

## THE EFFECT OF FEED ADDITIVES BASED ON THE MYCELIUM OF THE FUNGI *GANODERMA LUCIDUM* AND *LAETIPORUS SULPHUREUS* ON THE PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS OF POULTRY BLOOD

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### Abstract

*The possibilities of using the vegetative mycelium of xylotrophic fungi of the varnished tinder fungus (Curt.:Fr.) P. Karst. and tinder funnel of sulfur yellow (Bull.) Murrill as food additives for poultry of the parent flock. These types of mushrooms are known as promising biotechnology producers of compounds of sterol and carbohydrate nature, carotenoids, vitamins and other substances with high biological activity. In the process of growing the mycelium was enriched with trace elements germanium, which can partially stop oxygen starvation in tissues. Powdered feed additives were fed with the main diet and allowed to increase the total blood protein of poultry against the background of fraction growth by 5-7%, and the hemoglobin content - by 7-9%. A positive effect on the immune parameters of the parent flock blood and the antioxidant status of their body were established, and a decrease in the markers of oxidative processes was shown. Feed additives allowed to increase the safety of the parent poultry flock, improve incubation rates of eggs, increase the rates of chickens by 5-6% relative to the control.*

**Key words:** poultry farming, food additives, medical fungi

### INTRODUCTION

Large-scale poultry farming on different farms is associated with a number of serious problems. A large crowding of poultry causes stress, which entails a decrease in immunity, in addition, in such conditions there is a risk of the rapid spread of infections. In this regard, there is a need for the prevention these diseases, including mass immunization and other approaches to reducing morbidity and mortality.

In recent years, the attention of researchers has been attracted by the methods of non-drug maintenance of the bird's body resistance. Higher fungi are considered as sources of immunomodulating and prophylactic agents (Barneche et al., 2016; Jeong, Park, 2018; Koo et al., 2019).

The anti-inflammatory activity of *Laetiporus sulphureus* and the antioxidant, anti-inflammatory, anti-tumor and antiviral activity of compounds from *Ganoderma lucidum* (Jeong, Park, 2018; Sohretoglu, Huang, 2018; Khalilov et al., 2019) are indicated. Fungi *Lentinus edodes*, *Ganoderma lucidum*, *Pleurotus ostreatus*, and *Cordyceps* sp. they are

used to optimize the intestinal microflora of chickens, which helps to strengthen the immunity of birds (Robinson et al., 2018).

Of greatest interest in the context of our research are studies related to the role of higher fungi as inhibitors of oxidative stress in poultry (Li et al., 2019; Teng et al., 2019). It has been shown that fungi and their polysaccharides can play an important role in poultry farming: polysaccharides could act as immunity inducers or immunomodulators and exhibit antibacterial, antiviral, antiparasitic biological activity; fungal phenolic compounds can act as antioxidants; mushrooms in the broiler diet can be used as growth stimulants as an alternative to antibiotics; mushrooms in the diet could improve egg production and egg quality; mushrooms can be used as a safe and effective alternative for the induction of molting and enhancing immunity (Khan et al., 2019).

Growing consumer concerns about food safety and their residues have spurred the search for alternative prevention and control strategies, among which herbal medicine has become attractive due to a renewed interest in natural medicine. In this context, studies on the possibility of using natural ingredients derived

from mushrooms for the prevention and treatment of pathological conditions in productive birds seem relevant (Peek et al., 2013).

In the course of our research, the feasibility of using feed additives prepared on the basis of the mycelium *Ganoderma lucidum* and *Laetiporus sulphureus* in poultry practice was examined.

## MATERIALS AND METHODS

The investigations had been carried out on the basis of the Department of Biology, Biological Technologies and Veterinary Sanitary Expertise of Penza State Agrarian University (Russia), the Inter-Faculty Biochemical Laboratory and Kolysheyskaya Poultry Factory of the Penza Region from 2015 to 2019.

The objects of research were Hisex Brown hens, which make up the parent herd, eggs and young animals obtained in the experiment. The effects of the mycelium of the varnished tinder fungus (*Ganoderma lucidum*) and the sulfur tinder fungus (*Laetiporus sulphureus*) on the biochemical parameters of blood and serum of birds, the biochemical parameters of eggs, the productive qualities of the parent herd, the incubation quality of the eggs and the state of the young were studied.

The most productive fungal strains were used to prepare feed additives based on mycelium. The process of obtaining mycelium included three stages. The first stage was the deep cultivation of mycelium for its subsequent inoculation on solid-phase substrates. At the second stage, the obtained deep mycelium was transferred to solid-phase substrates (mixtures of straw, sawdust and grain), packaged in 4-5 kg packs in 10 l plastic bags or 10 l glass jars. At the third stage of preparation, an air-dry substrate overgrown with mycelium was

ground in a laboratory mill to obtain a fine-grained powder (particle diameter of not more than 100 µm). The finished product was mixed with the main diet of the bird at the rate of 0.1%.

After feeding the feed additive in the diet, on the 15th, 30th and 45th day, blood was taken for laying hens for analysis. The calculation of the number of red blood cells and white blood cells was carried out simultaneously in the Goryaev chamber according to the generally accepted method. The calculation of the number of cells was carried out according to generally accepted formulas. Blood hemoglobin was determined by the hemoglobin cyanide method. Statistical data processing was performed using the functions of Excel package Microsoft Office.

## RESULTS AND DISCUSSIONS

Feed additives based on the mycelium *G. lucidum* and *L. sulphureus* were fed to the bird of the parent herd for 45 days. The control group of chickens received the main diet. Analysis of the blood of birds on the content of serum proteins (total protein and albumin) was carried out three times during the experiment: on the 15th, 30th and 45th days. A positive effect on the dynamics of serum protein content was established, which is reliably shown for the variant using *G. lucidum* (Figure 1). Under the influence of a feed supplement based on *L. sulphureus*, the content of total protein in serum increases in comparison with the control by the 30<sup>th</sup> day, but then decreases below the control indicators. The dynamics of the albumin fraction indicates that the marked increase in the concentration of protein in serum occurs due to albumin. The results can be explained by the antioxidant effect of *G. lucidum*, which results in stabilization of protein metabolism in the cell.

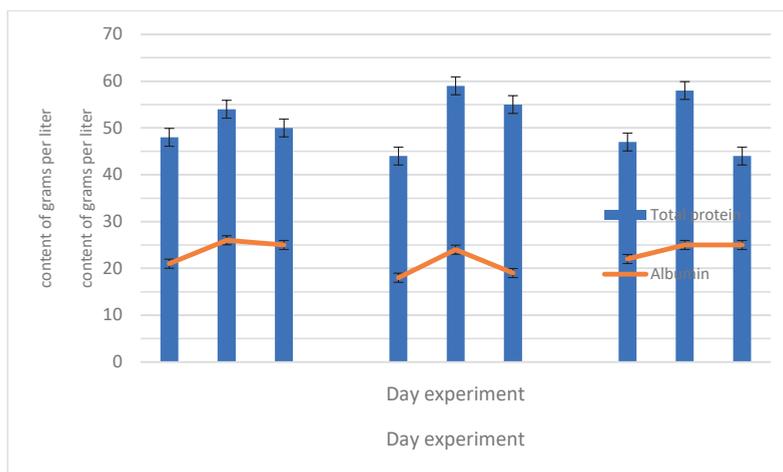


Figure 1. The effect of feed additives based on mycelium on the content of total protein and albumin fraction in the blood serum of birds of the parent herd: option 1 - *G. lucidum*, option 2 - *L. sulphureus* ( $p < 0.05$ , error bars - average error)

In order to assess the immune status of the organisms of the hens of the parent herd, the serum lysozyme content and serum bactericidal activity were also determined. In our studies, these indicators in the experimental groups

receiving feed additives based on the mycelium of the studied species of fungi were significantly higher compared to the control group of hens of the parent herd (Table 1).

Table 1. Indices of natural resistance of hens of the parent herd at the age of 135 days ( $n = 25$ ), ( $p > 0.05$ )

Groups	Indicators	
	The concentration of lysozyme in serum, $\mu\text{g} / \text{ml}$	Bactericidal activity in blood serum, %
Control group (normal diet)	24.2 $\pm$ 3.17	19.6 $\pm$ 1.33
Experimental group 1 (diet with feed additive <i>G. lucidum</i> )	41.5 $\pm$ 4.33	23.4 $\pm$ 1.97
Experimental group 2 (diet with feed supplement <i>L. sulphureus</i> )	38.2 $\pm$ 1.66	15.7 $\pm$ 2.31

Thus, the serum lysozyme content and bactericidal activity in the hens of the experimental groups receiving feed additives based on mycelium were significantly higher than in the control group ( $p < 0.05\%$ ). A morphological study of whole blood revealed that its composition increased the number of macrophages and mast cells.

Thus, the use of a feed additive based on the mycelium of the studied fungi has a positive effect on the immune parameters of the blood of chickens of the parent herd, stimulating the protective functions of the bird's body.

Indicative results were obtained by studying the effect of mycelial supplements on the antioxidant status of birds. In this case, two

indicators were used: the activity of the enzyme glutathione peroxidase and the content of malondialdehyde (MDA) -  $\text{CH}_2(\text{CHO})_2$  - in the blood serum of chickens of the parent herd. It was found that the activity of the glutathione peroxidase enzyme in the chicken serum of the experimental group receiving *G. lucidum* mycelium averaged 1022.0  $\mu\text{mol}$  glutathione/min, whereas in the control serum it was 861.3  $\mu\text{mol}/\text{min}$ . Thus, under the action of dietary supplements, GP activity increased by 18.7%. The feed supplement based on the *L. sulphureus* mycelium did not significantly affect the activity of glutathione peroxidase; the enzyme activity was 965.0  $\mu\text{mol}/\text{min}$  (Figure 2).

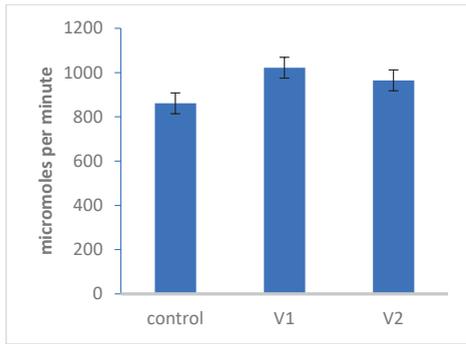


Figure 2. The effect of feed additives based on mycelium on the activity indicators of the glutathione peroxidase enzyme in the blood serum of birds of the parent herd: option 1 - *G. lucidum*, option 2 - *L. sulphureus* ( $p < 0.05$ , error bars - average error)

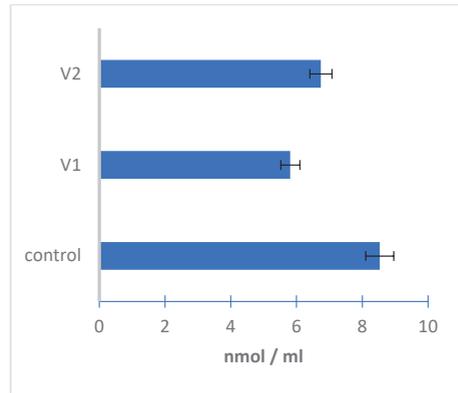


Figure 3. The effect of feed additives based on mycelium on the content of MDA in the blood serum of birds of the parent herd: option 1 - *G. lucidum*, option 2 - *L. sulphureus* ( $p < 0.05$ , error bars - average error)

MDA is formed in the body during the degradation of polyunsaturated fats by reactive forms of oxygen, and serves as a marker of oxidative stress. It was shown that the substance is able to react with DNA, forming DNA adducts, that is, leading to mutations. The concentration of MDA serves as a marker of the degree of endogenous intoxication.

The change in the MDA content in chicken serum under the influence of a feed additive based on *G. lucidum* mycelium in a positive direction is significant and is probably the most valuable of the noted facts of the effect of mycelium on serum biochemical parameters. The effect of using a feed supplement based on *L. sulphureus* mycelium was less pronounced. In the control serum samples, the average MDA content was 8.53 nmol/ml, and under the influence of the additive in the first experimental group it decreased to 5.81 nmol/ml, i.e. 32%, and in the second experimental group to 6.74 nmol/ml, i.e. 21% (Figure 3).

The effect of feed additives on the egg production of chickens of the parent herd was also studied.

The control group ( $n = 25$ ) received the main diet, and crushed mycelium was added to the diet of the hens of the experimental groups.

The experiment was carried out for 41 days, however, the eggs were counted for comparison, starting from the 7<sup>th</sup> day of research, taking into account that the additive had time to act, as well as the adaptation of the birds in the workshop. Statistical analysis allowed us to establish a reliable positive effect of a feed additive based on *G. lucidum* mycelium. Under its influence, the proportion of eggs that went for incubation increased significantly.

If in the control the proportion of rejected eggs reached an average of 14.1%, then in the experiment an average of 9.7%.

On some days in the first experimental group, rejection was not performed at all. The egg production of chickens of the second experimental group receiving a feed supplement based on the *L. sulphureus* mycelium remained at the control level, the results obtained were not significantly different from the control ones (Table 2).

Table 2. Average egg production rates of chickens of the parent herd under the influence of a mycelial dietary supplement ( $n = 25$ ), ( $p > 0.05$ )

Options	Egg production (n=25)		Defect (pcs, %)	Incubation (pcs, %)
	pcs	%		
Control group (normal diet)	20.6±3.2	82.4	3.34±1.05 (16.2)	17.3±1.1 (83.8)
Experimental group 1 (diet with feed additive <i>G. lucidum</i> )	23.1±2.6	92.4	2.21±0.77 (9.6)	20.9±0.8 (90.4)
Experimental group 2 (diet with feed supplement <i>L. sulphur</i> )	21.2±2.4	84.8	2.34±1.05 (11.0)	18.86±1.1 (89.0)

## CONCLUSIONS

The experiments performed have established the positive effect of feed additives based on the mycelium of the fungi *Ganoderma lucidum* and *Laetiporus sulphureus* on the anabolic processes and the immune status of chickens of the parent herd. The growth of indicators of the body's natural resistance has been established. It has been shown that the use of feed additives based on mushroom mycelium eliminates oxidative stress in birds, increases productive indicators and allows to reduce the proportion of rejected eggs, thereby increasing the incubation volume. The data obtained are consistent with the prophylactic potential and biological activity of these types of fungi described in the literature. Thus, the feasibility of using feed additives based on the mycelium *Ganoderma lucidum* and *Laetiporus sulphureus* in poultry practice has been identified.

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