ACTIVITY OF SELECTED ANTIOXIDANT ENZYMES IN LAYER HENS NON-INFESTED AND INFESTED WITH DERMANYSSUS GALLINAe

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Abstract

This paper presents the results of an experiment aimed to determine the activity of selected antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), conducted on 24 Hy-Line Brown layers raised in a battery cage system. The hens were divided into two groups, non-infested and infested with Dermanyssus gallinae. It was found that the exposure to the infestation significantly increased SOD and GPx activity and decreased CAT activity. Changes in the activity of the investigated enzymes may indicate the occurrence of oxidative stress in consequence of long-term infestation with the parasite.

Key words: hens, Dermanyssus gallinae, SOD, CAT, GPx.

Material and Methods

The experiment was performed on 24 Hy-Line Brown layer hens raised in a battery cage system. The investigated hens originated from a layer house with around 80,000 birds. The layer house was naturally infested with Dermanyssus gallinae. The severity of invasion was monitored weekly with the use of trap tubes. During 40 weeks, around 2000 various developmental forms of the parasite were found in 1 g of the litter sampled from the floor, including about 30% of females, 45% of larvae and nymphs, and 25% of eggs.

Randomly selected 49-week-old hens were divided into two groups, each of 12 birds. The first group comprised of hens twice sprayed with Butox 50 (deltamethrine, Hoechst) at a dose of 1 ml/l of water. The first spraying took place directly on the farm and the second – 6 d after the birds had been transferred to the animal house. This group was marked as non-infested with Dermanyssus gallinae. The other group comprised of hens infested with the parasite the invasion...
of which was monitored with the use of trap tubes until the completion of the experiment. In the animal house, the hens were placed in cages, six birds per cage, under conditions identical to those on the farm. Both groups were kept in separate compartments. They were fed farm-made feed and had free access to water. The hens were subjected to the same light programme, which was applied on the home farm.

At the age of 52 weeks, all the hens were sacrificed by quick decapitation. Prior to the slaughter, each bird was kept in a dark box for 5 min to reduce stress caused by handling. The hens were bled in around 10 s and blood samples were taken for enzyme determinations. In order to obtain erythrocyte lysate, blood was centrifuged at 2 500-3 000 g for 15 min to separate the plasma. Erythrocyte pellet was washed three times with saline and then diluted with 1.5 ml of cold water to lyse the erythrocytes.

The activity of SOD in erythrocytes was determined by the kinetic method with the use of the RANSOD reagent kit (RANDOX Lab. Ltd., UK), and the activity of CAT was assayed by the kinetic method of Aebi (2). The activity of GPx in blood was determined by the kinetic method, using the RANSEL reagent kit (RANDOX Lab. Ltd., UK). SOD, CAT and GPx assays were determined with the use of a UV/VIS BACKMAN DU 520 spectrophotometer.

The obtained results were statistically analysed by a one-way analysis of variance (ANOVA) followed by a Newman-Keuls test. The results were expressed as arithmetic means and standard errors of the mean (±SEM). The significance of differences between means was estimated at a level of P≤0.05.

Animal tests were carried out in accordance with the provisions of the “Act on Animal Protection” and the recommendations of the Local Ethics Committee for Animal Experiments at the University of Warmia and Mazury in Olsztyn.

**Results and Discussion**

Figs 1-3 present the activity of SOD, CAT, and GPx in hens non-infested and infested with *Dermanyssus gallinae*.

The obtained results indicate that long-term exposure of layer hens to *D. gallinae* invasion caused significant changes (P≤0.05) in the activity of antioxidant enzymes, in comparison with non-infested birds.

In the infested group, SOD activity increased by 19.9% in comparison with the group of birds free of the parasite (Fig. 1). Similar changes were observed with regard to GPx, the activity of which increased by 20.8% (Fig. 3). The blood concentrations of both enzymes usually increase simultaneously due to higher antioxidant use under oxidative stress conditions. Different results were reported in respect of CAT. The activity of this enzyme in the group infested with *D. gallinae* was by 24.3% lower than that in the group of non-infested birds (Fig. 2).

Due to the continued generation of reactive oxygen species, aerophobic organisms have developed a complex defence system to prevent its formation and impact. It comprises two coexisting systems: enzymatic (including SOD, CAT, and GPx) and non-enzymatic (including glutathione, ascorbic acid, and tocopherols) (17).

SOD is the main enzyme participating in the organism's complex defence against oxidative stress. This enzyme catalyses reactions, which eliminate superoxide anion radical and lead to the formation of hydrogen peroxide and molecular oxygen (22). Hydrogen peroxide is then removed by CAT and GPx. At low H₂O₂ concentrations, it is eliminated mostly by GPx, and when intracellular concentration of H₂O₂ is high – by CAT, which is not saturated even at very high H₂O₂ concentration (21). Since GPx reacts also with other hydroperoxides, it is believed that this enzyme plays a key role in antioxidant protection, in particular during oxidative stress of low intensity (22). Such stress occurred most probably in the current study, and the reported differences in CAT and GPx activity could result from different methods of H₂O₂ elimination.

Changes in the activity of the analysed enzymes could be indicative of the defence mechanism against reactive oxygen species, the generation of which may be a non-specific response against *D. gallinae*. This hypothesis is supported by the work of other researchers, who observed an oxidative imbalance in animals infested with parasites. Georgieva et al. (11) pointed to an increase in MDA levels and CAT activity and a drop in SOD activity in hens infested with *Eimeria tenella*. These authors suggest that changes in the analysed parameters are indicative of a shift in oxidative balance towards pro-oxidative effect. A significant increase in the peroxidation of erythrocyte membrane lipids in cattle infested with *Theileria annulata* was also reported by Asri Rezaei and Dalir-Naghadeh (3).

According to literature data, free radicals can play an important role in the pathogenesis of many diseases (6, 14). Oxidative stress may be also induced by other exogenous chemical substances (including drugs) (4, 10) as well as factors such as heat stress, anxiety, etc. (13, 19). Bouayed et al. (7) observed a significant correlation between the level of anxiety and reactive oxygen species in blood granulocytes in mice. Their findings support the previous observations made by Kuloglu et al. (18), who noted increased levels of antioxidant enzymes (GPx, SOD) in erythrocytes and malonodialdehyde (MDA) in patients suffering from obsessive-compulsive disorders. Changes in the activity of SOD, CAT, and GPx observed in this study, could therefore indicate that long-term anxiety caused by *D. gallinae* could induce oxidative stress in birds. By causing anxiety in birds, red mite infestation induces somatic stress, which is characterised by increased levels of hormones of the hypothalamo-pituitary-adrenal axis (16). In the present experiment, a nearly 2.5-fold increase in corticosterone plasma levels was recorded in hens infested with red mites, in comparison with the non-infested birds.
Fig. 1. SOD activity in layer hens non-infested and infested with Dermanyssus gallinae (* P<0.05)

Fig. 2. CAT activity in layer hens non-infested and infested with Dermanyssus gallinae (* P<0.05)

Fig. 3. GPx activity in layer hens non-infested and infested with Dermanyssus gallinae (* P<0.05)
This indicates that the rise in corticosterone levels could be at least partially responsible for the increased production of oxygen free radicals. Lin et al. (20) also demonstrated that a 2-week diet supplementation with corticosterone significantly increased lipid peroxidation in chickens. According to these authors, the increased generation of reactive oxygen species and the simultaneous, significant increase in SOD activity after three days of corticosterone administration could be indicative of a stronger antioxidant enzyme defence response.

The obtained results show that long-term exposure of layer hens to \textit{D. gallinae} invasion may result in oxidative stress, which is manifested in changes in the activity of SOD, CAT, and GPx. Since in the literature there is no information on the above problem, investigations should be continued on a wider range.

References