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Synthesis and study of iron ascorbates containing amino acids

Amrosi Chkuaseli [1, *], Avtandili Chagelishvili [1], Iamze Beshkenadze [2], Maia Khutsishvili- Maisuradze [1], Nazibrola Klarjeishvili [2] and Tamar Vasini [1]

[1] *Institute of Animal Science, Agricultural University of Georgia, Tbilisi, Georgia.*

[2] *Petre Melikishvili Institute of Physical and Organic Chemistry, Iv. Javakhishvili Tbilisi State University, Tbilisi, Georgia.*

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Abstract

A method was developed and iron ascorbates containing an amino acid (arginine or glutamine) with the general formula $Fe{\cdot}LX{\cdot}Asc_2\ {\cdot}nH_2O$ were synthesized, where L is glutamic acid (gl) or arginine (Arg), X = 1 or 2, and n = 0 or 2. The individuality of the chelates was determined by the diffractometric method. Composition - by analysis of trace elements. The mentioned chelates are solubility in water, and poor solubility in alcohol, acetone and dimethylformamide. Conductometric studies have been conducted, the value of the dissociation constant is quite low and it varies within the pKa range of 3.469-3.764. To determine the biological activity of iron ascorbate containing glutamic acid, the effect of the chelate on broiler productivity was studied. The use of iron chelate in broiler feed improved broiler growth and productivity

Keywords: Ascorbate; Arginine; Glutamic acid; Iron; Productivity index

1. Introduction

Malnutrition (undernutrition), including insufficient supply of microelements is a primary reason of metabolism disorder among agricultural animals and poultry. It is known that microelements in chelate form are featured by high quality of digestibility (high biological permeability), at that, while being in this form, they actively participate in various vitally important processes, that lays a basis for biological processes taking place in cells. At the same time, use of chelate form of microelements reduces the risk of ecological pollution, which predetermines ecological safety of nutritive process. Improvement of population health and environmental conditions is one of the most important challenges facing the modern agriculture. [1-8]

Interest in mixed-ligand chelate compounds has been strongly heightened recently, especially to those chelates, which contain organic ligands with different chemical-physical and biological activity. Among them it worth noticing the compounds simultaneously containing vitamins and amino acids. As the experiments showed, in such cases takes place change of properties of amino acids, vitamins and microelements included in chelates, and they acquire new, biologically active properties, which were not separately peculiar for ligands and microelements being in free state [9,10]. For instance, microelements' toxicity considerably decreases and they acquire the ability to catalyse biochemical processes. Respectively, there is an opportunity of creation of new-generation coenzyme preparations and biocatalysts, new medicinal preparations and biologically active additives. Taking these factors into account, we have selected a microelement iron as the object of our research, while ascorbic acid, and glutamine and arginine (among amino acids) has been taken as chelating agents.

The role of monobasic L-ascorbic acid $C_6H_8O_6$ (Asc) (vitamin C) having strong antioxidant properties lies in protection of biologically active substances from destructive action of free radicals and organism rejuvenation process. It promotes

* Corresponding author: Amrosi Chkuaseli

collagen synthesis, thereby playing an important role in preservation of healthy bones, skin and muscles. Vitamin C is an indispensable vitamin for human body, and plays the role of necessary coenzyme and antioxidant. In addition, in recent times the interest in vitamin C has been substantially increased in regard with cancer prevention and treatment, including treatment of gynecological cancer. Products rich in ascorbic acid and high plasma concentration of ascorbates are inversely proportional to cancer risk. It inhibits hemoglobin glycation, glucose transformation to sorbite. Due to anti-inflammatory properties it strengthens immune system, increases resistance to viruses and malignant bacteria. It participates in free radicals' neutralization and promotes formation of a cheerful mood. Its biological role is predetermined by participation in many reduction-oxidation processes. It promotes intracellular substances' maintenance in colloidal state and normal capillary permeability. In agriculture, chelates prepared on the basis of microelements and ascorbic acid [11-18] are successfully used in poultry feeding.

Glutamic acid is a dibasic amino acid containing nitrogen atoms of α-NH_2 group.

Glutamic acid is a potential energy source, especially for liver and intestines. Glutamic acid is one of the most important amino acids for brain exchange reaction, treatment of gastrointestinal diseases, neurological and mental disorder. It acts the crucial role in immune system functioning, as well. Glutamic acid is a brain fuel, it stimulates mental activity, promotes concentrating, and improves memory; glutamic acid has an unique feature of attaching additional nitrogen, thereby it organizes the synthesis of different proteins (nitrogen transport), or annexes excessive nitrogens (including ammonia), which cause malfunction of different organs (first of all, brain and liver)

Arginine (Arg) while containing amino groups in α-state and guanide groups in δ-state, is a basic amino acid:

Its biological effect – one of the most important discoveries, which implies arginine presence in the role of nitrogen oxide donor – was awarded a Nobel prize in medicine. Nitrogen oxide promotes blood vessel relaxation and elasticity, which is of great importance during anginal attack, it increases blood delivery of heart muscle and brain. It has a positive effect on genital organs, decreases intraocular pressure, reduces inflammatory processes and promotes struggle against ageing, strengthens immunity, accelerates cicatrisation processes, improves reproductive function, it is a preventive mean against atherosclerosis progression through reduction of bad cholesterol level in blood. [19-20]

2. Materials and Methods

Iron acetate, amino acid arginine, and glutamic acid were used in the synthesis of chelating compounds. Ascorbic acid was also used. The reagents were purchased from Sigma-Aldrich.

- Trace element analysis – for determination of chelate compounds' composition
- Melting temperature determination and X-ray diffractometric study – for establishment of chelate individuality
- Solubility – for study of qualitative solubility of compounds in different solvents
- Conductometric study – for determination of dissociation constant of solutions containing chelate compounds
- Weighing method – for determination of broiler's weight gain
- Counting technique – for establishment of poultry survival rate
- Method of consumed feed accounting – for determination of feed consumption and conversion.

3. Results and analysis

We have elaborated the method and synthesized iron ascorbates containing glutamic acid (arginine), in 1:1:2 and 1:2:2 ratio with general formula: $Fe{\cdot}L_X{\cdot}Asc_2{\cdot}nH_2O$, where L is glutamic acid (gl) or arginine (Arg), X = 1 or 2, while n = 0 or 2. Synthesis has been conducted under conditions of vigorous stirring, heating and incubation (exposure) in water bath.

The individuality of synthesized chelates has been established through melting temperature measurement, using the melting point determining device Melting point /SMP10/. Chelates melt within the range of temperatures 92÷105°C; qualitative solubility in different solvents has been determined as well, according to which chelates are featured by high water solubility, and poor solubility in alcohols, acetone and dimethylformamide (Table 1).

Conductometric study using the device pH and Conductivity Sensor LE703 has been conducted in order to determine dissociation constant of iron ascorbates containing amino acid (arginine or glutamic acid). For this purpose, solutions with molar concentration within the limits from 0.0001 to 0.00002M have been prepared for tested compounds. Experiment has been carried-out in the thermostat at 25°C. Experimental results are given in the Table 1.

R^2 is a regression assessment indicator, which shows how close are the experimental data to respective functions of the graph, and it varies within a range of 0.9593-0.8171. As is seen from the Table 1 data, value of dissociation constant is quite small (negligible) and varies within a range of pKa 3.469-3.764, which point at the fact that there is virtually no chelate dissociation.

Table 1 Some physical and chemical characteristics of iron ascorbates containing arginine (glutamic acid)

#	Compound formula	Molar mass, g/mol	Melting point. t ^{0}c	Humidity B (%)	Solubility				Results of conductometric studies	
					Water	Ethanol Ethanol	Acetone	DMSO*	R^2	pKa
1	$Fe{\cdot}gl{\cdot}Asc_2$	553.204	97	2.11	+	-	-	Slig.sol.	0.8922	3.714
2	$Fe{\cdot}gl_2{\cdot}Asc_2{\cdot}2H_2O$	736.374	197	0.52	+	-	-	Slig.sol.	0.9593	3.764
3	$Fe{\cdot}Arg{\cdot}Asc_2$	580.282	92	1.95	+	-	-	Slig.sol.	0.8144	3.469
4	$Fe{\cdot}Arg_2{\cdot}Asc_2{\cdot}2H_2O$	790.514	105	1.24	+	-	-	Slig.sol.	0.8171	3.496

DMSO- Dimethylsulfoxide, + Soluble, - Insoluble, Silig.sol.- Slightly soluble

Humidity B has been determined by the analyser AXIS ADGS50.

Individuality of chelate compounds $Fe{\cdot}gl{\cdot}Asc_2$ (1), $Fe{\cdot}gl_2{\cdot}Asc_2{\cdot}2H_2O$ (2), $FeArg{\cdot}Asc_2$ (3), and $Fe{\cdot}Arg_2{\cdot}Asc_2{\cdot}2H_2O$ (4), in addition to melting temperature determination, has been established via diffractometric method, as well. X-Ray-diffractometric study has been conducted using ДРОН-4.07 at $Cu_{K\alpha}$(λ=0.154184 nm) irradiation. During exposition, samples have been rotated in their eigenplane by means of special device – ГП-13. Diffractograms of the initial reacting compounds (glutamic acid (arginine) and ascorbic acid) have been made for comparison, as well. According to analysis of chelate diffractograms, the obtained compounds are in amorphous state, while the diffractograms of initial reacting compounds are characterized by diffraction maximums and intensities peculiar to them. As for the diffractogram of chelate compounds, obtained in case of chelate (2), it differs from that of initial reacting compounds. All this points at the fact that in all four cases, formation of new individual compounds takes place (Fig. 1-4).

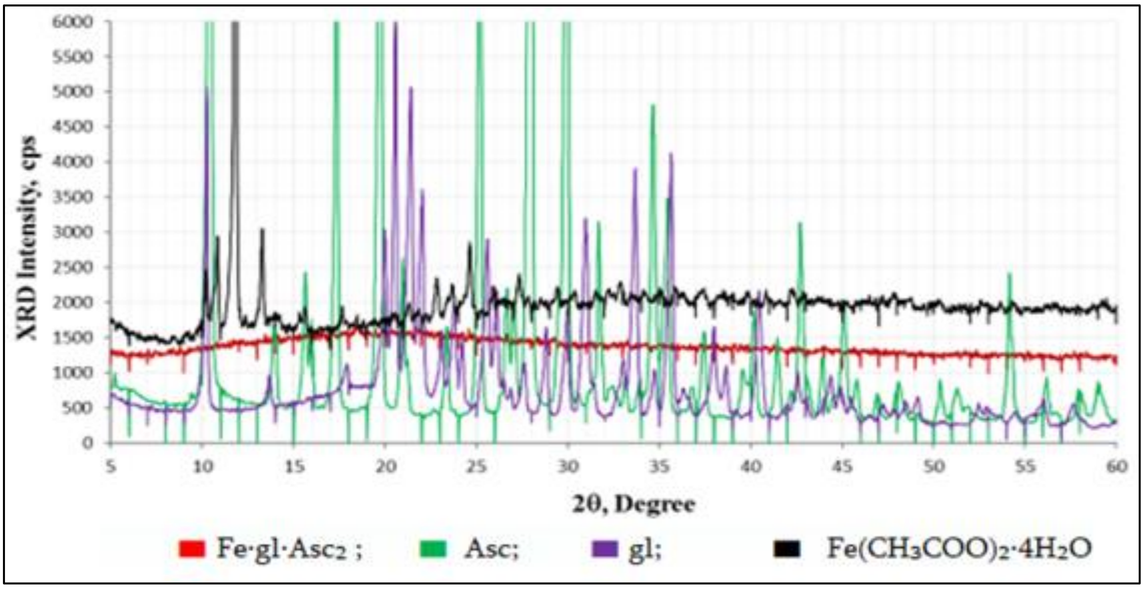

Figure 1 Diffractogram of the compound[Fe.gl.Asc$_2$

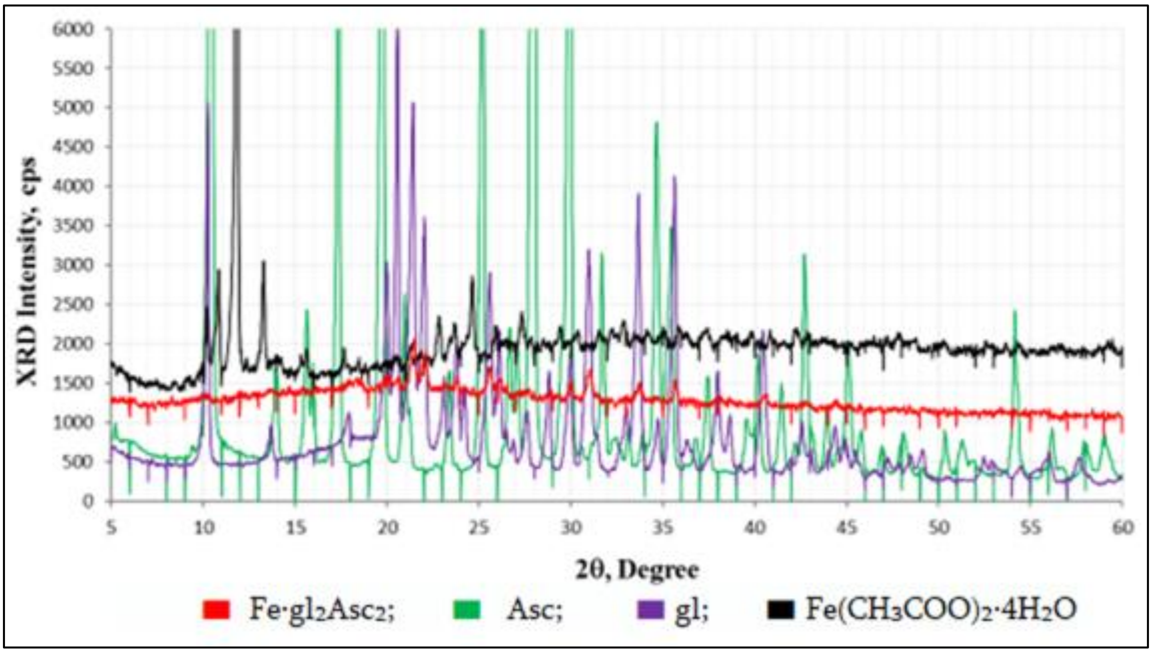

Figure 2 Diffractogram of the compound Fegl$_2$ Asc$_2$

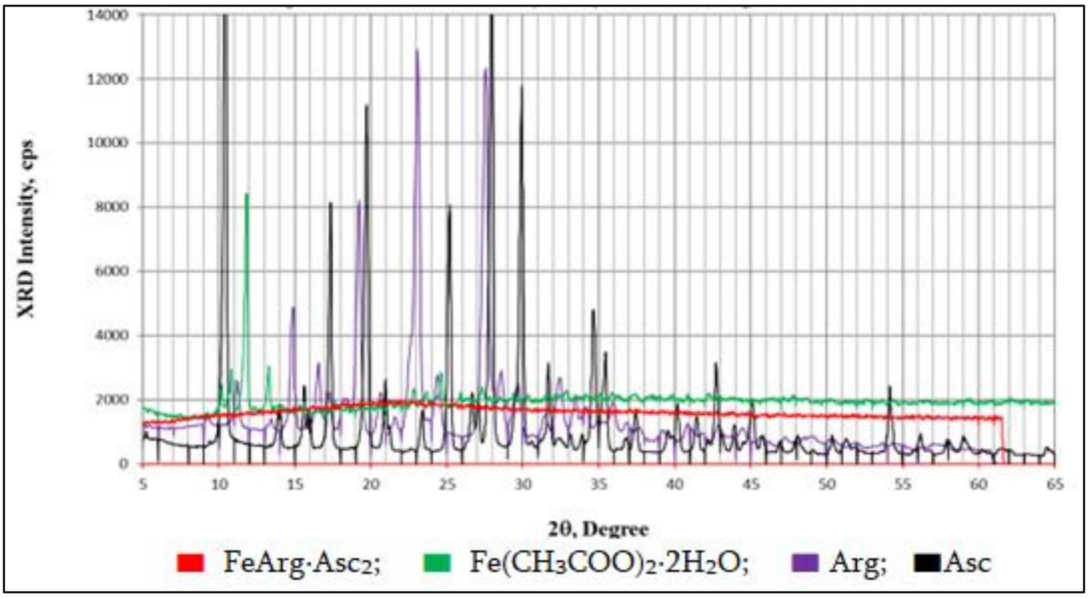

Figure 3 Diffractogram of the compound FeArg. Asc

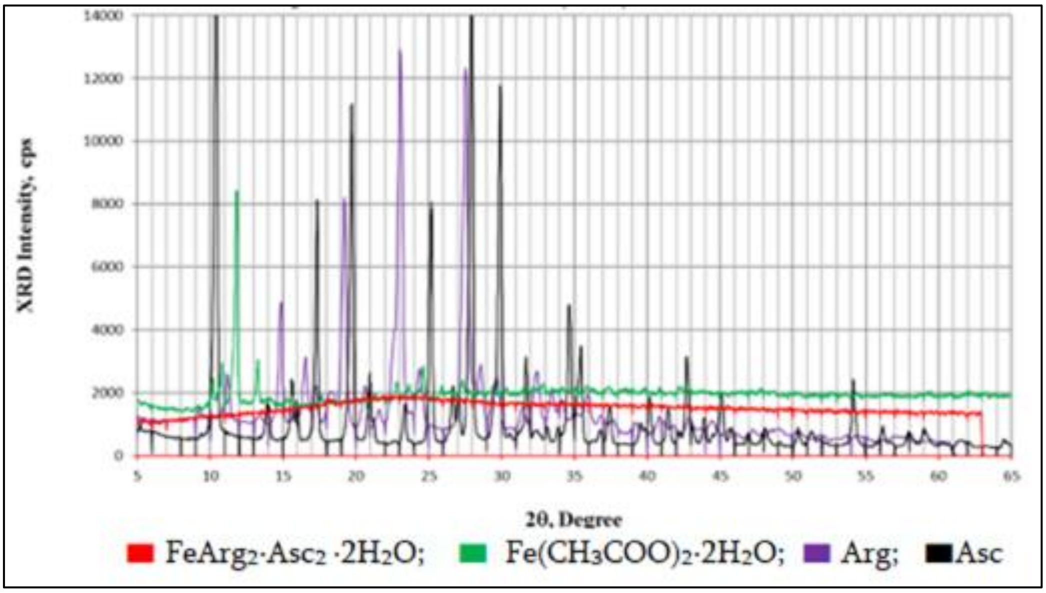

Figure 4 Diffractogram of the compound $FeArg_2Asc_2 .2H_2O$

3.1 Study of biological activity of glutamic acid-containing iron ascorbate under industrial conditions.

In order to study biological activity of iron ascorbate containing glutamic acid, we have studied chelate effect on broiler productivity. Experiment has been conducted according to the following design:

Table 2 Experimental design in case of iron chelate test (on meat-type cross "Ross 308" broiler)

Group	Number of broilers	Growing period (days)	Iron dosage per wing (mg)
Control	50	35	_
I Test	50	35	2
II Test	50	35	4
III Test	50	35	6

Industrial tests of meat-type poultry (broilers) have been conducted on the cross "Ross 308" broiler. The mentioned test set a goal of study of effect of iron chelate compound on poultry (broiler) productivity: daily, weekly and absolute weight gain and survival rate, feed consumption used in total per 1 bird and feed consumption for obtaining 1 kg weight gain, and poultry breeding efficiency (calculation of European index).

Main indices of productivity have been registered and studied for 0-35-day period in this case. Broilers have been fed according to phases (start – from 1st to 10th day, grower – 11-24 day and finish 25-35 day) using full-value combined feed applicable in farm enterprises, which fully corresponds the nutritional standards established for "Ross 308".

200 one-day chickens have been selected for a test according to principle of analogy and have been divided into four groups 50 birds each. In total, four groups of cross "Ross 308" broilers have been used for a test, namely: the control (which took full-value combined feed used by the poultry plant), I-test (with 2g iron chelate compound per chicken, added to the basic full-value combined feed), II-test (with 4g iron chelate compound per chicken, added to the basic full-value combined feed), and III-test (with 6g iron chelate compound per chicken, added to the basic full-value combined feed),

3.2 Iron chelate effect on broiler live weight

Table 3 Broiler live weight gain dynamics (iron chelate)

Group	Age (day)			
	1	14	28	35
Control	40.6 ±1.1	435.44 ±7.4	1412.24 ±22.45	1961.78 ±38.40
I Test	40.5 ±1.0	460.02 ±9.87	1484.17 ±27.40	2050.22 ±22.28
II Test	40.8 ±1.0	475.00 ±15.5	1490.83 ±27.57	2080.30 ±34.14
III Test	40.4 ±1.11	480.10 ±11.58	1505.19 ±29.04	2095.04 ±44.48

Based on test results it may be said that the weight of all one-day chicken taken on trial was almost the same in all groups and varied between 40,4 and 40,8 gr. Registered results were close to a standard weight of one-day chickens (42 gr). The mentioned fact points at high uniformity of tested chickens.

At 14-day age, the highest live weight, 480,10 gr was registered among broilers of the third test group, where iron chelate was added (iron dosage: 6 mg per chicken). The mentioned weight exceeds by 10,26% that of the control group (($P \geq 0.001$)). As for the same-period tested broilers of the first and second test groups, where iron chelate dosage was 2 and 4 mg per chicken, their weight exceeded the control group by 5.64-9.09% ($P \geq 0.01$-0.001). At 28-day age, a positive effect of iron chelate compound on live weight compared to the control one is also observed, the highest live weight is recorded in the third test group and it is 6,58% higher compared to the control one ($P \geq 0.01$), while a live weight of the first and second test groups exceeded that of the control group by 5.09-5.56% (in both cases the difference is significant $P \geq 0.05$).

At the final 35th day of upbringing (slaughter age), the live weight of the second and third test groups comprised 2080-2095 gr, which is 119-134 gr (6-6.8%) higher compared to live weight of the control group, as for the first test group, the broiler's live weight exceeded that of the control group by 4,5% (significant difference $P \geq 0.01$).

As is seen from the Table given above, broilers of the first group were slightly behind that of the second and third groups in terms of live weight.

3.3 Effect of chelate additive on absolute weight gain

From the tables given above, it is clearly seen that during upbringing period (0-35 days) the highest weight gain, both absolute and daily, was registered in the second and third test groups (58,27-58,70).

Table 4 Absolute weight dynamics (iron chelate)

Absolute gain (g)				
Group	**0-14 day**	**14-28 day**	**28-35 day**	**0-35 day**
Control	394,84	976,8	549,54	1921,18
1 Test	419,52	1024,15	566,05	2009,72
2 Test	434,20	1015,83	589,47	2039,5
3 Test	439,70	1025,09	589,85	2054,64

3.4 Effect of iron chelate additive on daily weight gain

Table 5 Daily weight gain (iron chelate)

Daily weight gain (g)				
Group	**0-14 day**	**14-28 day**	**28-35 day**	**0-35 day**
Control	28,20	69,77	78,51	54,89
1 Test	29,97	73,15	80,86	57,42
2 Test	31,01	72,56	84,21	58,27
3 Test	31,41	73,22	84,26	58,70

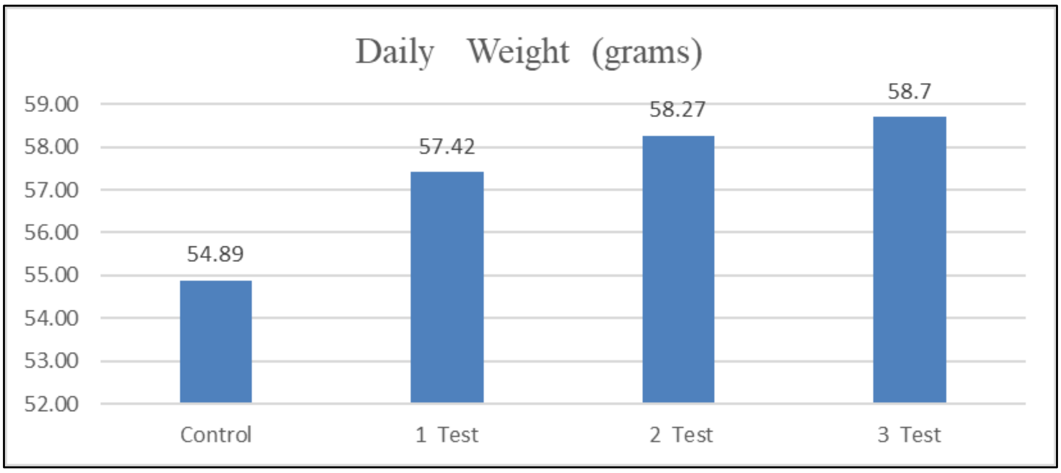

Figure 5 Daily weight gain (iron chelate)

3.5 Effect of iron chelate additive on broiler survival rate

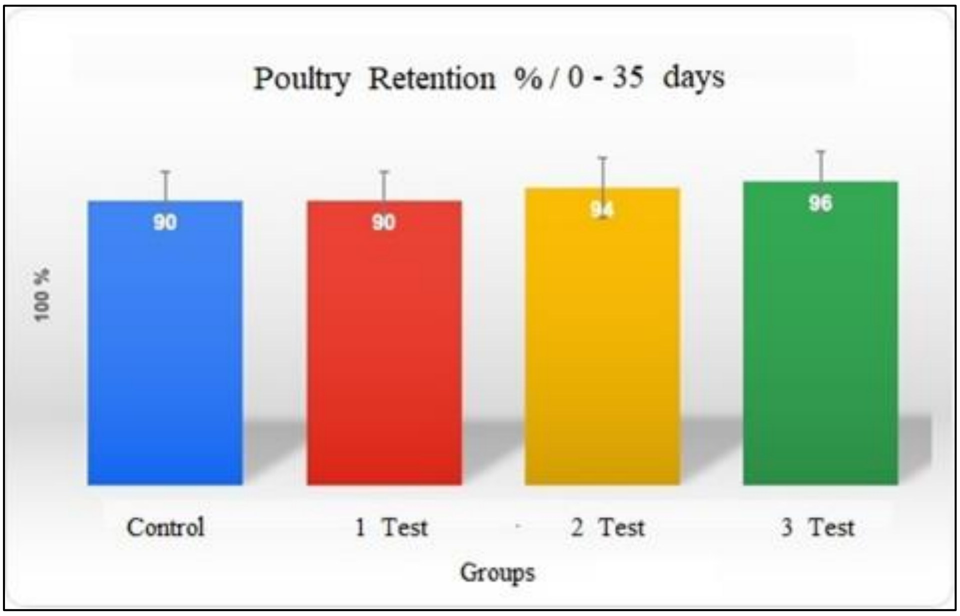

Figure 6 Poultry survival rate % (iron chelate)

The highest survival rate during upbringing period – 94-96,0%, was registered in the second and third test groups, while in the control one it was equal to 90%, which is at least 4% and at the most 6% lower compared to the second and third test groups. No significant mortality has been recorded during any week of poultry upbringing.

3.6 Broiler upbringing efficiency (European productivity index)

Meat-type poultry productivity indicators have been calculated according to the European index formula given below:

$$\textbf{European index} = \frac{\textbf{Survival rate } (\%) \times \textbf{ live weight } (\textbf{kg})}{\textbf{Poultry slaughter age } (\textbf{day}) \times \textbf{ conversion}} \times \textbf{100}$$

Obtained results are united in the Table given below.

Table 6 Productivity index for iron chelate test:

Indicator	Control	I Test	II Test	III Test
European index (Unit)	253	293	323	355

Productivity indicators are shown in the .

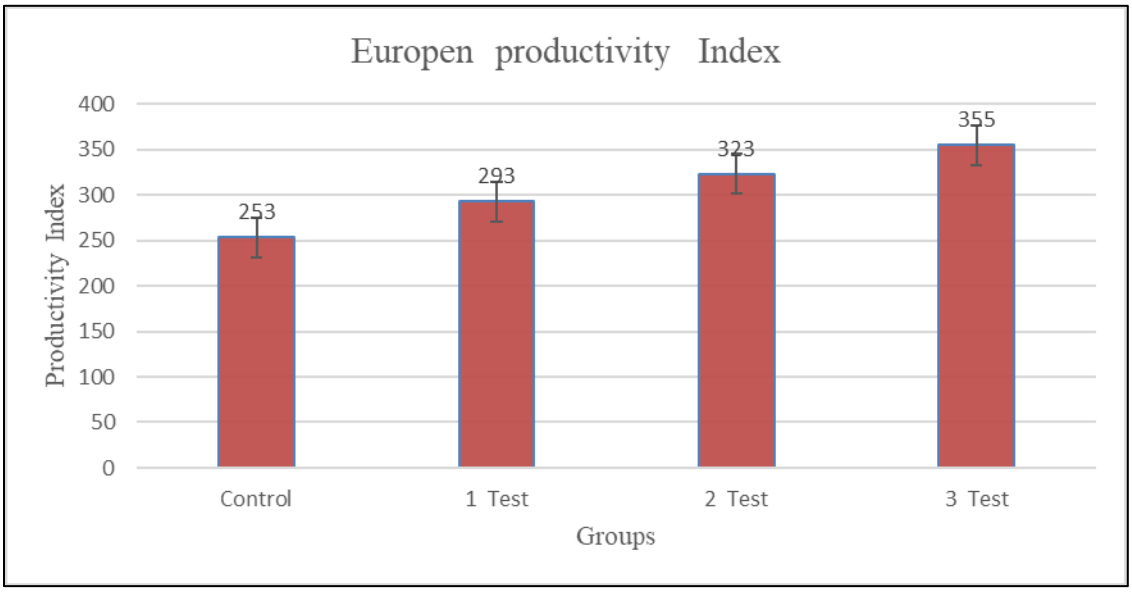

Figure 7 European productivity index (iron chelate)

Productivity index was the highest in the third test group – 355 units, which is 102 units higher compared to the control one. Productivity index is also higher by 40-70 units in the first and second groups, compared to the control one.

As is seen from the Diagram, despite the fact that broiler meat production was effective (profitable) in all four groups, the European index of broiler's upbringing efficiency was nonetheless highest in the second and third groups – 323-365 units, which is mainly explained by the use of iron chelate form.

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

- Synthesis conditions have been established and chelate compounds of iron ascorbates containing arginine (glutamic acid) have been synthesized;
- Chelates are the individual compounds featured by high solubility in water, and poor solubility in alcohol, acetone and dimethylformamide;
- According to conductometric study of chelates, their dissociation constants vary within a range of 3.469-3.764, which points at the fact that they are quite poor electrolytes;

- It has been established through melting temperature measurement and diffractographic study method that they are the individual compounds;
- Application of chelate form of iron in broiler feed showed that during upbringing period (35 days), addition of 6 mg iron chelate per each chicken to full-value combined feed has improved broiler growth-development and productivity;
- At the end of upbringing, at the 35-day age, iron chelate addition has increased broiler's live weight in test groups by 4.51-6.79%, compared to the control one;
- During upbringing period (0-35 days), inclusion of 6 mg of iron chelate used in feed of broilers of the 2nd and 3rd test groups has increased daily weight gain by 6,16-6,94% compared to broilers of the control one;
- Insertion of 6 mg of iron chelate to the test group broiler's feed has reduced feed consumption by 9.5-18.6% per 1 kg of weight gain, compared to broilers of the control one;
- The European index is the highest in the second and third groups and equals to 323-365 units, which is mainly explained by the use of iron chelate form.

4. Conclusion

A method was developed and iron-containing chelates were synthesized. The physico-chemical properties were studied. In order to determine the biological activity of synthesized chelates, the effect of chelate on broiler productivity was studied. The use of iron chelate in broiler feed has been shown to improve broiler growth and development and increase the European Productivity Index. It is known that microelements in chelate form are featured by high quality of digestibility (high biological permeability), reduces the risk of ecological pollution. Considering these factors, chelates have great scientific and practical importance

Compliance with ethical standards

Disclosure of conflict of interest

None of the authors of this work has a conflict of interest.

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