Part 1. Veterinary medicine

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INFLUENCE OF DIFFERENT DOSES OF FEED ADDITIVE BASED ON SILKWORM PUPAE ON CLINICAL INDICATORS OF RAT BLOOD

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Summary. The paper presents data on the effect of different doses of feed additive based on silkworm pupae. A positive correlation between the number of leukocytes and erythrocytes, as well as an increase in the number of agranulocytes compared with granulocytes in the analysis of leukocyte formula, indicate the activation of hematopoiesis, which in turn affects the natural resistance of animals. Under the conditions of the vivarium of the NSC 'IECVM' the experimental part of the work on studying the effect of different doses of feed additive based on silkworm pupae was carried out on male Wistar rats (n = 49) aged four months, weighing 175–190 g. Three groups of rats were formed following the principle of analogs. Rats of the control group received a standard diet with free access to water, rats of group I were fed a standard diet with the addition of the feed additive at a dose of 8.0 ml/kg body weight, group II — 12 ml/kg body weight. On days 21 and 41 of the experiment, blood samples were taken from rats during light chloroform anesthesia. The level of hemoglobin in the blood was determined using reagent kits manufactured by PJSC 'Reagent' (Ukraine), the total number of leukocytes and erythrocytes was performed according to the generally accepted methods of counting in Goryaev's chamber. The calculation of the leukocyte formula of the blood of rats was performed on blood smears. Smears were stained by Romanowsky–Giemsa method. Based on the obtained data, integrated hematological parameters and color index were calculated following the methodological recommendations. There was a positive correlation between the number of leukocytes by 13.4% ($p \leq 0.05$) and erythrocytes by 39.2% ($p \leq 0.05$), as well as an increase in the number of agranulocytes to 50.0% ($p \leq 0.05$) than granulocytes in the analysis of leukocyte formula — this indicates the activation of hematopoiesis, which in turn affects the natural resistance of animals. In addition, the index of immunoreactivity according to Ivanov et al. (2002) in the group II of animals had an increase of 61.4% ($p \leq 0.05$) relative to control. The obtained results allow us to expand the current knowledge about the effect of the drug based on silkworm pupae on the body of animals, in particular on the clinical parameters of the blood. A positive correlation between the number of leukocytes and erythrocytes, as well as an increase in the number of agranulocytes than granulocytes in the analysis of the leukocyte formula, indicates the activation of hematopoiesis, which in turn affects the natural resistance of animals

Keywords: feed additive, clinical indicators, blood, rats

Introduction. Modern medicine uses a wide range of organic drugs for the treatment and prevention of diseases such as pathologies caused by abnormal hemodynamics, they are also used as metabolic activators, antihypoxants, antioxidants, and neuroprotectors. However, today the demand for organic drugs is constantly growing despite the development of traditional medicine. It determines the need to develop and create new drugs. This is evidenced by the rapidity of their implementation (Ohar and Chernykh, 2013; Anon., 2010; Rolik, 2004; Sergienko et al., 2009b).

In this regard, the safety of such drugs deserves special attention. It is known that a quarter of all drugs of biological origin, approved in the United States and Europe, cause side effects, including immune system disorders, post-injection reactions, cancer, etc. (Theurer, 2007).

Our work aimed to study the effect of different doses of feed additive based on silkworm pupae on the clinical parameters of the blood of rats.

Materials and methods. Experimental studies in rats were approved by the Bioethics Commission of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (NSC 'IECVM') and conducted following the recommendations of the 'European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes' (CE, 1986) and Council Directive 86/609/EEC (CEC, 1986), standards of maintenance, care, and feeding.

Under the conditions of the vivarium of the NSC 'IECVM' the experimental part of the work on studying the effect of different doses of feed additive based on silkworm pupae was performed on male Wistar rats (n = 49) aged four months, weighing 175–190 g. By the principle of analogs, three groups of rats were formed.

After keeping experimental animals of all groups on a standard diet for 15 days (equalization period), rats of the control group received a standard diet with free access to water, rats of group I were fed a standard diet with the addition of the feed additive with silkworm pupae at a dose of 8.0 ml/kg body weight, animals of group II — 12 ml/kg body weight.

Every 7 days experimental and control groups of animals were weighed on an empty stomach. On days 21 and 41 of the experiment, four rats were removed from each group, respectively, to take blood samples from the carotid artery under mild chloroform anesthesia.

The level of hemoglobin in the blood of rats was determined using reagent kits manufactured by PJSC 'Reagent' (Ukraine), the total number of leukocytes and erythrocytes was performed according to the generally accepted methods of counting in Goryaev's chamber (Prystupa, 2019). The calculation of the leukocyte formula of the blood of rats was performed on blood smears. Smears were stained by the method of Romanowsky–Giemsa (Prystupa, 2019; Levchenko, 2010). Based on the obtained data, integrated hematological parameters and color index were calculated, according to the methodical recommendations (Horalskyi, Radzykhovskyi and Dyshkant, 2018).

Digital data were processed biometrically using conventional statistical methods using Student's *t*-test and computer programs Statistics 6.0 (StatSoft Inc., USA) and Microsoft Excel 2007.

We used an experimental sample of additive from silkworm pupae which was made by the staff of the Laboratory of Clinical Biochemistry of the NSC 'IECVM' by extraction and filtration and has the following chemical composition: protein — 5.55 g/l, vitamin A (retinol acetate) — 43.2 mg/l, vitamin E (α-tocopherol acetate) — 200 mg/l; microelement composition of the drug: zinc — 1.318 mg/l, copper — 0.781 mg/l, iron — 1.018 mg/l, manganese — 0.382 mg/l, nickel — 0.011 mg/l, strontium — 0.119 mg/l, bromine — 0.019 mg/l.

Results and discussions. Analyzing the clinical data of rat blood (Table 1) on the 21st day of the study, the following changes were noted: decrease in the number of erythrocytes and leukocytes in group I (8 ml/kg) by 17.6% and 13.5% in group II (12 ml/kg) — by 35.7% ($p \le 0.05$) and 9.0%, as well as a decrease in hemoglobin concentration by 19.5% ($p \le 0.05$) and 27.6% ($p \le 0.05$) respectively relative to the control level.

Table 1 — Clinical parameters of rat blood under different doses of the additive with silkworm pupae (M ± m, n = 3–4)

Indicator	Day of experiment	Experimental groups		Control group
		I	II	III
Erythrocytes, × 10^{12}/l	21	7.31 ± 0.89	5.70 ± 0.17*	8.87 ± 0.05
	41	8.21 ± 0.51	8.31 ± 0.51*	7.33 ± 0.98
Hemoglobin, g/l	21	82.12 ± 4.51*	73.80 ± 3.81*	101.98 ± 4.51
	41	108.30 ± 2.41*	105.89 ± 4.81*	98.67 ± 3.61
Leukocytes, × 10^{9}/l	21	10.72 ± 0.96	11.28 ± 0.80	12.40 ± 0.40
	41	16.64 ± 0.53*	13.55 ± 0.53*	11.95 ± 0.75
Color indicator, conventional units	21	0.94 ± 0.11	1.04 ± 0.06	0.92 ± 0.04
	41	1.05 ± 0.09	1.02 ± 0.03	1.11 ± 0.16

Note. * — $p \le 0.05$ relative to control.

It should be noted that on the 41st day of the study in the blood of rats of these experimental groups there was an increase in the number of erythrocytes and leukocytes in group I (8 ml/kg) by 12.0% and 13.4% ($p \le 0.05$) and in group II — by 39.2% ($p \le 0.05$) and 13.4% ($p \le 0.05$), which led to an increase in the concentration of hemoglobin by 9.7% and 7.3% in the blood of rats of groups I and II respectively. At the same time, the calculated hemoglobin saturation index of erythrocytes — a color indicator on the 21st day of the study increases in the blood of group II rats by 13.0%.

It is known that the leukocyte formula is an integral indicator of the balance of all homeostatic systems of the body. Due to the effect of the additive based on silkworm pupae on the body of rats of group I on the 21st day, there was a decrease in the number of rod-shaped neutrophils to 16.6% and eosinophils to 35.7%, an increase in segment nuclear neutrophils by 8.4%. In the blood of group II rats during this period the number of rod-shaped neutrophils increased by 50.0% and lymphocytes by 10.3% ($p \le 0.05$), while the number of segment nuclear neutrophils and eosinophils reduced by 33.7% and 35, 7%, respectively, relative to control (Table 2).

On the 41st day of the study, the leukocyte formula of rat blood changed by groups: in group I, we observed an increase in rod-shaped neutrophils by 11.1% and monocytes by 72.7%, a decrease in segment nuclear neutrophils by 24.0% ($p \le 0.05$) and eosinophils by 30.2% ($p \le 0.05$) relative to control. In the blood of rats in group II — a decrease in rod-shaped neutrophils by 14.8%, segment nuclear neutrophils by 35.0% ($p \le 0.05$), eosinophils by 53.5% ($p \le 0.05$), and monocytes by 30.3%, instead, lymphocytes increased by 16.7% ($p \le 0.05$).

The dynamics of the leukocyte composition of the blood reflects the activity of the cellular immune response with the participation of innate resistance factors and specific immunity both in inflammatory processes and in the action of drugs on the body (Sydorchuk et al., 2015). The most important information is the indicators of integrated leukocyte indices, which are presented in Table 3.

Table 2 — Leukocyte formula of rat blood under the action of different doses of the feed additive with silkworm pupae (M ± m, n = 3–4)

Indicator	Day of experiment	Experimental groups		Control group
		I	II	III
Rod-shaped neutrophils, %	21	1.25 ± 0.25	2.25 ± 0.25	1.50 ± 0.25
	41	3.00 ± 0.66	2.30 ± 0.33	2.70 ± 0.33
Segment nuclear neutrophils, %	21	22.50 ± 1.50	13.75 ± 1.00	20.75 ± 0.75
	41	16.70 ± 1.33*	14.30 ± 1.00*	22.00 ± 0.66
Eosinophils, %	21	2.25 ± 0.25	2.25 ± 0.50	3.50 ± 0.50
	41	3.00 ± 0.00*	2.00 ± 0.66*	4.30 ± 0.33
Monocytes, %	21	3.75 ± 0.50	4.00 ± 0.50	3.75 ± 0.50
	41	5.70 ± 0.66	2.30 ± 0.66	3.30 ± 1.00
Lymphocytes, %	21	70.00 ± 0.75	77.75 ± 0.50*	70.50 ± 1.75
	41	71.70 ± 0.33*	79.00 ± 0.66*	67.70 ± 1.33

Note. * — $p \leq 0.05$ relative to control.

Table 3 — Integral indices of rat blood under the action of different doses of the feed additive with silkworm pupae (M ± m, n = 3–4)

Indicator	Day of experiment	Experimental groups		Control group
		I	II	III
Lymphocyte to monocyte ratio	21	18.66 ± 0.16	19.44 ± 0.15	18.80 ± 0.10
	41	12.58 ± 0.12*	34.35 ± 0.17*	20.51 ± 0.14
Immunoreactivity index by Ivanov et al. (2002)	21	19.27 ± 0.08	20.00 ± 0.10	19.73 ± 0.06
	41	13.10 ± 0.24*	35.22 ± 0.20*	21.82 ± 0.12
Allergization index	21	3.73 ± 0.10	5.51 ± 0.12*	4.44 ± 0.11
	41	4.39 ± 0.09	5.77 ± 0.11*	4.31 ± 0.10

Note. * — $p \leq 0.05$ relative to control.

The ratio of lymphocytes and monocytes reflects the relationship of affector and effector parts of the immunological process (Glushko and Fedorov, 2014). This indicator on the 41st day of the study was reduced by 38.6% ($p \leq 0.05$) in rats of group I, while in group II was reliably higher than control by 67.5%.

Additionally, we determined the index of immunoreactivity by Ivanov et al. (2002), which on the 41st day of the study in rats of the group II was reduced by 40.0% ($p \leq 0.05$), and in group II of animals, in contrast had an increase of 61.4% ($p \leq 0.05$) regarding control. According to the results of our studies on days 21 and 41 of the experiment, the index of allergization increased only in the body of group II rats (additive at a dose of 12 ml/kg body weight) by 24.1% ($p \leq 0.05$) and 33.87% ($p \leq 0.05$), respectively.

The effect of the additive on the body of rats at a dose of 12 ml/kg body weight had an advantage over the effect of this additive at a dose of 8 ml/kg body weight. It consisted of an adaptogenic effect, namely: immunomodulatory effect, manifested by an increase in the ratio of lymphocytes and monocytes, because of the increase in the number of blood lymphocytes, and a high level of allergization index by reducing the number of neutrophils. Note that according to the literature tissue drugs began to disappear from the arsenal of drugs, and recently they are rarely mentioned. The market of drugs for veterinary medicine is represented mainly by foreign synthetic drugs and antibiotics. The additive developed by us on the basis of silkworm pupae and the obtained data on its effect on the body of laboratory animals were consistent with the results of research by many scientists.

Thus, Sergienko et al. (2009a) developed a technology for obtaining a finely dispersed powder based on silkworm caterpillars, the chemical composition of which (per 100 g of powder) contains 53.5 g of protein, 6 g of lipids, vitamins B and A, C, E; enriched with minerals (sodium, potassium, calcium, magnesium, copper, iron, zinc), and contains chitin-melanin complex.

They studied the general condition of the body of rats, the functional state of the basic physiological systems, biochemical and morphological parameters of blood and internal organs. Prolonged administration (28 days) of a dry mixture of caterpillars *per os* provided an increase in hemoglobin, erythrocytes, reticulocytes, hematocrit, eosinophils, osmotic resistance of erythrocytes, and a decrease in the total number of leukocytes.

There was a decrease in glucose levels, a slight increase in total cholesterol and antiatherogenic lipoproteins, decreased urea. The average water consumption of the experimental group of rats was

3.87 times higher than that of animals on the standard diet, feed use decreased by 22.1%.

The body weight of rats receiving a mixture of caterpillars was 34.8% higher than in the comparison group, which received tap water. The indicators of organ indices adequately changed. They noted the stimulation of physical and mental activity of experimental rats in the first week with decrease by the end of the fourth week to the initial level. An experimentally proven pharmacological substantiation of the use of silkworm larvae in a short course as an anabolic and actoprotective agent, without general toxic action, which will allow the use of a mixture of caterpillars for therapeutic and sports nutrition.

It is known from foreign scientific sources (Baimishev et al., 2018; Wang, Wang and Zhang, 2014; Wattanathorn et al., 2012), that drugs based on silkworm pupae are used in Alzheimer's disease to improve the condition of patients with memory impairment and neurodegenerative processes. In experiments on rats, it was proved that the protein hydrolysates from silkworm pupae with a molecular weight of the peptide less than 5,000 Da reduces systolic blood pressure in 1.5 h to 21.5 mm Hg. In physiologically healthy rats, the hydrolyzate did not cause pressure changes. In the study of acute toxicity, it was found that the hydrolyzate is harmless and does not have side effects.

Ukrainian scientists obtained and patented the hydrophilic extract from the pupae of Chinese oak tussar silkworm (*Antheraea pernyi* G.-M.) and studied its effectiveness (Trokoz et al., 1997, 2018). However, studies on the dynamics of hematological and biochemical parameters of the blood of experimental animals had unidirectional character — increase within the physiological norm. Thus it was necessary to study the blood parameters of experimental animals under the influence of different doses of extract from silkworm pupae on laboratory animals.

The dynamics of clinical, hematological, biochemical and productive parameters in pigs under the influence of hydrophilic extract from Chinese oak tussar silkworm pupae was studied.

Animals in the control group were given one subcutaneous injection of saline at a dose of 0.1 ml/kg, and the experimental group — according to the same scheme was injected with native hydrophilic extract from the pupae of Chinese oak tussar silkworm. In all pigs, body temperature, heart rate and respiration were recorded 1, 2, 5 h and 1 and 10 days after injection.

Before injection of the extract, 1 and 10 days after, the number of erythrocytes and leukocytes, the content of hemoglobin, total protein, and its fractions in serum were determined in the blood. The color index of blood, the average content of hemoglobin in one erythrocyte, and protein ratio were calculated.

It has been established that the effect of hydrophilic extract from Chinese oak tussar silkworm pupae on the body of productive animals is to stimulate hematopoiesis (increase in hemoglobin content in the blood), protein synthesis (increase in total serum protein, mainly due to increased content of γ-globulins), which contributes increase weight gain. The introduction of a complex of biologically active substances of the extract does not have a reaction from the cardiovascular and respiratory systems of the body, as well as a pyrogenic effect.

Hydrophilic extract of Chinese oak tussar silkworm pupae, when administered subcutaneously to productive animals, stimulates hematopoiesis (increase in hemoglobin in the blood by increasing its concentration in one erythrocyte at normal values of color), and proteinaceous processes (increase by 7.5% of total protein in blood serum mainly by increasing the content of γ-globulins by 29.6% in 10 days after administration of the extract.

As the additive from silkworm pupae belongs to tissue drugs, the received data were consistent with the results of other researchers in this direction. Thus, Baimishev et al. (2018), by analysis of morphological parameters of cow blood depending on the use of drugs STEMB and Uteromastin, has found that the hemoglobin content in the blood of animals of the first experimental group was 112.42 g/l, which is 2.19 g/l higher than in animals of the second experimental group and 3.86 g/l lower than in animals of the third experimental group ($p < 0.01$).

The number of erythrocytes in the blood of animals of the third experimental group, which used drugs Uteromastin and STEMB in combination, was 6.75×10^{12}/l, which is 0.7×10^{12}/l higher than when using the drug Uteromastin alone, and 0.46×10^{12}/l more than when using the drug STEMB. The number of leukocytes in the blood of animals of the second experimental group on the 15th day after calving is less by 0.17×10^{9}/l than in the blood of animals of the first experimental group, and 0.40×10^{9}/l less than in the blood of animals of the third experimental group.

Analysis of the leukocyte formula showed that, depending on the drugs used, there are significant differences between groups of animals between individual forms of leukocytes. According to the content of basophils and eosinophils, no significant differences between groups of animals were found.

The number of segment nuclear neutrophils in the blood of animals of the third experimental group is 1.27% higher than in the blood of animals of the second experimental group and 0.54% higher than in the blood of animals of the first experimental group. The number of lymphocytes in the blood of animals of the third experimental group is less by 2.07% than in the blood of animals of the second experimental group, and 1.22% less than in the blood of animals of the first experimental group. However, the number of monocytes, important for the function of phagocytosis, in animals of the third experimental group was higher than those in the first and second experimental groups by 0.4% and 0.63%.

Bagdanova (2013) studied the effect of the drug 'Biostim', which was administered subcutaneously to cows of the second experimental group at a dose of 20 ml/cow twice with an interval of 7 days on the

background of the accepted method of treatment of infertile animals (vitaminization with 'Tetravit' at a dose of 5 ml/cow twice, active regimen, rectal massage of the uterus and ovaries). Cows of the first experimental group received a tissue drug in the same dose without the use of vitamins. Control animals did not receive 'Biostim'.

At the end of the experiment, under the influence of the drug 'Biostim', the number of leukocytes in the blood of cows of the first experimental group significantly increased by 1.06×10^9/l and 1.17×10^9/l in the second experimental group, where the tissue drug was used on the background of vitamin fortification. The content of erythrocytes in the blood of cows increased by 0.62×10^{12}/l in the first experimental group and 0.71×10^{12}/l in the second experimental group. The hemoglobin level increased by 11.07 and 11.46 g/l in the first and second experimental groups, respectively, compared with the control.

Tiutiun et al. (2018) studied the effect of the STP drug on pigs. The results of hematological studies in piglets treated with the STP tissue drug before vaccination with the inactivated vaccine 'Hemofilosan' against swine hemophilia indicate its positive effect on animals and more intensive stimulation of the cellular immune system, as the absolute number of lymphocytes increased by 13.7% ($p < 0.05$), compared with their content in animals of the control group.

In experiments on cows using the tissue drug 'Fetoplacentant', an increase in the number of erythrocytes in the blood of experimental animals by 5.2%, hemoglobin content by 3.8%, and a decrease in leukocytes by 19.8% was determined (Hryshchuk, 2013).

Thus, based on our research and the research of other scientists, it can be argued that under the influence of modern tissue drugs, including our preparation based on silkworm pupae, there is an increase in some components of the blood. And this fact may indicate that under the drug activates some biochemical processes without general toxic effects occurs.

Conclusions. Thus, our results allow us to expand modern knowledge about the effect of the feed additive based on silkworm pupae on the body of animals, particularly on the clinical parameters of the blood.

A positive correlation between the number of leukocytes and erythrocytes, as well as an increase in the number of agranulocytes than granulocytes in the analysis of the leukocyte formula, indicates the activation of hematopoiesis, which in turn affects the natural resistance of animals.

The next stage of work will be an in-depth study of the effect of feed additive based on silkworm pupae on the factors of nonspecific humoral immunity of birds.

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