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(RESEARCH ARTICLE)

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Synthesis and study of arginine-containing iodine chelates

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Abstract

Synthesis conditions have been established and arginine (Arg) containing iodine chelate compounds with the general formula: $I_XArg_Y \cdot mH_2O$ (where x = y = 1 or 2; m=1 ÷3) have been synthesized. The synthesized chelates have been studied using a number of physical and chemical research methods. Composition has been determined by means of trace element analysis, while individuality – via melting temperature measurement and diffractographic method. It has been established via study of qualitative solubility in different solvents that chelates are highly soluble in water and poorly soluble in organic solvents. Using the conductometric method there has been calculated the dissociation constant for solutions containing chelate compounds.

As a results of studies, conducted under production conditions it has been established that addition of chelate form of microelement (iodine) to broiler feed has a positive impact on all zootechnical parameters (live weight, daily weight gain, survival and feed consumption).

Keywords: Arginine; Chelate; Microelement; Broiler; Fee

1. Introduction

Nowadays, population provision with safe, high-quality agricultural products (poultry and animal meet) and improvement of environmental state is one of the relevant problems. Important role in solution of this problem is played by so-called essential microelements. This circumstance can be explained by the fact that microelements are essential for normal growth and development of living organisms. That is why their deficit or abundance in living organisms causes disruption of vital processes and number of pathologies. Their content in living organisms varies within the limits of 10^{-3}–10^{-5} %. As far as microelements perform their functions in chelate form, it is preferrable to enter them in this form. Our scientific team continues with research oriented at creation of essential microelements, their physical-chemical study and their use in poultry feed [1-6], the essential microelement iodine is selected as the subject of research.

Microelement iodine is ranked among the indispensable biogenic elements. In living organisms, more than half of available iodine is contained by thyroid gland. According to the scientists of the World Health Organization, an intellect quotient IQ is directly depended on the iodine content in organism. Iodine deficit causes retardation of physical and mental development and contraction of an endemic disease – goiter. Iodine is one of the basis elements for synthesis of thyroxine and thyroid thyroxine. These hormones are produced by thyroid gland. Thyroxine regulates (adjusts) energy exchange and thermal balance in organism. At the same time thyroxine performs the catalyst's function during energy

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production in cells. If poultry portion doesn't contain selenium, iron, and vitamins in sufficient quantity, animals and poultry always experience iodine deficiency since iodine intake is not managed. Entry of chelate form of iodine into portion (due to high digestibility degree) reduces its dose to be digested and increases iodine content in organism.

Iodine deficiency causes reduction of thyroxin excretion from thyroid gland, which in it turn stimulates hypophysis formation and excretes increased quantity of thyroid gland stimulating hormone (TSH). This increased TSH production causes further enlargement of thyroid gland, which is usually called goiter. Enlarged gland is formed as a result of thyroid gland follicles' hypertrophy and hyperplasia, which increases secretory surface of follicles. Lack of thyroid gland activity or thyroid gland suppression among birds via administration of thiouracil or thiorea causes chickens standing and obesity. It also causes growth of abnormally long lacy floccus. Uptake of thyroxine or iodinated casein by egg-laying birds increases egg production and improves egg shell quality. Iodine content in eggs depends on iodine dose taken by a chicken. The degree of chicken egg-laying and hatching reduces when applying by iodine deficit-related diet. Retarded iodine absorption takes place as well.

Iodine biological role is as follows:

- Stimulates organism growth and development;
- Adjusts tissue growth and differentiation;
- Increases arterial blood pressure and strengthens heart cardiac performance;
- Adjusts (increases) rate of many biochemical reactions
- Adjusts exchange reactions, increases body temperature
- Adjusts protein, fat metabolism and electrolytic exchange
- Adjusts vitamin exchange
- Expands oxygen consumption by tissues [7-10]

Despite advantages related to chelate form, microelement iodine is used in medicine, veterinary and as an additive in premixe composition in non-chelate state – mainly KI, NaI, iodates or organic iodine (iodinated yeasts, milk protein etc.) [11-14]; this circumstance is explained by the fact that iodine is characterized by high affinity to formation of anion-type compounds, that is why receipt of chelate iodine compounds is associated with problems. Amino acids, which are basic structural units of protein molecules in living organisms, are most frequently used as chelating agents during chelate synthesis, therefore they are of especial importance when used in poultry and animal feeding [15-17]. They are nutriceuticals, which correct organism resistance, that is why amino acid-containing chelates may be successfully used not only in premix composition, but also in veterinary as medications. Among amino acids, we have selected basic amino acid arginin as a research subject. It is a nitrogen oxide donor, that promotes blood-vascular system enervation and elasticity. This fact is of great importance for treatment of number of diseases (cardiovascular system, brain, immune and nervous system, atherosclerosis, genital system etc.). According to new investigations, very abundant arginine consumption by immune cells (which protect brain) is a reason of Alzheimer disease contraction [18-22].

Taking these factors into account, our scientific team has established synthesis conditions and has synthesized arginine-containing iodine chelate compounds. Physical-chemical study of synthesized compounds has been conducted and their biological activity has been explored.

2. Method

- Trace element analysis – for establishment of chelate compounds composition;
- Melting temperature determination and X-ray-diffractometric study – in order to establish chelates' individuality;
- Solubility – for study of compounds' qualitative solubility in different solvents;
- Conductometric study – for determination of dissociation constant for solutions containing chelate compounds;
- Weighing method – in order to determine broiler weight gain;
- Count method – for establishment of birds' survival rate;
- Consumed feed accounting method for determination of feed consumption and conversion.

3. Results and Discussion

We have elaborated the method and have synthesized iodine chelate compounds containing arginine (Arg), with iodine and arginine 2:1; 1:2 and 1:1 molar ratio, in minimum water volume in alkaline medium, under conditions of vigorous mixing and heating, through evaporation over the bath. Individuality of synthesized chelates has been established by

means of melting temperature measurement at the melting temperature determining device – melting point /SMP10/. Chelates are easily fusible substances and virtually melt at the room temperature. Qualitative solubility of compounds in different solvents has been determined as well, according to which they are characterized by high solubility in water, and poor solubility in alcohol, acetone and dimethylformamide (Table 1).

In order to determine the dissociation constant of chelate compounds containing arginine a conductometric study on the device pH and Conductivity Sensor LE703 has been conducted. For this purpose, for compounds there have been prepared solutions with molar concentration within the limits from 0.0007 to 0.00002M. Experiment has been conducted in thermostat at 25 °C. Experimental results are given in Table 1. R^2 – regression estimate indicator, which shows how close are the experimental data to the corresponding graph function, it varies within 0,41-0,75 limits. In our opinion, an increased numerical value **of R^2** in case of 2:1 ratio of Arg : I (arginine and iodine), may be explained by formation of stable five-membered cycles around iodine atoms by two mole arginin atoms.

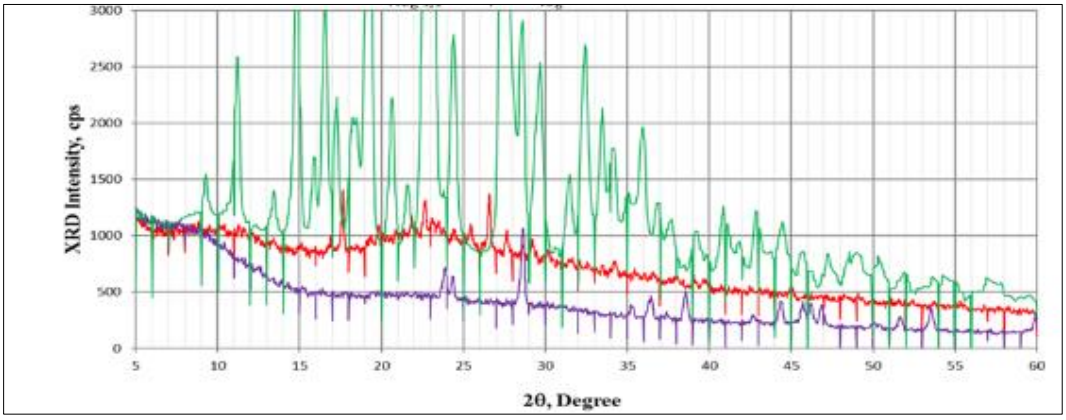
Table 1 Some physical-chemical characteristics of iodine chelate compounds containing arginine

#	The formula of the Compound	Mol Mass	Melting t⁰c	Humidity B (%)	Solubility				Conductometric Survey Results	
					Water	Alcohol	Acetone	Dmf *	R^2	pKa
1	$I_2Arg{\cdot}1{,}5{\cdot}H_2O$	455.5	18	2.49	+	-	-	sl. sol.	0.41	2.97
2	$IArg_2{\cdot}3H_2O$	529.8	22	0.97	+	-	-	sl. sol.	0.75	3.69
3	$IArg{\cdot}H_2O$	319.1	20	0.46	+	-	-	sl. sol.	0.42	3.22

DMFA* - dimethylformamide, + soluble, - insoluble, sl. sol. – slightly soluble

The humidity B has been determined using the analyser AXIS ADGS50. With study of their solubility in different solvents it has been established that all chelates are highly soluble in water, in ethanol and acetone these compounds are virtually insoluble, while in dimethylformamide they are featured by slight solubility.

Except for melting temperature determination, the individuality of the compounds: $IArg{\cdot}H_2O$ (1), $IArg_2{\cdot}3H_2O$ (2) and $I_2Arg{\cdot}1{,}5{\cdot}H_2O$ (3) has been established by the diffractometric method, as well. X-ray-diffractometric study was conducted using ДРОН-4.07 at $Cu_{k\alpha}$(λ=0.154184 nm) irradiation. During exposure, samples were rotated in their own plane by means of special device – ГП-13. Diffractograms of the initial compounds have been recorded for comparison, as well (Fig. 1-3).



■ $IArg{\cdot}H_2O$ ■ J; ■ Arg

Figure 1 Diffractogram of the compound $IArg.H_2O$

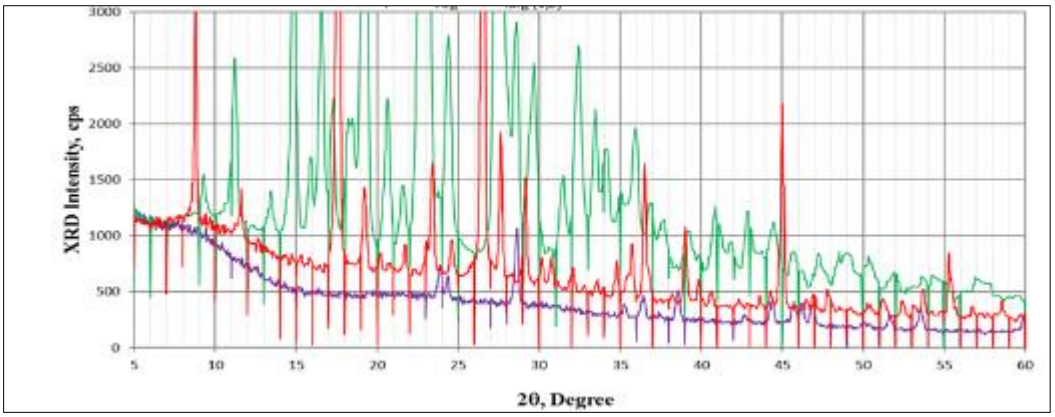


Figure 2 Diffractogram of the compound $IArg_2.3H_2O$

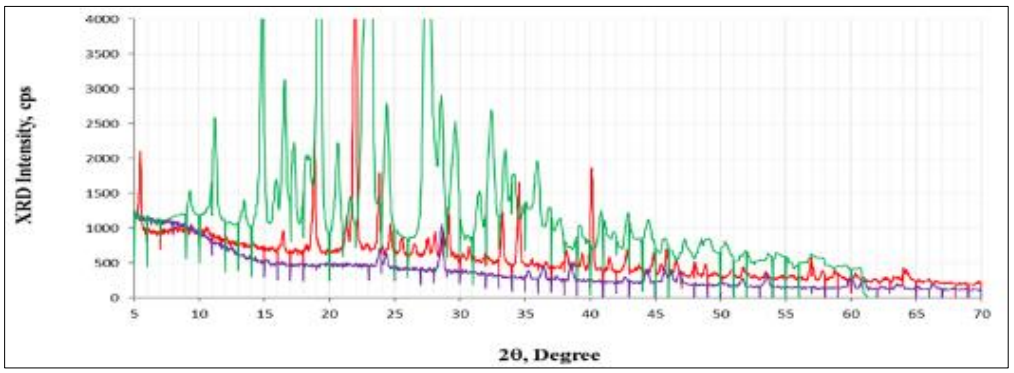


Figure 3 Diffractogram of the compound $I_2Arg.1,5H_2O$

As is seen from the diffractograms' analysis, in all three cases (Fig. 1, 2 and 3) the reacting substances (iodine and arginine), as well as the obtained products are in crystalline state. At that, as far are the diffractogram of each of the obtained chelates differs both from each other and from diffractograms of reacting components and are featured by location and intensity of diffraction maximums, which are characteristic for them. From there, one may conclude that new, individual chelates are obtained in all three cases (Fig. 1, 2 and 3).

3.1. Research results under factory conditions

Table 2 Zootechnical analysis (percentage composition) of pre-prepared combined broiler feed for iodine chelate test

Research setting (%)	Start (0-10 days)	Grower (10-25 days)	Finish (25 dais +)
Raw protein	23.2	21.5	19.7
Raw fat	5.0	5.2	4.9
Humidity	10.6	10.4	10.1
Ash	5.5	5.1	4.2
Raw cellulose	3.1	2.7	3.4
Starch	37	36	39
Sodium	0.18	0.18	0.18
Calcium	1.0	0.75	0.67

In order to establishe the biological activity of iodine chelate compounds containing arginine, we have studied effect of arginine-containing chelate on broiler productivity.

During a test, all technological parameters of breeding, such as illumination, ventilation, stocking density, feeding line, drinking space meet the requirements for cross „ROSS 308". Analysis results for the feed used during a test are obtained by the Near-infrared spectroscopy (NIRS) using FOSS-device.

Table 3 Experimental design

Groups	**Iodine dose per 1 wing (mkg)**
Control	0
I Experimental	300
II Experimental	200
III Experimental	100

Experiment was carried out on the meat-type cross „ROSS-308"in broiler breeding factory located in the Karaleti Village of Gori Municipality. Chelates' effect on growth and development of broilers and meat quality has been studied. Main productivity indices have been recorded and studied within a period of 0-35 days. Broilers have been fed phase-by-phase by combined feed intended for farm enterprise. 200 one-day chickens have been selected for a test and have been divided into four groups 50 birds each. Control (first) group took basis feed portion, while the first, second and third test groups have been fed by the same portion to which an iodine chelate compound has been added. During a test, technological parameters of broilers survival rate were similar for all groups and complied with the requirements for cross „ROSS-308"breeding. Broilers have been fed phase-by-phase: start – 1-10 days, grower – 10-25 days and finish – 25-35 days. Feed portions satisfied the nutritional standards for „ROSS-308".

The goal of the first stage of tests conducted on meat-type cross „ROSS-308"broiler was a study of effect of feed additive iodine chelate on poultry (broiler) productivity – daily and absolute weight gain, survival rate, total feed consumption by 1 bird, feed consumption per 1 kg of weight gain and breeding efficiency (European productivity index).

In total, four groups of cross „ROSS-308" broiler have been tested: control one (which took a combine feed used by the poultry enterprise); I test group (basic full-value combined feed plus iodine chelate compound 300 mkg per bird); II test group (basic full-value combined feed plus iodine chelate compound 200 mkg per bird); III test group (basic full-value combined feed plus iodine chelate compound 100 mkg per bird); main productivity index has been recorded and studied within a period of 0-35 days

Table 4 Broiler live weight dynamics

Groups	**The live weight (g) according to days**			
	1 days	14 days	28 days	35 days
Control	41.6	450.13	1450.06	1960.33
I Experimental	41.6	455.16	1470.39	1980.44
II Experimental	41.5	465.41	1495.14	2070.02
III Experimental	41.5	460.21	1480.46	2030.36

Based on trial results one may state that a weight of one-day chicken subject to test was almost the same in all groups and varied between 41.5-41.6 gr. Results are in close proximity to standard weight of one-day chicken (42 gr). The mentioned fact points at the high homogeneity of chickens tested.

At the age of 14 weeks the highest live weight was recorded among broilers of the second test group – 465.41 gr, to which iodine chelate was added (iodine dose: 200 gr per 1 bird). The mentioned weight is 3.4% higher than that of the

control group (P≥0.01). As for the same-period broilers of the first and third test groups, where chelate dose was 300 and 100 gr per bird, respectively, their weight exceeded the control group by 1.12% and 2.24% (P≥0.05).

At 28-day age, the surplus of live weight of broilers of the second test group over the control one was slightly reduced down to 3.11%. At that, the difference between live weight of the first and third test groups and the same index of the control one comprised 1.4-2.10%.

At the end of breeding (at slaughter age), at 35-day age, the highest live weight 2070.02 gr was recorded in the second test group, which exceeded the control one by 5.60%.

Table 5 Absolute weight dynamics

Groups	Absolute weight dynamics			
	0-14 days	14-28 days	28-35 days	0-35 days
Control	408.53	999.93	510.27	1918.73
I Experimental	413.56	1015.23	510.05	1938.84
IIExperimental	423.91	1029.73	574.88	2028.52
III Experimental	418.71	1020.25	549.9	1988.86

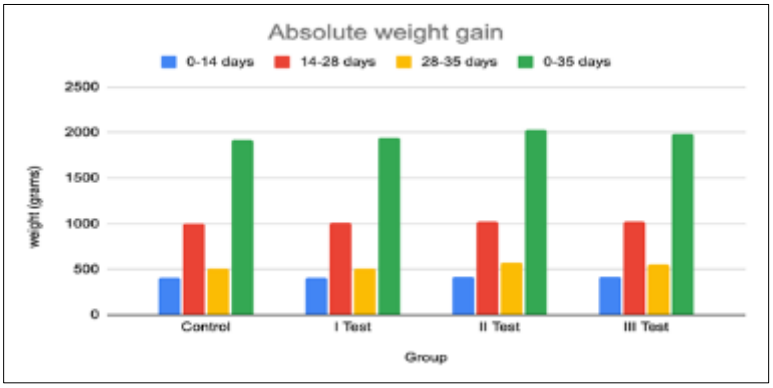


Figure 4 Absolute weight gain

By calculation of the absolute weight gain we established that a weight gain is different at the different breeding periods. The maximal absolute weight gain was observed at 14-28-day age and comprised 999-1030 gr. The highest weight gain for this period was obtained in the second test group and was equal to 1029.73 gr, which exceeded the control one by 2.98%.

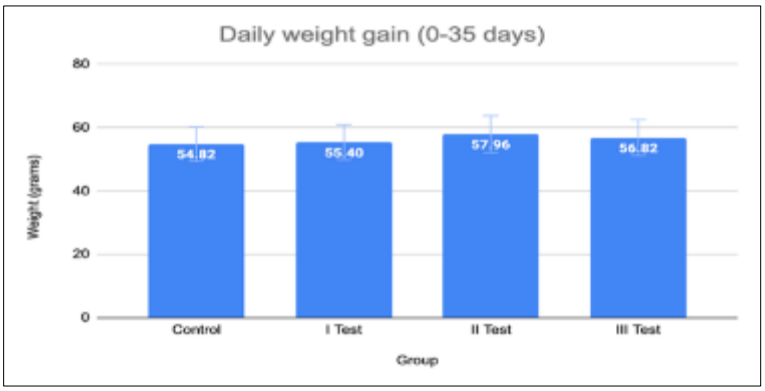


Figure 5 Daily weight gain (0-35 days**)**

The highest weight gain was recorded in the test groups. During a breeding period (0-35 days) daily weight gain varied between 54-58 gr in all groups and was the highest – 57.96 gr in the second group and the smallest in the control one – 54.82 gr.

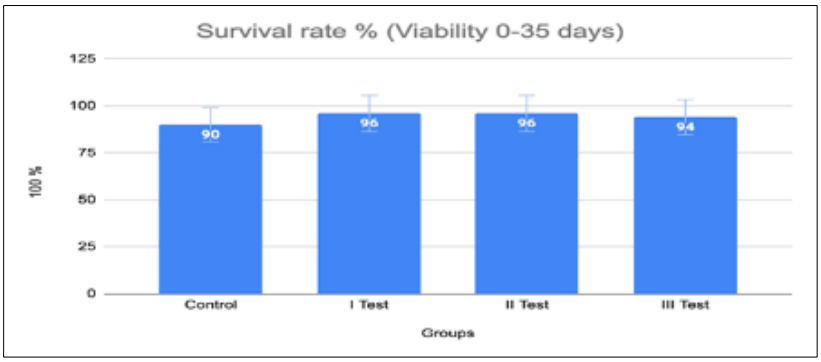


Figure 6 Survival rate % (viability 0-35days)

Broilers' survival rate during the experiment comprised 94-96% in the test groups; survival rate is the highest one in the first and second test groups – 96%, which exceeded the index of the control one by 6%.

3.2. Feed consumption and conversion

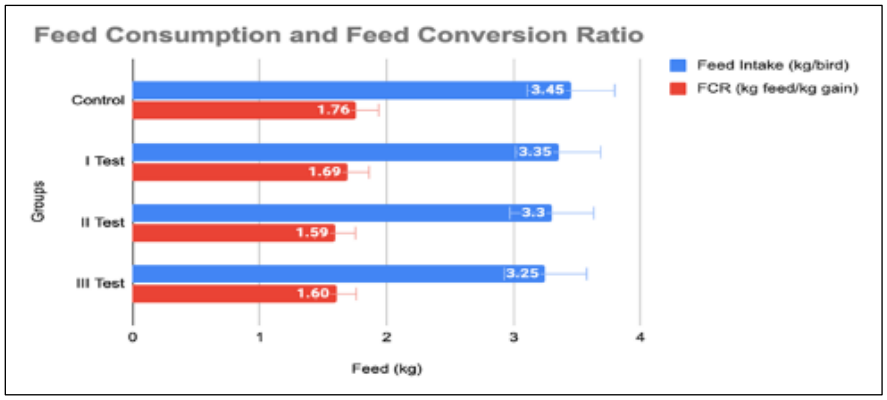


Figure 7 Feed consumption and feed conversion ratio

Feed consumption in the broiler's breeding period comprised 3,25-3,45 kg per 1 bird, and 1,59-1,76 in terms of one kilogram of weight gain. The lowest feed consumption per 1 kg of weight gain was recorded in the second test group – 1,59 kg, which is 9.66% lower than in the control one. The feed consumption per 1 kg of weight gain in the first and third test groups was lower by 3.98-9.09%, as well.

3.3. Broiler breeding efficiency index (European productivity index)

Productivity indices for meat-type poultry have been calculated according to European index formula, given below:

$$\text{European Index} = \frac{\text{Viability (\%)} \times \text{Body Weight (kg)}}{\text{Age (d)} \times \text{FCR (kg feed/kg gain)}} \times 100$$

Table 6 European index results

Indicators	Control	I Experimental	II Experimental	III Experimental
European Index (unit)	286	321	357	341

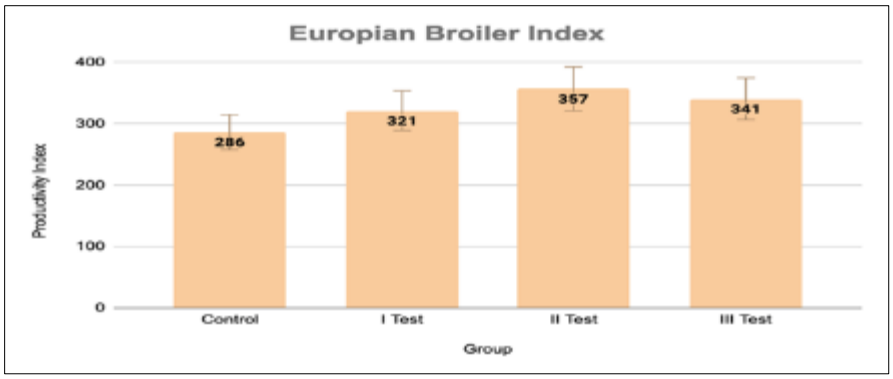


Figure 8 European Productivity Index

Productivity index was the highest one in the second test group and was 71 units higher compared to the control group. Productivity index is 35-55 units higher in the first and third groups, as well.

4. Conclusion

Based on the carried-out studies the following conclusions can be made:

- Synthesis conditions have been established and arginine-containing iodine chelate compounds have been synthesized; chelates are individual substances, which are featured by high water solubility, poor solubility in ethanol and acetone and are insoluble in dimethylformamide. The individuality of synthesized compounds has been established through melting temperature measurement and diffractographic method of study;
- Dissociation constants of compounds have been calculated using conductometric method of study;
- Addition of chelate form of microelement (iodine) to poultry feed has had a positive impact on all zootechnical parameters;
- The live weight of 35-day age broiler has been increased by 1.03-5.60%, and daily weight gain – by 1.06-5.73%, compared to the control one.
- Survival rate in the test groups was 4-6% higher, compared to the control one, and feed consumption has been reduced from 3.98 to 9.66%, in comparison with the control group.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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