) in healthy broiler chickens (negative controls) and in *E. acervulina* infected broiler chickens. Results are expressed as Mean ± SEM.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Initial body weight (12-days old), g</th>
<th>Final body weight (20-days old), g</th>
<th>Weight gain, g/chicken</th>
<th>Weight gain, %</th>
<th>FCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy (negative controls)</td>
<td>25</td>
<td>138.7 ± 2.0</td>
<td>411.0 ± 3.5</td>
<td>272.0 ± 4.2</td>
<td>100</td>
<td>1.35 ± 0.05</td>
</tr>
<tr>
<td>Infected with <em>E. acervulina</em></td>
<td>25</td>
<td>146.0 ± 3.1</td>
<td>288.0 ± 5.0 ¹</td>
<td>142.0 ± 2.1 ²</td>
<td>52.2</td>
<td>2.33 ± 0.02 ²</td>
</tr>
</tbody>
</table>

¹ p<0.05 ; ² p<0.01 vs. healthy controls.

**Table III.** — Blood MDA (malonedialdehyde) concentrations, SOD and CAT activities in *E. acervulina* - infected broiler chickens and in healthy broiler chickens. Results are expressed as Mean ± SEM.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>MDA, µmol/L</th>
<th>SOD, U/g Hb</th>
<th>CAT, U/g Hb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy (negative controls)</td>
<td>25</td>
<td>2.55 ± 0.07</td>
<td>3486.5 ± 63.6</td>
<td>1218.4 ± 117.6</td>
</tr>
<tr>
<td>Infected with <em>E. acervulina</em></td>
<td>25</td>
<td>2.76 ± 0.12 ¹</td>
<td>2759.4 ± 106.2 ²</td>
<td>2092.0 ± 115.2 ²</td>
</tr>
</tbody>
</table>

¹ p<0.05 ; ² p<0.01 vs. healthy controls.
rence of an oxidative stress due to infection and the impairment of antioxidant/pro-oxidant equilibrium in favour of pro-oxidants. The concomitant increase of CAT activity would be a compensatory mechanism in infected birds.

It is known that carotenoids are a group of liposoluble antioxidants, whose efficacy was lower to vitamin E [3]. NAGLER et al. [25] observed that vitamin A and β-carotene increased systemic antioxidant properties and decreased lipid peroxidation. In our study, blood carotene and vitamin A concentrations were dramatically lowered in infected birds. The E. acervulina-induced injury of the intestinal wall classically described with this eimeria species [20] and confirmed by histopathological studies probably reduced carotene and vitamin A absorption. Besides, the impairment of liver function observed in birds with eimeriosis [20, 21] would also contribute to reduce blood concentrations by decreasing hepatic storage and release of carotenoids. Consequently, the observed deficiency in carotenoids in E. acervulina infected birds would strengthen the intensity of the oxidative stress. Moreover, the more severe course of E. acervulina infection in chickens were observed when vitamin A concentrations were depressed [8, 12, 36].

The role of vitamin C as the most effective water-soluble antioxidant, found primarily in plasma, is well known [32]. In our experiment, blood vitamin C concentrations in infected chickens were significantly lower compared to healthy birds (Table IV), probably consequently to post infection oxidative stress. Similar results were also reported by TAKAHASHI and AKIBA [35].

As early as the 5th post infection day, E. acervulina caused significant damage to the intestinal tract. Although less significant, the changes persisted some days. As expected, oocysts were released in considerable amounts up to the end of the study, evidencing a rather severe infection in chickens. The intestinal alterations, manifested by the number of lesions per bird and the oocyst index (Table I), were indicative for the severity of the invasion. In Eimeria infected birds, growth performances were markedly altered and were probably related to the worsened feed conversion ratios. These results are in agreement with previous studies which reported a stunted growth in chickens infected by E. acervulina on the 4th - 5th days after infection [15, 31].

In conclusion, the observed increased blood MDA concentrations and the alteration in the activities of antioxidant enzymes SOD and CAT as well as the lower blood concentrations of carotene, vitamin A and vitamin C in 20-days old broiler E. acervulina - infected chickens provided evidence for the occurrence of an oxidative stress and of antioxidant status impairment consequently to the infection. The studied parasitic and economical parameters indicated a severe infection, in which oxidative stress was most probably involved. The deviations in the antioxidant status of E. acervulina-infected birds compared to healthy controls allowed us to extend the studies upon the mechanism of avian eimeriosis, and how decreasing the oxidative stress in broiler chickens using a combination of coccidiostatics and substances with proved antioxidant properties.

**References**


