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CHELATE COMPLEXES OF MALIC OR CITRIC ACIDS WITH IRON, MANGANESE AND ZINC AS A BIOLOGICALLY ACTIVE SUPPLEMENT FOR BROILER CHICKEN DIET

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Abstract. The aim of the present study was to define the effects of the various doses of biologically active supplement, which was consisted of iron, manganese, zinc malates and citrates, on muscle mass formation, blood morphological composition and protein metabolism in broiler chickens during the entire period of their growth. It is shown, that chelate complexes of the life-critical trance elements stimulated muscle mass accumulation, which was associated with more efficient expenditure of amino acids for protein biosynthesis in myocytes. Researched morphological blood parameters were within the normal range for broiler chickens. From the physiological and biochemical point of view, the using of citrate malates is preferable to citrates.
Key words: iron, manganese, zinc malates, citrates, broiler chickens, growth extensity, muscle mass, blood morphological and biochemical parameters.

Introduction.

Bird feeding is often associated with the shortage of many mineral and biologically active substances. Traditionally for the correcting of micronutrient deficiencies their inorganic derivatives are used in the feeding, which are not always sufficiently effective and the bioavailability of them is lower in many cases [1]. Currently conducted research in the field of veterinary pharmacology associated with the study of the influence of various factors on broiler chicken [2, 3, 4]

It is known, if biometal is tightly bound to the chelating agent that is a participant of metabolism, it can ensure targeted absorption of biometals, in that regard the interest is the use of chelate complexes of trace elements with biologically active substances: vitamins, amino acids, organic acids [5, 6].

The main criterion for the development of new drugs is their pharmacological efficacy, so the purpose of the research was the studying of the influence of citrates and malates of biogenic metals on physiological and biochemical parameters of the tissues and organs of broiler chickens in order to justify the optimal conditions for their use to stimulate the productivity of the birds.

The structure of the obtained agents was identified based on the content of the metal by atomic absorption spectroscopy, on the basis of data of infrared spectroscopy, data, H-element analysis.

The studied citrates of manganese, zinc, iron and cobalt are characterized by a strong absorption band at 1558 cm^{-1} and 1560 cm^{-1}, which characterises the complex compounds with a chelate bond.

The assignment of frequencies to one or another group was carried out on the basis of the known literature data [7].

The data of IR-spectra shows that citrates of zinc, iron, manganese, and cobalt, obtained in the given technological conditions practically don't contain free carboxyl groups, i.e., all three carboxyl groups are linked with the ion of the transition metal. The carbonyl groups of acidic residues and the tertiary hydroxyl are also associated diversely. Atom of the transition metal coordinates the donor atoms, where in the formed

complexes have a chelate (cyclic) structure due to coordination of metal ion simultaneously by several donor-active groups of citric acid (ionized carboxyl groups and hydroxyl group).

The major determining factors the composition and the proportion of the dominant forms in the products of the interaction of transition metal ions with citric acid are the pH of the solution at the final stage of synthesis and the ratio of the concentrations of the reacting substances.

Regardless of the composition of the initial samples (in solid state), its dissolution, including in the gastrointestinal tract of animals, causes the conversion the of initial forms in the form which are the most stable at a pH characterising the acidity of the solution.

The high stability of the dominant forms of malate and citrate complexes of the studied metal ions should determine the prolonged effects of drugs on the basis of the study samples [8].

Material and methods.

The experiment was conducted using a total of 175 "Ross-308" broiler chickens at the age interval of 1 to 42 days. Broilers were randomly allotted into seven groups, 25 birds in each.

In the first control group chickens fed the inorganic compounds like iron sulphate, manganese carbonate and zinc oxide (FeSO4, Mn $(CO_3)_2$, ZnO) in the combination fodder. In the feed for the chicken of the second, third and fourth groups the inorganic compounds of the trance elements were replaced by malate; in the fifth, sixth and seventh groups - by citrate metals. Doses are presented in table 1.

Table 1.

Scheme for microelement chelate complexes application in the composition of broiler chicken combination fodder.

Dose, g/ton	Group					
	2	3	4	5	6	7
Malates:						
Fe	19	25	31	–	–	–
Mn	75	100	125	–	–	–
Zn	53	70	88	–	–	–
Citrates:						
Fe	–	–	–	19	25	31
Mn	–	–	–	75	100	125
Zn	–	–	–	53	70	88

Chickens of all experimental groups were in the similar conditions and they were fed by standard mixed feed. The chicken states were assessed by the daily clinical observation. After 42-daily growing time six broilers were chosen from each group and live weight, mass of carcase after killing were determined.

The obtained experimental data were statistically processed with the use of indicators of variation statistics of Microsoft Office Excel 2010. Student's t-test and table values of student's criterion were used to assess the significance of differences. The differences were considered significant at $p \leq 0.05$.

The results of the study and their discussion.

In previously conducted studies, it was found that the complexes of micronutrients increased the average daily weight gain by 2.0-6.0% ($p \leq 0.05$; $p \leq 0.01$) and the preservation by 4.0% [9, 10].

Blood analysis was conducted to evaluate the effects of new supplements on the health of the animals. The data are presented in table 2.

Table 2.

Biochemical parameters of broiler chicken blood, n = 6.

Parameters	Groups						
	1	2	3	4	5	6	7
Hemoglobin, g/l	76,0±3,21	79,9±2,43	82,6±2,96	78,3±3,30	79,4±2,64	79,3±3,12	80,4±2,92
Erythrocytes, 10^{12}/l	2,1±0,04	2,3±0,07	2,3±0,04*	2,3±0,09	2,3±0,06	2,3±0,03	2,3±0,1
Leucocytes, 10^9/l	39,1±3,01	42,4±3,20	41,0±6,33	41,3±3,52	39,6±2,91	38,9±2,94	39,1±3,42
Total protein, g/l	29,9±1,26	33,6±1,51	32,9±1,23	33,1±1,30	31,4±1,34	31,1±1,67	30,4±1,42
Albumin, g/l % to the total protein	17,7±0,91 59,2	19,3±0,90 57,4	18,6±0,93 56,5	17,9±0,90 54,1	14,7±0,71* 46,8	14,3±0,73* 45,9	14,3±0,70* 47,0
Globulins, g/l % to the total protein	12,2±0,3 40,8	14,3±0,51* 42,6	14,4±0,64* 43,5	15,2±0,70* 45,9	16,6±0,71** 53,2	16,8±0,62** 54,1	16,1±0,51** 53,0
Albumin-globulin ratio	1,45	1,35	1,29	1,18	0,89	0,85	0,88

Note: * $p \leq 0,05$; ** $p \leq 0,01$

In the end of the experiment it was indicated a rising trend in hemoglobin contents and red blood cell count by 6,1-7,5% (p>0,05) in all experimental groups. White blood count had a rising trend only in the groups where malates were used and it was in the frame of the physiologically normal state. In these experimental groups the concentration of total protein in blood serum of chickens was increased by 1,8-12,4% (p>0,05). Decreasing of the concentration of albumins and increasing of the proportion of globulins in the blood by 32,3-37,7% (p≤0,01) were observed in the blood of chickens which consumed citrates. The proportion of albumins in the protein had a rising trend in the groups where chickens fed malates.

The data showed that the organic forms of biometals had the positive effect on the morphologic and biochemical blood composition. Malates had the advantage of citrates according to the changes in the proteinogram, albumin-globulin ratio and number of red blood cells.

At the external examination of carcasses was not detected any significant differences among the groups. Carcasses were yellowish colour, the surface of the skin was dry, subcutaneous fat was yellowish tinge. The consistency of the organs was elastic and their cutting edges were not separated. Pectoralis was white with a pinkish tinge and elastic [11]. Broilers from the first (control) group had the lowest body weight. The differences of the control among the experimental groups (except the fourth group) and control were confirmed statistically.

The results of the research confirmed statistically valid positive effect of the biocoordination complexes of trance elements on meat productivity (table 3).

Table 3.

The data of anatomical butchering broiler chicken carcasses of, n=6.

Parameters	Groups						
	1	2	3	4	5	6	7
Body weight, g	1853 ±16,0	1970 ±17,0**	1936 ±17,2*	1905 ± 17,1	1957 ±16,4*	1944 ±16,1*	1935 ±16,5*
Mass of the eviscerated carcasses, g	1335 ±14,5	1419 ±15,9*	1415 ±14,2*	1392 ±14,8*	1377 ±14,5	1401 ±15,4*	1365 ±15,5
Mass of muscles, g	639,0 ±9,14	688,1± 8,5*	692,0± 9,25*	669,2 ±9,00	674,1 ±10,98	676,6 ±8,45	657,3 ±9,20
Mass of bones, g	312,1±5,3	302,0±7,2	306,1±4,2	312,1±9,4	305,8±7,1	306,1±5,5	305,2±2,4

Note: * p≤0,05; ** p≤0,01

It is known that liveweight gain of broliers is happen due to the unique of biosynthesis muscle proteins. We point out that the substitution of inorganic salts of iron, manganese and zinc on their chelate forms promoted the increasing the muscle mass.

Preslaughter weight in the groups was distributed in decreasing order of body weight as follows: second-fifth-sixth-third-seventh-fourth. Body weight of chicken fed malates has changed depending on doses: in the variant with the minimal dose it was higher than in the variant with middle and maximal concentration. Similar dependence was identified in the case with citrates. The given differences are statistically significant.

The study found a positive correlation between the mass of the eviscerated carcasses and preslaughter weight. However there was a little difference in slaughter yield among the control and experimental groups. It demonstrates that experimental trance elements did not disturb the normal relation among somatic and visceral parts of the body.

At the end of the study muscle mass by using malates in diet was higher than in the control group by 45 g. per carcass (on average by 7.1%); when using citrates the parameter was only 4,9%. Mass of bones was changed slightly and it didn't have statistically significant differences.

Conclusion.

Thus, the investigation of whole blood and its serum confirmed not only the safety of the new supplements for the health of birds, but also the positive effect of the new agents on the biochemical functions of the body.

The used metal organic biometal complexes with malic and citric acids stimulated growth of broilers more effectively than their inorganic compounds.

More intensive increase of the body mass of the chickens can be attributed to the fact that trance elements are absorbed in the intestine easily and

quickly from organic compounds and they are transported on the specific enzymes for them, that determine the intensity of metabolic processes in the growing organism.

In previously conducted studies [9] it was showed that the content of the proteins and the proportion of tryptophan in protein were increased, the ratio of this amino acid and oxyprolin is the qualitative indicator of meat.

Designed supplements can be used for animal microelementosis prophylactic and treatment and also for stimulation of the productivity.

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