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Serum lysozyme concentrations in broilers treated with Sel-Plex® and sodium selenite and infected with *Eimeria tenella*

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Abstract: The aim of the present study was to elucidate the potential role of humoral factors of innate immunity in broilers infected with *Eimeria tenella* under the influence of inorganic and organic Se cations. It was found that the chickens treated with Sel-Plex® had higher serum lysozyme concentrations compared to those treated with sodium selenite in infected and noninfected groups. The authors consider that Sel-Plex® has a better expressed immunostimulating effect on serum lysozyme concentrations than sodium selenite.

Key words: Sel-Plex®, sodium selenite, lysozyme, broilers, *Eimeria tenella*

Introduction

Selenium (Se) as a chemical element was discovered by Swedish chemist Berzelius nearly 200 years ago. Since then, many publications have appeared describing its chemical properties and biological activity. In the 1930s, it was found that selenium is a toxic element and can even be carcinogenic. The next 20 years of research was devoted mainly to selenium toxicity; however, in 1957 it became clear that selenium was an essential nutrient in animal nutrition. This discovery was expanded by the knowledge that selenium is an integral part of an antioxidant enzyme, glutathione peroxidase (GSH-Px), as it was described in 1973 by Rotruck et al. (1).

Selenium exists in 2 chemical forms in nature: organic and inorganic. Inorganic selenium can be found in different minerals in the form of selenite, selenite, and selenide as well as in the metallic form. In contrast, in vegetable feed ingredients selenium is an integral part of amino acids including methionine and cysteine where it substitutes for sulphur. Therefore, animals receive selenium mainly in the organic form. Inorganic selenium (mainly in the form of selenite) has been widely used for the last 20 years to supplement diets of farm animals. The experience of using selenium in animal nutrition has given us today some important information necessary for further understanding the biological role of this element. The limitations of using inorganic selenium are well known: toxicity, interactions with other minerals, poor retention, low efficiency of transfer to milk and meat, and poor ability to maintain selenium reserves in the body. Consequently, a high proportion of the consumed element is excreted. In addition, the selenite ions

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have pro-oxidant properties (2). Recently, the use of sodium selenite in animal diets has been questioned (3). The development and commercialisation of organic selenium (Sel-Plex®, Alltech, USA) opened up a new way in animal nutrition by providing new opportunities not only for improvement of animal health and productivity but also for production of selenium-enriched meat, milk, eggs, and other foods. Like in other animals, broiler diet is a major factor in determining susceptibility to different diseases, and organic selenium can also have a great impact on human health. Selenium is an important element in poultry nutrition, and participates in the cellular antioxidant system. In the chicken, selenium deficiency, especially in combination with low vitamin E supply, is responsible for the development of various diseases including exudative diathesis (4), nutritional encephalomalacia (5), and nutritional pancreatic atrophy (6).

The aim of the present study was to compare the serum lysozyme concentrations as a marker of innate immunity in broilers experimentally infected with *Eimeria tenella* supplemented with inorganic or organic Se cations.

Materials and methods

Birds and experimental design

Eighty newly hatched broilers (♀White Plymouth Rock × ♂White Cornish) were obtained from a poultry hatchery controlled in respect to bacterial and viral diseases. Their bacteriological status was monitored prior to the study. During the experiment (from day 1 to 23), the chickens were fed a standard mixed diet without antibiotic or coccidiostatic supplements but containing Sel-Plex® (Alltech, USA) or sodium selenite (0.3 ppm/kg). They were housed on a slat floor under conditions minimising the risk of spontaneous infection with *Eimeria*.

Broilers were divided into 4 groups:

Group I – non-infected and treated with Sel-Plex® (n = 20); Group II – non-infected and treated with sodium selenite (n = 20); Group III – infected with *E. tenella* and treated with Sel-Plex® (n = 20); Group IV – infected with *E. tenella* and treated with sodium selenite (n = 20). Chickens from groups III and IV were individually infected on day 15 after hatching with 8×10^4 sporulated *E. tenella* oocysts using an ingluvial tube according to the method of Lozanov (7).

The challenging agent was an *E. tenella* strain, isolated from naturally infected birds, enriched through 3-week-old birds and cultivated according to Gawain and Baker (8). The oocyst culture used for infection was tested for sterility by inoculation of 2 mL in MacConkey agar in petri dishes.

Methods

Blood was sampled from *v. ulnaris profunda* and allowed to clot for 1 h and then centrifuged for 10 min at 2000 × *g* at room temperature. Serum lysozyme concentrations were determined by the method of Lie et al. (9). Briefly, 20 mL of 2% agarose (ICN, UK, Lot 2050) dissolved in phosphate buffer (0.07 M Na_2HPO_4 and NaH_2PO_4, pH 6.2) was mixed with 20 mL of suspension of 24 h culture of *Micrococcus lysodeicticus* at 67 °C. This mixture was poured out in a petri dish (14 cm diameter). After solidifying at room temperature 32 wells were made (5 mm diameter). Fifty microliters of undiluted sera were poured into each well. Eight standard dilutions (from 0.025 to 3.125 mg/L) of lysozyme (Veterinary Research Institute, Veliko Tirnovo) were used in the same quantity as well. The samples were incubated for 20 h at 37 °C and lytic diameters were measured. Lysozyme content was calculated using a special computer program developed in Trakia University.

Statistical analysis

Data were analysed using ANOVA (STATISTICA, StatSoft, Inc., USA). Differences were considered as significant when P values were less than 0.05.

Results

Data presenting the dynamics of serum lysozyme concentrations are presented in the Table. Serum lysozyme level was the highest in the chickens in the non-infected and treated with Sel-Plex® group. This group was significantly higher ($P < 0.05$) than the other 3 groups. Compared to it serum lysozyme concentration in the non-infected but treated with sodium selenite group was almost 50% less. In the infected with *E. tenella* and treated with Sel-Plex® group serum lysozyme concentration was also

Table. Effect of Sel-Plex®, Sodium selenite and *E. tenella* infection on lysozyme blood serum concentrations in chickens. SE - standard error; VC - variation coefficient.

Groups	Mean ± SE	VC%	n
Non-infected + Sel-Plex®	0.76 ± 0.21*	57.93	20
Non-infected + Na_2Se	0.39 ± 0.09	68.54	20
Infected + Sel-Plex®	0.61 ± 0.27	50.18	20
Infected + Na_2Se	0.19 ± 0.04	107.64	20

*P < 0.05 vs. healthy controls

higher than it was in the infected and treated with sodium selenite group. These results indicated that Sel-Plex® influenced the enzyme concentrations more effectively compared to sodium selenite. The chickens from the non-infected groups had higher lysozyme concentrations compared to the infected groups, showing that lysozyme was involved in the defence of the organism against *E. tenella*.

Discussion

It is known that *E. tenella* causes serious damage to the *caecum mucosa,* leading to the crossing of bacteria into the blood stream, and decreasing lysozyme concentration in the blood (10). In the same way lysozyme concentrations in the sera of chickens infected with *E. acervulina* were 2 to 3 times lower than those of non-infected birds (11). Suteu et al. (12) reported that lysozyme concentration in caecal content of chickens infected with *E. tenella*, *E. maxima*, or *E. hagani* increased significantly compared to non-infected control chickens; the serum lysozyme concentrations were lowest in infected chickens and highest in infected birds treated with sulfaquinoxaline and olaquindox. Khovanskikh (13) observed decreases in lysozyme and properdin plasma concentrations in chickens, infected simultaneously with *E. tenella* and *E. maxima* completed to increased IgM and IgG production. The latest studies show that lysozyme is effective not only against gram-positive bacteria, as supposed until recently (14), but also against some viruses such as avian fowl-pox virus (15) and HIV (16). Elevated selenium intake may be associated with reduced cancer risk and may alleviate other pathological conditions including oxidative stress and inflammation. Selenium appears to be a key nutrient in counteracting the development of virulence and inhibiting HIV progression to AIDS. It also improves sperm motility and may reduce the risk of miscarriage. Selenium deficiency has been linked to adverse mood states and some findings suggest that selenium deficiency may be a risk factor in cardiovascular diseases (17). Fluctuations in nutrient availability can dramatically affect organs involved in immune cell development and consequently immune cell populations. For example, the thymus is responsible for the maturation and selection of T lymphocytes and is especially sensitive to fluctuations in the nutritional state. As early as 24 h into acute starvation, the thymus undergoes a rapid decline in weight accompanied by a decline in cellularity (18,19). Starvation and protein energy malnutrition also dampen the cell mediated and humoral immune responses by lowering the total number of CD8+ and CD4+ T cells, respectively (18-22). Fewer CD4+ T helper cells induce depression of IgG production by B cells, while fewer CD8+ cytotoxic T lymphocytes diminish delayed-type hypersensitivity response (21,22). In addition, refeeding induces a slow replenishment of thymus weight and cellularity, demonstrating the long-term effects of nutrient fluctuation on T lymphocytes (23). Understanding the interactions between nutrition and immunity is essential for improving animal welfare and production. The development of the immune system requires primarily energy for the differentiation of the various leukocyte lineages, while immune system maintenance requires adequate substrate to maintain cell populations and macromolecule production. Activation of the immune response results in the largest nutritional demand due to the increased leukopoiesis and acute phase protein production (24).

Activities of antioxidant enzymes are regulated by gene expression and can respond to environmental changes, thereby making regulation of the antioxidant system more sophisticated (25). It is interesting to note that selenium supplementation of the maternal diet increased GSH-Px activity in the liver of newly hatched chicks to such an extent that during the next 10 days it remained at this plateau level with a further increase noted at day 20 of age (Surai, unpublished data). Tissue susceptibility to peroxidation significantly decreased in 1- to 5-day-old chickens and liver MDA accumulation was significantly reduced in chickens from antioxidant-supplemented hens. When either 200 ppm vitamin E or 40 ppm vitamin E + 0.2 ppm Se was given to hens, differences remained through 10 days of age. Therefore, liver susceptibility to lipid peroxidation substantially decreased even if supplementation with vitamin E and carotenoid decreased. This can be explained as a result of increased concentration of glutathione and GSH-Px activity as well as of lipid composition changes (26). In fact, MDA accumulation in livers of chicks in treatments 3-6 was similar and significantly lower than in the controls (commercial diet). This means that antioxidant protection afforded by increased GSH-Px activity is equal to dietary inclusion of 40-100 mg/kg vitamin E. Similarly, in another experiment dietary inclusion of 0.5 ppm Se or its combination with vitamin E for 5 weeks increased activities of GSH-Px and SOD and decreased MDA contents in tissues, confirming the antioxidant protective effect of selenium (27). In rats, an inverse linear correlation was found between lipid peroxide concentration and Se-GSHPx activities in various tissues (28). The benefit of organic selenium in breeder diets lies in its efficient absorption, transport, and accumulation in egg and embryonic tissues. This results in improved antioxidant status of the newly hatched chick. As the levels of major natural antioxidants (vitamin E and carotenoids) in tissues progressively decline after hatch, the antioxidant enzymes become a critical arm of antioxidant defence. Therefore, enhanced GSH-Px activity in tissues as a result of organic selenium supplementation of the maternal diet may have a positive impact on chick viability in the first few weeks post-hatch.

According to Surai et al. (29), antioxidant systems of the living cell include 3 major levels of defence. The first level of defence is responsible for prevention of free radical formation and consists of 3 antioxidant enzymes, namely superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase plus metal-binding proteins. It is generally accepted that the superoxide radical is the main free radical produced in living cells and that the electron transport chain in the mitochondria is responsible for its generation (30). SOD dismutases this radical throughout formation of hydrogen peroxide (H_2O_2), but the latter is still toxic to the cell and must be quickly removed. This important step in antioxidant defence is provided by GSH-Px and catalase (23). GSH-Px is found in different parts of the cell, but catalase is mainly located in peroxisomes. As a result, the efficacy of hydrogen peroxide removal from the cell is higher in the case of GSH-Px (30). Therefore, selenium, as an integral part of the antioxidant enzyme GSH-Px, belongs to the first line of antioxidant defence.

In conclusion, our results demonstrated that, firstly, serum lysozyme concentrations decrease in chickens infected with *E. tenella*, and secondly Sel-Plex® as source of selenium ion influences serum lysozyme concentrations more than sodium selenite.

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