ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Scientific and Practical Aspects of the Application of a Biologically Active Adaptogenic Additive in Poultry Farming.

Elena V. Kuzminova[1], Marina P. Semenenko[1]*, Inna S. Zholobova[2], and Daria V. Antipova[1]

[1]Krasnodar Research Center for Animal Husbandry and Veterinary Medicine, Krasnodar, Russia
[2]Kuban State Agrarian University named after I.T. Trubilin, Krasnodar, Russia

ABSTRACT

The aim of the research was to evaluate the biological activity of the adaptogenic complex additive and the effectiveness of its application in poultry farming. As a result of the conducted studies on the cellular-level test systems, membrane-stabilizing and antioxidant properties were revealed in the complex additive. Its application in broiler chickens contributes to the increase of the poultry adaptive potential, which allows obtaining high rates of their preservation and meat production.

Keywords: humic substances, succinic acid, infusoria *Paramecium caudatum*, broiler chickens, biochemistry, blood.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Currently, poultry farming is the most dynamically developing branch of the agro-industrial complex, which provides production of such valuable food products as eggs and meat. The increase in the efficiency of growing poultry is largely determined by the use of highly effective biologically active complexes that have a stimulating and adaptive effect [8, 10].

These requirements are met by the composite additive, containing humic substances and succinic acid, which was developed in Krasnodar Research Center for Animal Husbandry and Veterinary Medicine.

Humic substances, formed in the biosphere for thousands of years, are an indispensable component that ensures the existence of modern life forms. However, directly in soils humates are presented in inactive form. With a certain technological preparation their functional groups become bioactive and have the opportunity to participate in a variety of chemical and biological processes. The main raw material for the production of humic preparations is peat, some varieties of brown coal, vermicompost, organic waste, etc. The advantage of this raw material is its wide availability, large volume of resource base, low toxicity and production costs. In addition to the above positive qualities, this class of compounds has a pronounced pharmacological activity, showing adaptogenic, antioxidant and immunostimulating properties [1, 3, 6, 9].

Inclusion of succinic acid in the biologically active additive is caused by results of numerous studies that determined its beneficial effect on the organism of animals and poultry due to the activation of metabolic processes, stimulation of immunity, enhancement of preservation and productivity. In addition, succinic acid has adaptogenic ability, improving the supply of tissues with oxygen, removing the state of stress, normalizing the energy and plastic metabolism of the organism. When the load on any of the body systems increases, their adequate functioning is provided mainly by oxidation of succinic acid [2, 5, 11].

The aim of this research was to evaluate the biological activity of the adaptogenic complex additive and its effectiveness in poultry farming.

METHODOLOGY OF RESEARCH

The object of the research is a biologically active additive containing humic substances and succinic acid which composition and physico-chemical properties are presented in Table 1.

The study of the quantitative composition of humates was carried out according to the method of I.V. Tyurin in the modification of Ponomareva and Plotnikova (1968).

The amino acid composition of the biohumate was determined by the method of capillary electrophoresis on the Kapel-105 instrument. The measurement method is based on conducted acid hydrolysis of samples, separation, identification and determination of the mass fraction of amino acids. The components were recorded by their own absorption in the wavelength area of 190-200 nm.

In evaluation of the biological activity of the complex additive, the infusorians *Paramecium caudatum* were used as the test system. The culture of paramecia was in the phase of stationary equilibrium, the working concentration of the cell suspension was 100 cells /ml. In each series of experiments, tests were carried out in parallel in control samples (culture of infusorians with the addition of distilled water in equivalent volumes of studied substances) and test samples that contained solutions of humic substances and succinic acid in various concentrations. The level of changes in the viability of infusorians was visually observed with the help of a microscope on the motor activity of paramecia and the duration of their life (cessation of all types of motor activity). The effectiveness of the additive was estimated as the difference with the control in percent [4].

The toxic effect was modeled by introducing peroxide into the hydrogen paramecia environment, creating a pathological model of damage to the cell membrane by the type of lipid peroxidation. The damaging concentration and volume of the toxicant solution were previously experimentally selected.

ISSN: 0975-8585

Table 1 – Composition and physico-chemical properties of a biologically active additive

Indicator name	Indicator value
Appearance	finely divided powder of brownish-black color with particle size ≤ 2.0 mm
pH of the aqueous solution	8-8.5
Transparency	when diluted 5 times is transparent
Sterility	not sterile
Moisture, %	10-15
Humic substances, g/kg	200.0
Amino acids, g/kg including:	
proline	0.5-0.7
arginine	2-2.2
the sum of glycine, leucine and isoleucine	2.4-2.5
threonine	5-5.2
methionine	4-4.3
cysteine	2.9-3.2
tyrosine	2.5-2.7
aspartic acid	8.6-9.0
Macroelements, g/kg including:	
potassium	1-2.5
magnesium	0.5-2.3
calcium	5.0-7.0
phosphorus	1.5-2.2
zinc	0.28-0.35
manganese	0.8-1.1
cuprum	0.5-2.1
iron	0.2-2.5
succinic acid, g/kg	80.0
Filler - bran	the rest up to 1000 g

Solutions of the studied substances were added to the test samples at various concentrations – 1:100; 1:1000; 1:10000 and 1:100000, for which a series of successive dilutions of the initial 0.1% solutions was carried out. On glass slides 0.05 ml of the medium containing paramecia (in the field of view of at least 5 individuals) was applied, one drop served as a control. To all the others additives were added tangential corresponding volumes of the test substances at an exposure time of 10 minutes. After this time 0.05 ml of the pharmacological indicator – hydrogen peroxide – was added to all drops and further functional and structural changes of paramecia were recorded in comparison with the control.

Evaluation of biological activity of the additives was carried out by observing the increase in tolerance and ability of paramecia cells not to change their functional and structural characteristics when adding a toxicant on the background of a complex of humates and succinic acid.

The effectiveness of the adaptogenic additive in poultry farming was studied on broiler chickens. By the principle of analogues two groups of chickens of ROSS 308 crosses of seven-day age were formed with 50

heads each with an average initial body weight of 136 ± 2.5 g. The conditions of keeping and feeding were the same for all groups and corresponded to the zootechnological requirements of broilers' growing.

According to the scheme of the experiment, the first group of chickens received a composite supplement daily at a dose of 0.01% of the total mass of the feed. The second group was intact, receiving only the feed of the main diet. Feeding of the additive was done in two courses of 10 days with intervals of 10 days, starting from the seven-day age.

Control over the safety and the health of the experimental poultry was carried out by daily clinical observations. The growth rate was determined by the weight characteristics of the body weight of broilers when comparing the weight of the entire number of experimental chickens on the 10th and 30th day of the study.

Adaptogenic action of the additive was studied by determining the biochemical parameters of the poultry blood, which characterize the main metabolisms (protein, carbohydrate, vitamin-mineral) and the intensity of the processes of lipid peroxidation (LPO) when determining serum levels of malondialdehyde (MDA).

Blood for biochemical studies was selected from 10 chickens from each group on the 15th and 30th day of the experimental period. Laboratory studies were carried out on an automated biochemical analyzer Vitalab Flexor with the help of ELITech Clinical Systems kits.

Statistical processing of the results was carried out using special software packages. The study of quantitative characteristics was conducted by comparing the mean values of two sample populations with the definition of the Student's test and the significance level (p).

RESULTS

In evaluation of the biological activity of the additive *in vitro* using *Paramecium caudatum*, it was determined that in the control sample the average lifetime of infusoria in the damaging effect of hydrogen peroxide was 128.7 ± 3.16 seconds. The preventive effect of the additive was confirmed by a significant increase in the resistance of the protozoan to the damaging effect of the indicator solution, with the most pronounced efficiency manifested in concentrations of 0.001% (with an optimum ratio of humic acid and succinic acid components as 2.5:1) with the difference of 28.6% ($P < 0.05$) in relation to control.

Therefore, the primary biopharmaceutical research on the cell level test system – the culture of infusorians *Paramecium caudatum* – was determined that humic substances in combination with succinic acid provide an increase in the stability of paramecia to the action of toxicants, indicating that the additive has adaptative properties (membrane-stabilizing and antioxidant). The ability to change the structural and functional properties of biological membranes under the action of damaging factors can be one of the mechanisms for realizing the pharmacological activity of the biologically active additive.

When determining the effectiveness of the adaptogenic additive in poultry farming, it was found out that the addition of the complex additive consisting of humates and succinic acid in feed diets has a positive effect on the gravimetric parameters of the body weight of broilers (Table 2).

Table 2 – Dynamics of the body weight of broiler chickens when using an adaptogenic additive (M±m; n=50)

Age of poultry / Day of experiment	Body weight, g	
	1 experimental	2 control
7/1	135.9±2.3	136.1±2.8
17/10	643.2±4.7	605.1±3.9
37/30	2189.1±12.6*	2035.7±11.4

Note: * – degree of reliability $P \leq 0.05$ in relation to control

The data presented in the table indicate that by the 10th day of the experiment the body weight of the 1st group of poultry exceeded the control by 6.3%, and after the experiment the significant difference was 7.5%. Wherein, the additive had a positive effect on the safety of broilers, which in experimental poultry was 98% in comparison with 94% in control.

Adaptive potential of the poultry organism is a measure of health, reflecting the functional state of its physiological systems, and, as a consequence, it determines the degree of realization of productive qualities. To study the adaptogenic properties of the additive the biochemical status of the broilers was evaluated according to the number of indicators characterizing the state of the main metabolic processes and the functional activity of the liver cells of the chickens' organism (Table 3).

Table 3 – Influence of the adaptogenic additive on the biochemical parameters of the blood of broiler chickens (M±m; n=10)

Days of the study	1 group – experimental	2 group – control
Total protein, g/l		
15	34.2±0.28*	31.1±0.56
30	36.8±0.95**	32.8±0.73
Urea, mmol/l		
15	3.6±0.17	3.4±0.21
30	3.8±0.23*	3.5±0.34
Glucose, mmol/l		
15	9.5±0.26	8.4±0.15
30	10.1±0.43	9.8±0.34
AST, U/l		
15	155.9±3.81**	173.7±4.29
30	148.8±5.27***	176.8±7.5
ALT, U/l		
15	10.9±0.14	11.3±0.4
30	11.8±0.05*	13.4±0.11
Total calcium, mmol/l		
15	2.6±0.03	2.5±0.07
30	2.7±0.09	2.6±0.02
Phosphorus inorganic, mmol/l		
15	2.3±0.01	2.4±0.03
30	2.4±0.04	2.5±0.02
Vitamin A, µmol/l		
15	0.38±0.08	0.37±0.06
30	0.73±0.09	0.69±0.17
MDA, mmol/l		
15	20.4±0.18*	22.5±0.34
30	19.6±0.44**	23.2±0.52

Note: * P ≤ 0.05; ** P ≤ 0.01; ** P ≤ 0.001 – degree of reliability in relation to control

Factors determining the effectiveness of adaptation of the organism to the action of stress factors include blood proteins which mobility reflects the degree of resource costs of the organism. It occurs due to the fact that blood proteins are closely related to tissue proteins and very sensitive to changes in metabolic processes in poultry organs [7]. In our studies the difference of the increase in the concentration of the total protein by the middle of the experiment was 9.7%, (P ≤ 0.01), and at the end of the experiment it was 12.2% (P≤ 0.001) relative to the control poultry.

On the formation of a positive nitrogen balance in the chickens' organism indicated also the increase of the urea concentration in the blood level on 30th day of the experiment to 3.8 ± 0.23 mmol/l against 3.5 ± 0.34 mmol/l in the control with a difference of 8.6% (P ≤ 0.05).

Optimization of protein metabolism under the influence of the additive is a factor of increasing the adaptive potential and providing the genetic fund of the poultry organism. The realization of these properties occurs due to the composition of the biohumate, which is the source of all essential amino acids in an accessible form. This circumstance makes it possible to include it in animal diets not only as a good protein supplement to feed, but also, taking into account the unique biological functions of amino acids, to use humic substances as the means used to nonspecific stimulation of metabolic processes.

The results of our studies showed that the effect of the additive components on the chicken organism was manifested at the cellular level. The use of catalytic proteins as cell markers of activity of catalytic proteins characterized by different intracellular localization (ALT – in the cytoplasm and AST – in the mitochondria) allows assessing the degree of stability of the cellular structures under the influence of the adaptogen.

Thus, in the blood serum of the first test group the concentration of AST and ALT enzymes was significantly less than in the control broilers, with a difference in AST on the 15th day of 11.4% ($P \leq 0.01$) and on the 30th day – of 18.8% ($P \leq 0.001$). In ALT activity a significant difference with the control was recorded only after the end of the experiment, which was 13.6% ($P \leq 0.05$), that indicates an improvement in the rate of use of free amino acids in the processes of anabolism and catabolism, since AST provides a cycle of tricarboxylic acids and ALT provides gluconeogenesis by amino acid substrates, supporting the energy balance in the body of poultry [7].

When evaluating the vitamin A content in the blood serum of the poultry participating in the experiment, the difference of 5.6% (at the trend level) was determined only at the end of the experiment.

One of the main indicators of the antioxidant effect of the substance is its ability to reduce the level of aldehydes in the body, in particular malondialdehyde (MDA). This indicator as a whole reflects the level of free radical oxidation in the body, so a decrease in its concentration indicates the presence of adaptogenic properties in the studied preparations. It was found out that in the experimental group in comparison with the control poultry the MDA decreased by 10.3% ($P \leq 0.05$) on the 15th day of studies and decreased by 18.4% on the 30th day of studies ($P \leq 0, 01$).

CONCLUSION

As a result of the studies carried out on cell-level test systems, the adaptive properties (membrane stabilizing and antioxidant) of the complex additive containing humic substances and succinic acid were identified. Its application to broiler chickens contributes to the increase in the adaptive potential of the poultry due to the optimization of biochemical processes and the associated functions of organs and systems (protein and vitamin exchanges, stability of cellular structures, lipid peroxidation processes). The course application of the additive to chickens during the whole growing period allows obtaining high safety and meat productivity of the poultry.

REFERENCES

[1] Buzlama AV, Chernov Yu.N. Analysis of pharmacological properties, mechanisms of action and prospects for the use of humic substances in medicine. Experimental and Clinical Pharmacology. 2010; 73(9): 43-48.

[2] Gasanov AS, Shabiev LF. Succinic acid and its derivatives in veterinary medicine and medicine. Uchenye zapiski of Bauman Kazan State Academy of Veterinary Medicine. 2009; 197: 194-197.

[3] Gostishcheva MV. Characteristics of the chemical and biological properties of various fractions of humic acids of peat and sapropel. Materials of the 5th scientific school "Mires and Biosphere". Tomsk: CNTI. 2006; 168-175.

[4] Ivanova NG, Telesmanich NR, Andreeva IN *et al*. Methodical recommendations "*Paramecium caudatum* - as a model *in vitro* for the study of biological activity of substances of different nature". Rostov-on-Don, 2001, 24 p.

[5] Lebedev AF, Shvets OM, Evglevskaya EP *et al*. Scientific and practical development of new immunometabolic preparations on the basis of succinic acid, their clinical effectiveness: amber biostimu-

lant, metal-losuccinate and metal-oxycinate-plus. Kursk: Kursk State Agricultural Academy Publishing House, 2010, 113 p.

[6] Orlov DS. Properties and functions of humic acids. Humic substances in the biosphere: Moscow, 1993, pp. 6-27.

[7] Sereda TI, Derkho MA. Evaluation of the role of aminotransferases in the formation of productivity in laying hens. Agricultural Biology. 2014; 2: 72-77.

[8] Tyapkina EV, Khakhov LA, Semenenko MP, Kuzminova EV *et al*. Rational use of medicinal products in veterinary medicine. Tutorial: Krasnodar, 2014, 57 p.

[9] Nardia S, Pizzeghelloa D, Muscolob A, Vianelloc A. Physiological effects of humic substances on higher plants. Soil Biology & Biochemistry. 2002; 34: 1527-1536.

[10] Semenenko MP, Zabachta NN, Sokolov MN, Kuzminova EV. A study of the pharmacodynamic effects of a complex hepatoprotector on broiler chickens. Journal of Pharmaceutical Sciences and Research (JPSR). 2018; 10(1): 146-147.

[11] Van Immersial F, Bussell B, Flythe MD *et al*. The use of organic acids to combat Salmonella in poultry; a mechanistic explanation of the efficacy. Avian Phathology. 2006; 35(3): 187-188.