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Original scientific paper

DEVELOPMENT OF LITHIUM–CONTAINING FEED ADDITIVE AND ITS USE FOR FORTIFICATION OF CHICKEN BROILERS MEAT AND BY-PRODUCTS

Elena A. Miftahutdinova[1], Sergey L. Tikhonov[2]*, Natal'ya V. Tikhonova[2]

[1] South Ural state agrarian University, Troitsk, Russia

[2] Ural state economic University, Ekaterinburg, Russia

Key words: *feed additive, lithium, broiler chickens, meat, minced meat, fortified food products*

Abstract

The article presents the researches on the development of a feed additive for broiler chickens, including succinic (amber) acid, zinc sulfate, manganese sulfate, copper sulfate, lithium carbonate, L-carnitine, betaine. It was found that the introduction of the developed feed additive to mice food in a dose exceeding 5000 mg / kg per body weight did not affect the general condition of the animals. The experimental results did not allow determining the LD50 of the researched feed additive. The lithium-containing feed additive does not have chronic toxicity, does not cause pathological processes in the internal organs of mice and it belongs to hazard class IV (low-hazard substances). It was found that the introduction of a feed additive in amount of 2,350 g per 1 ton of mixed forage for broiler chickens (the concentration of ionized lithium is 66 mg per 1 kg of feed) allows increasing the content of lithium in cooked meat, minced meat and by-products in comparison with samples of products taken from the experimental group. The lithium content in boiled white meat is 0.418 mg / 100 g, which is 211% higher than in the control sample; 0.452 mg / 100 g in boiled red meat which is 426.4% higher than in the control sample. Eating of 300 g of boiled chicken meat fortified with lithium obtained in the process of broiler chickens cramming, this meat provides the recommended daily dose of the specified trace element. The obtained data allow using the raw meat and by-product for the production of lithium fortified food products.

Introduction

Food fortification with essential elements is a reliable way to prevent nutritional-related diseases. Particular attention is paid to the development of the mass-market food fortified with irreplaceable micronutrients in a biologically accessible form, which is refereed to meat products also.

One of the directions of scientific activity in fortified food technology is the introduction of a biologically active component at the stage of production of food raw materials — i. e. biological fortification when growing crops or cramming/fattening farm animals (in vivo formation of the raw materials composition). The use of mineral zinc-containing fertilizers when growing plants allows increasing the content of this microelement in plants and increases its bioavailability [1,2].

Gyro T. M. and Gorlov I. F. (2018) developed a method for fortifying of mutton by incorporating organic forms of iodine and selenium into the animals forage in the form of feed additives [3].

One of the important trace elements in human nutrition is lithium.

Lithium belongs to the range of essential elements, it prevents stress development, reduces the risks of oncology, prevents the formation of atherosclerotic lesions in blood vessels and is characterized by other pharmacological features. The therapeutic doses of lithium are 70–280 mg per day. The recommended prophylactic dose is up to 5.6 mg of elemental lithium per day or 600 mg of lithium carbonate 3–4 times / day [4]. The maximum daily intake dose for adults is 2.4 g. Lithium is a part of dietary supplements: KAL, Lithium Orotate 5 mg, 60 VegCaps, Solaray, Lithium Aspartate 5 mg, 100 Capsules [5].

In 1985 the Environmental Protection Agency (EPA) of the United States of America proved that the daily intake of Li by the adults weighing 70 kg should be 650–3100 mcg. While monitoring the diets of adult people it was found that in East Germany the average lithium intake is 0.85 mg / day, in the USA — 2.0 mg / day. But in certain regions of the planet the intake of lithium at a dose of 10 mg per day does not cause any negative consequences. It is currently accepted that a vital dose of lithium for an adult weighing 70 kg is 1 mg / day. This dose provides important neurogenesis processes, and also protects neurons from toxic substances and influences the activity of stem cells at the level of nerve tissue and bone marrow [6].

In psychiatry lithium salts are used in high doses — from 600 to 2100 mg per day. In equivalent of ionized lithium this dose ranges between 110 and 400 mg. High doses lithium therapy of pregnant women does not affect the intrauterine development of the fetus; there is also no reason to stop taking lithium-containing pharmacological agents during breastfeeding [7].

The aim of the research is to develop and evaluate the toxicity of the lithium-containing feed additive «Peak

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Antistress» and its use for broiler chickens meat and by-products fortifying.

Materials and methods

The pilot batches of the «Peak Antistress» feed additives were produced in the laboratory of the innovative research center of the South Ural Agricultural University by mixing the components, their grinding and evaluating the stability of the mixture. Before application each experimental batch underwent the solubility test and toxicity assessment in white mice and rats.

The toxicological properties of the «Peak Antistress» feed additive were studied by determining the parameters of its acute and chronic toxicity. Acute toxicity experiments were done on white nonlinear mice weighing 19–22 g in July-August 2018 at a temperature of 14 to 28 °C. Mice were kept indoors in standard cages. Light mode: 14 hours of light, 10 hours of dark. The animals were fed in accordance with generally accepted diets and feeding standards. The selection of the method of introduction of the additive into the body was determined by the physicochemical properties of the biologically active feed additive «Peak Antistress», as well as the methods of introduction of feed additives into the body of animals. The insolubility of the components of the feed additive does not allow using the parenteral routes of the feed additive administration. The dose of the feed additive for the initial administration was selected in the basis of body weight, and in all experiments it was more than 5000 mg per 1 kg of live weight.

The toxicity was studied according to the «Methodological guidelines for determining the toxic properties of drugs used in veterinary medicine and animal husbandry». In this case the most acceptable way to introduce a feed additive into the body of mice is via pills.

Mice were divided into two groups of 10 animals in each. The first group is experimental one, the second is control one. The experimental group was given pills weighing 0.5 g, 1 pill to each mouse. Each pill contained 0.115 g of «Peak Antistress» supplement, and wheat flour was used as filler. The pills were dried by natural ventilation and temperature. The control group of mice used a placebo consisting of wheat flour only. Before the pills administration the mice were not fed for one day.

The chronic toxicity of «Peak Antistress» feed supplement was studied in 30 male mice. The mice were divided into 3 groups of 10 animals each. The first group is control one, the mice of the second group were given «Peak Antistress» at 5 therapeutic doses, which are 5% of the feed weight or equal to 6 grams of «Peak antistress» per whole group, which it its turn is 0.6 grams per head. The third group was given the feed additive in a dose 10 times exceeding the therapeutic dose, or 10% of the feed weight or 12 grams per whole group, which is 1.2 grams per head. The feed additive was given in the form of pills, divided by a multiple of 10 for more uniform eating by mice. The control group received a placebo consisting of pills without the studied feed supplement. The drug was given for 30 days.

Before the experiment and on the 30th day of the experiment the mice were weighed. On the 30th day of the experiment, blood samples were taken and autopsies were made on experimental and control groups mice, their internal organs were weighed.

6 mice from each group were sacrificed by incomplete decapitation.

The mass of the liver, heart, kidneys and spleen was determined using torsion weights. After the autopsy the organs were isolated by removal the associated tissues. Large vessels were cut off from the heart, coagulated blood was removed from the cavities, the liver was weighed without a gall bladder, and the kidneys were weighed without a capsule. To remove blood organs were washed in physiological saline of sodium chloride, and then dried up with filter paper. Having obtained absolute indicators of organ weight we determined the relative mass (weight coefficients), which are expressed as ratio of the organ weight in grams to the body weight of the animal, expressed in kilograms.

Lithium concentration in meat, minced meat and by-products was determined by atomic absorption spectroscopy (AAS). Sample were prepared for AAS determination of metals content by dry mineralization according to the guidelines for atomic absorption methods for the determination of toxic elements content in food products and food raw materials (refer to the standard method of the State Committee of Sanitary and Epidemiological Surveillance of the Russian Federation (GKSEN RF) No. 01–19 / 47–11–92 dated from 25 December 1992). Meat, minced meat, by-products samples were ground, mixed and dried in an oven at a temperature of 100 °C. Meat samples were obtained by grinding the carcass and obtaining minced meat including muscles, skin and bones to obtain data on the average lithium content in the poultry carcass. A 100 g sample of mincemeat was burned in a muffle furnace with step-by-step heating (50 °C every 0.5 hours) up to 500 °C. The ash was moistened with a HNO3 solution in a 1:1 dilution ratio and dried up. The dry residue was diluted with 1N solution of HCl, filtered through a «blue ribbon» filter and the volume of the acid extract was adjusted to 25 ml. The metal content was determined on a flame-absorption spectrophotometer «AAS-1» («Karl Zeiss Jena», Germany). The state standard reference sample of lithium ion was used as calibration solutions. LT-6M lamp from LLC «Technoquant», KORTEK with a wavelength of 670.8 nm was used for determination of lithium content.

Results and discussion

The developed feed additive includes succinic (amber) acid, zinc sulfate, manganese sulfate, copper sulfate, lithium carbonate, L-carnitine, betaine in the following proportions: succinic (amber) acid 37.0–38.0; L-carnitine 5.0–6.0; copper sulfate 2.6–2.8; zinc sulfate 11.5–11.7; manganese sulfate 11.5–11.7; lithium carbonate 16.5–16.7; betaine 14.0–15.0.

It is advisable to consider the effect of biologically active substances that are part of the developed feed additive «Peak Antistress» on the body of poultry.

Succinic acid is a universal intermediate metabolite formed during the interconversion of carbohydrates, proteins and fats, and an antioxidant. It helps to strengthen the central link of intracellular energy — it increases the oxidation of succinic acid and the activity of succinate dehydrogenase of mitochondria respiratory chain; provides significant acceleration of ATP formation and its reducing equivalents, as well as stabilization of the membrane potential of both mitochondrial and cell membranes. Succinic acid compounds are the adaptogenes for hypoxia and intoxication. In poultry farming the succinic acid is recommended to mitigate the effects of various kinds of poultry stresses, it is used as growth and productivity stimulator, as well as an immunity protector [8,9].

The anti-stress activity of certain trace elements and their salts is proven. In particular the efficiency of lithium as anti-stress drug in industrial poultry farming is known [10,11,12,13].

Zinc is part of more than 200 metal enzymes, it influences on cell growth and division, skin and feather condition, osteogenesis, wound healing, reproductive function, immune system, cellular respiration, brain development, behavior, etc. Zinc deficiency causes growth stasis, testis atrophy, decreased egg production, infringement egg shell formation [14].

Manganese in birds activates numerous enzyme processes, influences blood formation, acts as antioxidant, takes part in fat utilization, counteracts liver degeneration, improves the quality of egg shells, improves the condition of embryos, affects the action of vitamins B, E, C and minerals — iron, calcium, phosphorus, improves the functioning of the endocrine glands, helps to maintain reproductive function. It was found that in case of manganese content decrease in a body, the process of ossification increases. Lack of manganese leads to a decrease in insulin synthesis, decrease or loss of reproductive ability, anemia, infringement of bone formation processes, i. e. promotes the development perosis disease. In an adult poultry herd the egg production and hatchability are reduced. Being an integral part of metalloproteins, copper regulates the oxidation-reduction processes in the body. As part of the hormones, copper affects the metabolism, growth and development, the content of vitamin B_{12} and C in the liver, enhances the action of insulin and pituitary hormones. As part of the enzymatic link of the antioxidant system, copper exerts a pronounced effect on processes of lipid peroxidation and the undesirable formation of proteins oxidative modifications [15].

L–Carnitine is a natural compound that takes a key place in animal energy metabolism. Lack of this substance leads mainly to a weakening of the conversion of energy and membrane functions. The main role of L-carnitine in energy production is the delivery of fatty acids from the cell cytoplasm to the inner space of mitochondria, moreover it participates in ketones metabolism of a body, in the regulation of glycogen synthesis and ATP formation, it stimulates the oxidation of acetoacetate, and is involved in ketogenesis and thermoregulation in fatty tissues. The use of carnitine in laying hens feed makes it possible to obtain and increases hens preservation in the productive period by 2.8%, increases egg production rate by 2.5% [16].

Betaine helps maintaining the osmotic balance in the intestines and cells exposed stress, prevents disturbances caused by osmotic shock at high temperatures, and reduces the negative effects of feed stressors and mycotoxins [13].

The components, that make up the feed supplement, complement and synergistically enhance each other's action — the pronounced anti-stress effect of trace elements is observed: lithium, copper, manganese and zinc is enhanced by osmoprotecting betaine and antioxidant activity of succinic acid; succinic acid, L-carnitine, lithium carbonate, zinc sulfate, manganese sulfate and copper sulfate have pronounced effect on metabolism, have adaptogenic properties and allow mitigating the body's costs, which increase significantly along with the development of stress.

The feed additive «Peak Antistress» looks as a white powder with inclusions of tiny grains of different colors– blue, pink. The additive is poorly soluble in water.

The experiment showed that the introduction of the «Peak Antistress» feed additive to mice in a dose exceeding 5000 mg / kg body weight did not affect the general condition of the animals. The experimental results did not allow determining the LD_{50} of the researched feed additive.

Measurements of the live weight of mice before and after the experiment (refer to Table 1) in the experimental group correspond to the indices of the control group and prove the absence of a pronounced toxic effect.

Table 1. Change in live weight of mice before and after the experiment

Group	Experimental group	Control group
Before the experiment, g	21.00 ± 0.22	21.28 ± 0.24
After the experiment, g	21.66 ± 0.25	21.91 ± 0.21
Difference, g	0.66 ± 0.34	0.63 ± 0.32
Difference,%	3.05	2.88
Before the experiment, g	0.10	0.10

While determining the chronic toxicity no animals died. Appearance and behavior were consistent with the norm and did not differ in the mice of the experimental and control groups. Data on changes in live weight of white mice before and after the experiment are shown below in Table 2.

Table 2. Change in live weight of mice before and after the experiment

Group	1 Control group	2 Experimental group	3 Experimental group
Before the experiment, g	21.3 ± 0.59	21.5 ± 0.90	22.0 ± 0.86
After the experiment, g	23.7 ± 1.21	25.1 ± 1.34	24.6 ± 1.23
Difference,%	10.1	16.7	10.6

Intergroup differences before and after the experiment are not pronounced. Comparing after the experiment the parameters of the control group and the first experimental group, it is necessary to note that the difference is statistically significant and is P = 0.029872. When comparing the control group and the third group, the difference is not statistically significant and is P = 0.109371. This indicates a stimulation of live weight gain in mice that received the «Peak Antistress» dietary additive at a dose of 5% of the feed weight, in the group where the «Peak Antistress» was given at a dose of 10% of the feed weight no similar effect was found. Estimating overall body weight indices, it is necessary to note that «Peak Antistress», when administered in large doses for 30 days, did not have a toxic effect, but rather stimulated the live weight gain in mice, possibly due to amino acids and enzymes included in the feed supplement.

Pathological diagnostics revealed that in mice of all groups the larynx, trachea, and esophagus mucous membranes are pale pink, the skin is elastic, the fur is smooth, and subcutaneous tissue is well defined. The stomach contains a small amount of fodder masses; the mucous membrane is of a pale gray color. The mucous membrane of the small intestine is gray-pink, covered with mucus. In the lumen of the colon feces are observed. Internal organs have no signs of pathological changes; have the color, shape, location and texture inherent to healthy organisms.

The difference in the coefficients of the internal organs between the control and experimental animals has no significant differences (Table 3). Therefore, it is possible to talk about the absence of visible damaging effects on the internal organs of white mice.

Table 3. The relative mass of mice internal organs, (mg / 100g body weight)

Organ	Group		
	1 Control group	2 Experimental group	3 Experimental group
Heart	6.56 ± 0.44	6.16 ± 0.31	6.54 ± 0.28
Liver	72.18 ± 2.12	73.26 ± 1.82	71.78 ± 1.16
Kidney	16.62 ± 0.38	17.14 ± 0.28	16.62 ± 0.21

When analyzing the results of research of toxicity of the biologically active feed additive «Peak Antistress» for animals, no symptoms of acute poisoning were found in all experimental animals, there was no lethality. The administration of high doses of a feed supplement is well tolerated by animals. With the introduction of single doses for mice, no changes in the gain in live weight were detected. The Peak Antistress feed additive does not have chronic toxicity to mice and does not cause pathological processes in the internal organs of mice. The results of testing the biologically active feed additive for animals «Peak Antistress» give reason to believe that the toxicity of this feed additive can be classified as unexpressed. The feed additive for animals «Peak Antistress» must be classified according to GOST 12.1.007–76 to hazard class IV (low-hazard substances). The «Peak Antistress» feed additive does not have chronic toxicity and does not cause pathological processes in the internal organs of mice.

The «Peak Antistress» feed additive was used in the maximum tolerated dose of 2.350 g per 1 ton of feed, which corresponds to intake of 25 mg per 1 kg of body weight of lithium carbonate, or to 6–7 mg of lithium ion per 1 kg of body weight per day.

Table 4 presents the content of the ionized form of lithium in the organs and tissues of chickens of the experimental group and control group.

Table 4. The content of lithium ion in cooked meat, minced meat and by-products

Name	The lithium content, mg / 100 g	
	Experimental group	Control group
White meat	0.418	0.198
Red meat	0.452	0.106
Minced meat	0.376	0.106
Skin	0.271	0.125
Heart	0.352	0.106
Liver	0.340	0.132
Head	0.218	0.130

Conclusion

In result of research it was found that the introduction of the «Peak Antistress» feed additive in amount of 2.350 g per 1 ton of feed for broiler chickens (ionized lithium concentration is 66 mg per 1 kg of feed) allows increasing the lithium content in cooked meat, minced meat and by-products in comparison with product samples of the experimental group. Thus the lithium content in boiled white meat is 0.418 mg / 100 g, which is 211% higher than the control sample; the lithium content in boiled red meat is 0.452 mg / 100 g, which is 426.4% higher than the control sample. Similar results were obtained in determining the lithium content in the skin, heart, liver and head of poultry. Therefore when using 300 g of boiled meat fortified with lithium given in the process of broiler chickens cramming, it provides the recommended daily consumption amount of the specified trace element. The obtained data allow using of raw meat and by-products for the production of lithium fortified food products.

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AUTHOR INFORMATION

Elena A. Miftahutdinova- post-graduate student, South Ural state agrarian University,
457100, Chelyabinsk region, Troitsk, Gagarin str., 13, Tel.: +7–951–470–31–80, E-mail: nirugavm@mail.ru
ORCID: https://orcid.org/0000–0002–1999–4063

Sergey L. Tikhonov-doctor of technical Sciences, professor, head of the Department of food engineering, Ural state University of Economics, 620144, Yekaterinburg, 8 March str., 62. Tel.: +7–912–276–98–95, E-mail: tihonov75@bk.ru
ORCID: https://orcid.org/0000–0003–4863–9834
*corresponding author

Natal'ya V. Tikhonova — doctor of technical Sciences, docent, Professor of the Department of food engineering, 620144, Ural state University of Economics, Yekaterinburg, 8 March str., 62. Tel.: +7–912–276–98–95, E-mail: tihonov75@bk.ru
ORCID: https://orcid.org/0000–0001–5841–1791

All authors bear responsibility for the work and presented data.

All authors made an equal contribution to the work.

The authors were equally involved in writing the manuscript and bear the equal responsibility for plagiarism.

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