ANTIOXIDANT PARAMETERS IN EIMERIA ACERVULINA INFECTED CHICKS AFTER TREATMENT WITH A NEW ZINC COMPOUND

VENTSISLAV KOINARSKI, MARGARITA GABRASHANSKA3, NEDJALKA GEORGIVA1 AND PETKO PETKOV 2

Department of Veterinary Microbiology, Infectious and Parasitic Diseases,  
1Department of Pharmacology, Veterinary Physiology and Physiologic Chemistry,  
2Department of Internal Diseases and Clinical Toxicology,  
Faculty of Veterinary Medicine, Trakia University, 6000 Stara Zagora, Bulgaria  
3Institute of Experimental Pathology and Parasitology, Bulgarian Academy of Sciences,  
1113 Sofia, Bulgaria  
e-mail: v_koin@yahoo.com

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Abstract

The effect of 2Gly.ZnCl2.2H2O compound on the antioxidant status in chicks infected experimentally with Eimeria acervulina was studied. Antioxidant status was measured via determination of blood plasma malonyl dialdehyde (MDA) reactive products, activity of superoxide dismutase (SOD) and catalase (CAT) as well as blood concentrations of carotene, vitamins A, C, and E, and zinc. The results showed increased MDA and CAT-activity, decreased SOD activity, hypovitaminosis C, A and E, and reduced Zn-level in the infected chicks. An antioxidant imbalance was developed due to the E. acervulina infection. The 2Gly.ZnCl2.2H2O oral administration restored vitamin E and zinc losses, and reduced CAT-activity. However, SOD activity, vitamins C and A, carotene and MDA levels in the infected chicks were not statistically changed. The observed changes in the small intestine, lesion and oocyst index, and economical parameters (body weight gain and feed conversion ratio) were indicative for a severe E. acervulina infection. They were correlated with the oxidative stress. Administration of 2Gly.ZnCl2.2H2O enhanced the antioxidant balance and performance of chicks with eimeriosis.

Key words: chicks, Eimeria acervulina, zinc, antioxidants.

Reactive oxygen species (ROS) are implicated in several pathophysiological conditions. ROS are known to attack almost all molecules of the cells including membrane lipid peroxides. The cellular system is equipped with both enzymatic and non-enzymatic defense system to neutralize ROS. The group of vitamins, comprising vitamins E and C, and provitamin A (exogenous antioxidants), need to be provided by the diet in sufficient amounts to ensure optimal antioxidant protection. Enzymes which typically contain trace elements as structural parts of the molecules, play another most important role in the antioxidant protective system, like glutathione peroxidase, catalase (CAT) and superoxide dismuthase (SOD). Trace elements (Se, Fe, Zn, Mn and Cu) associated with an active site or occurring in antioxidant enzymes as structural elements also play an important role in the antioxidant defense. Lipid peroxidation (LPX) is taken as an index of oxidative stress in tissues (14).

Avian eimeriosis is one of the most common diseases in countries with industrial poultry breeding. One of the most frequently encountered protozoas in chicks is Eimeria acervulina. Its pathogenic influence on avian organism has been studied in various aspects. Metabolic disorders take important place in the pathogenesis of eimeriosis (16, 17). It has been demonstrated that the concentrations of ROS are increased in fasciolosis, trichostrongylosis and eimeriosis in sheep (7). Reduction of some small molecular weight antioxidants (vitamins C, E, and A) was established in chick eimeriosis by Coles et al. (5). Metal enzyme-antioxidants are changed in the infection too (18). Zinc supplementation has been shown to enhance the activity of antioxidant enzymes, notably CAT and SOD (21). By this means, zinc inhibits the free radical chain reaction and is known to possess antioxidant properties (11).

The present study the effect of 2Gly.ZnCl2.2H2O compound on the oxidant-antioxidant balance ( vitamins E, C, and A, β-carotene, activity of SOD and CAT, and levels of Zn and MDA ) in chicks
experimentally infected with *E. acervulina* was investigated.

**Material and Methods**

**Experimental animals, design, and treatments.** The study was performed on 100 clinically healthy 20-d old broiler chicks, Cobb 500 hybrids, weighing 288.0 - 411.0 g. Up to the age of 10 d, they were housed in cages on slat floors under conditions excluding an additional *Eimeria* infection and received a standard diet without antibiotics or coccidiostats. At the age of 11 d, 4 equal groups of the birds were formed. The first group was non-treated and non-infected (negative controls). The second group was control and received 2Gly.ZnCl₂·2H₂O compound added to the feed – 0.12 g Zn²⁺/kg of feed. The compound was given for 10 d, starting 2 d before the oocysts inoculation. The third group was infected three times with 3 × 10⁵ sporulated *E. acervulina* oocysts, at 2-d intervals (at the 12th, 14th, and 16th d), using an ingluvial tube (8). The fourth group was experimentally infected with *E. acervulina* and received 2Gly.ZnCl₂·2H₂O at the mentioned before dose.

2GlyZnCl₂·2H₂O was synthesized from the aqueous solution using the method of isothermal decrease in the supersaturation (2).

The experiment was approved by the Committee on Animal Experimentation at Trakia University, Stara Zagora, Bulgaria, and was performed according to the recommendations of the Directive 86/609/EC of November 24, 1986.

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

**Biochemical investigations.** Blood samples were taken from the *v. subcutanea ulnaris* or *v. brachialis* on the day 8 after the first inoculation for MDA, SOD and CAT assays. Ethylenediamine tetraacetic acid (EDTA) was used as anticoagulant.

The whole blood was centrifuged for 15 min at 2 000 - 3 000 g to obtain the plasma. The proteins from the plasma were removed by 25% trichloroacetic acid by continuous mixing for 5 min and centrifugation at 2 000 g for 15 min. The received deproteinized plasma was used to establish a lipid peroxidation.

The erythrocyte pellet was washed three times with cold saline solution. The cells were lysed with distilled water (13). The haemolysate was centrifuged and the supernatant was used for the determination of SOD and CAT activities and haemoglobin concentration. SOD activity was assayed using the xanthine/xanthine oxidase system for superoxide anion (O₂⁻) generation. This anion reduced nitroblue tetrazolium (NBT) to formazan, which was monitored at 560 nm (25). The activity of CAT was determined at 25°C and pH 7 by the method of Beers and Sizer (3). The decrease in H₂O₂ concentration was determined at 240 nm. Haemoglobin concentration was assayed spectrophotometrically at 546 nm by the cyanmethaemoglobin method of Mahoney *et al.* (19).

The total amount of lipid peroxidation product in plasma was assayed using the thiobarbituric acid (TBA) method, measuring spectrophotometrically MDA reactive products at 532 nm (22).

Plasma β-carotene, and vitamins A, C, and E concentrations were determined by HPLC methods using a fluorescence detector as described by Bieri *et al.* (4).

Plasma Zn concentration was determined using an atomic absorption spectrophotometer Varian Techran model AA 220 (1).

**Parasitological studies.** In order to determine some parasitological parameters - lesion scores per bird (15) and the oocyst index (6) 8 d after the infection, 8 chicks from each group were sacrificed by cervical dislocation.

**Determination of production traits.** At the beginning and at the end of the experiment (8 d after the infection) the determination of body weight and forage expenditure were done in order to establish the body weight gain (BWG) and feed conversion ratio (FCR).

The data were statistically processed by the one way analysis of variance (ANOVA). The differences were considered significant when P values were less than 0.05.

**Results**

The vitamin levels are presented in Fig. 1. The levels of the investigated vitamins were reduced after the *Eimeria* infection. Carotene concentration was more than twice lower in the infected chicks than in the controls. 2Gly.ZnCl₂·2H₂O application did not show any effect on carotene level in controls but it was increased in the infected chicks (Fig. 1).

Vitamin E level was reduced in infected chicks by 42%. The Gly-Zn supplementation almost normalized vitamin E level increasing it by 45% in the infected chicks. Vitamin E level in the control chicks was increased after Gly-Zn treatment too. Vitamin C level was not influenced by the compound in the control chicks but it was slightly increased in the infected ones (Fig. 1). Reduced vitamin A level in the infected chicks was not significantly changed by the Gly-Zn supplementation (Fig. 2).

Vitamin A reduction in the infected and treated chicks was higher than in only infected chicks. Reduced vitamin A level in the infected chicks was not significantly changed by the Gly-Zn supplementation (Fig. 2).

In chicks infected with *E. acervulina* a reduced serum zinc concentration was observed. The Gly-Zn application increased the serum Zn in control and in infected chicks (P < 0.01) (Fig. 3). CAT activity was increased in the infected chicks (1 218±117 vs 2 092±115; P<0.05). The Gly-Zn addition reduced the CAT-activity only in the infected chicks to the value of controls (Fig. 4).
Fig. 1. Plasma β-carotene, vitamin C, and vitamin E levels in chicks non-infected or infected with *E.acervulina* and non-treated or treated with GlyZn -compound. Group 1 - healthy, non-treated; group 2 - healthy, treated; group 3 – infected; group 4 - infected, treated.

Fig. 2. Plasma vitamin A level in chicks non-infected or infected with *E. acervulina* and non-treated or treated with GlyZn-compound. Group 1 - healthy, non-treated; group 2 - healthy, treated; group 3 – infected; group 4 – infected, treated.

Fig. 3. Plasma Zn content in chicks non-infected or infected with *E. acervulina* and non-treated or treated with GlyZn-compound. Group 1 - healthy, non-treated; group 2 - healthy, treated; group 3 – infected; group 4 – infected, treated.
Fig. 4. Plasma SOD and CAT-activity in chicks non-infected or infected with *E. acervulina* and non-treated or treated with GlyZn-compound. Group 1 - healthy, non-treated; group 2 - healthy, treated; group 3 – infected; group 4 – infected, treated.

Fig. 5. Plasma MDA content in chicks non-infected or infected with *E. acervulina* and non-treated or treated with GlyZn-compound. Group 1 - healthy, non-treated; group 2 - healthy, treated; group 3 – infected; group 4 – infected, treated.

Fig. 6. BWG in chicks non-infected or infected with *E. acervulina* and non-treated or treated with GlyZn-compound. Group 1 - healthy, non-treated; group 2 - healthy, treated; group 3 – infected; group 4 – infected, treated.
SOD activity was significantly decreased in blood samples from the infected chicks (3 486.5 ± 63.6 vs 2 759±106.2; P<0.01). There were no significant changes in SOD activity after Gly-Zn application in the infected chicks but it was slightly increased in the control ones (Fig. 4).

Blood MDA was significantly increased in the infected chicks vs the healthy ones (2.76 ± 0.12 vs 2.55 ± 0.07; P<0.05). Zn-supplementation statistically significantly decreased MDA concentration only in the infected chicks (2.76± 0.12 vs 2.60± 0.05) (P< 0.01) (Fig. 5).

Figure 6 presents the BWG in chickens infected with *E. acervulina* and in the healthy ones. 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 the infected birds (P<0.01). This decrease was obviously due to the relatively low weight gain (52.2%) in the infected chicks compared to healthy controls. The Gly-Zn compound increased the body weight gain in the infected chicks by 7% compared with the non-treated infected ones (P<0.01).

The FCR in infected birds was higher (2.33) than in healthy controls (1.35) (Fig. 7). Gly-Zn application did not influence the FCR in the healthy and infected chicks (Fig. 7).

The number of lesions in the duodenum of infected birds and oocyst index are shown in Fig 8. As early as on the 5th d after infection, *E. acervulina* caused considerable injuries to the intestine, which persisted until the 8th d although at a lesser extent. The Zn-salt application did not influence significantly the number of lesions and the oocyst index (P < 0.05).
The results presented in this work clearly showed that *E. acervulina* infected chicks were under oxidative stress. Concentrations of non-enzymatic and enzymatic antioxidants as well as the levels of free radical scavengers were found to correlate with various physiological (BWG and FCR) or pathological conditions (number of lesions). Our results confirmed the finding that stress leads to a series of biochemical, physiological and behavioural changes, thus altering normal body homeostasis (10). 2Gly.ZnCl₂·2H₂O supplementation has been shown to influence the activity of antioxidants enzymes, notably CAT and SOD (21). Zn inhibits the free radical chain reaction and it is known to possess antioxidant properties (11). The increased CAT activity indicates the highly induced capacity to scavenge hydrogen peroxide produced in the red blood cells in response to oxidative stress due to the infection. This situation may reflect the persistent oxidative stress. The increased CAT-activity may be a compensatory mechanism to get rid of excess of peroxides (10). 2Gly.ZnCl₂·2H₂O application almost normalized it up to the control. SOD activity was reduced in the infected chicks. The fall of its activity may be due to the inactivation by interaction with oxygen radicals. Hodson and Fridovich (12) and Pigeolet et al. (23) reported a decrease in SOD activity caused by hydroxyl radicals and H₂O₂. The depression in SOD activity may result in cellular injury by superoxide radicals. The 2Gly.ZnCl₂·2H₂O supplementation did not influence the SOD activity in control and infected chicks. One of the main blood lipid peroxidation products (MDA), a marker of radical-induced damage, was statistically significantly increased in the *E. acervulina* infected chicks. Increased concentrations of lipid peroxidation end-products have been used as indicators of ROS-derived damage in biological systems (10). There were no differences between MDA in infected chicks after Zn-application.

The plasma Zn concentrations in all the chicks supplemented with 2GlyZnCl₂·2H₂O compound were found to be significantly higher than those of control groups.

Developed hypovitaminoses A, E, and C as well as reduced β-carotene content in infected chicks manifested the oxidative stress due to the eimeriosis. Our results are in a good accordance with those presented by Koinarski et al. (18). They clearly show that chicks infected with *E. acervulina* are under oxidative stress, which is manifested primary via alterations of antioxidant enzyme activities of SOD and CAT and the reduction of some non-enzymatic antioxidants (vitamins A and C) and increased plasma MDA concentration. Vitamin E losses restored by the Zn-application showed the positive interaction between these antioxidants. Vitamin E is an excellent biological chain-breaking antioxidant that protects cells and tissues from lipoperoxidative damage induced by free radicals (9). Vitamin C was slightly increased in the infected and treated with 2Gly.ZnCl₂·2H₂O compound chicks. It was reduced in infected chicks because the vitamin, as a water soluble compound, is the front-line of defense against free radicals created by metabolism. Vitamin C has been demonstrated to enhance the antioxidant ability of vitamin E by reducing the tocopheroxyl radicals back to their active form of vitamin E (14) or sparing available vitamin E (24). Regarding antioxidant property, there is a synergistic effect of vitamins E and C on the immune response. Reduced vitamin A level in the *E. acervulina* infected chicks was established by many authors (5, 7). Vitamin A deficiency develops in diseases of the intestines and liver as both the conversion of β-carotene into vitamin A in the epithelial cells of the intestines and the storing of vitamin A in the liver are disturbed. If the content of vitamins C and E is reduced, there is a considerable loss in the precursors of vitamin A and the vitamin itself during digestion, especially in the presence of unsaturated fatty acids.

Ascorbic acid, tocopherol, and carotenes are important non-enzymatic antioxidants which could potentially reduce the rate of initiation or prevent the propagation of free radicals, and thereby inhibit the oxidation of low-density lipoproteins. It is suggested that the tocopherols play the most effective role in the *E. acervulina* infection. The antioxidant properties of vitamin E are considered to have a role in the development of immune response. The vitamin protects lymphocytes, macrophages and plasma cells against oxidative damages and enhances the proliferation and activity of these cells in immune response (20).

Zinc is an essential trace element being an integral component of many enzymes and proteins needed in a wide range of metabolic processes. Zinc may work as an antioxidant through superoxide dismutase. So, it is very important in protecting animal tissues from oxidative destruction. This protective benefit results in an improved immune response which decreases infectious diseases incidence in poultry, as well as in BWG increase. Administration of oral 2Gly.ZnCl₂·2H₂O compound has been shown to be useful in chicks infected with *E. acervulina* by its improvement of their antioxidant status.

**References**

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