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Synthesis and study of Arginin-containing iron and cobalt chelates

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Abstract

Synthesis conditions have been established and arginin-containing iron and cobalt chelate compounds with general formula: $Me(Arg)n(CH_3COO)_2 \cdot mH_2O$, where Me = Fe, Co; Arg – neutral arginin molecule, n = 1÷3; m = 1÷4 have been synthesized. Synthesized chelates have been studied using a number of physical and chemical methods. In particular, the content has been established via trace element analysis, while individuality has been identified using melting temperature measurement and diffractographic method. According to study of qualitative solubility chelates are well soluble in water, though they are poorly soluble in organic solvents. Using the conductometric method there has been calculated the dissociation constant of chelate compound containing solutions.

On the basis of preliminary trial tests conducted with a purpose of biological activity study, there has been expressed an opinion that entry of arginin-containing iron and cobalt chelates into composition of broilers' combined feed premixes has had a positive impact on poultry live weight gain and poultry survival rate. An opinion on the reasonability of basic tests conduction has been given in order to determine the doses of arginin-containing iron and cobalt chelate compounds optimal for broilers.

Keywords: Iron; Cobalt; Chelate; Arginin; Broiler; Premix; Combined Feed

1. Introduction

Manufacture of ecologically safe, high-quality food products (mainly, poultry and animal meet) is one of the most topical problems. Important role in this problem settlement is attached to essential microelements that is explained by the fact that microelements have diverse functions in the living organism, in particular they:

- Participate in the supporting tissue formation
- Provide internal environment homeostasis preservation
- Provide cell membrane preservation
- Secure biochemical reactions activation acting on enzyme system
- Have a direct and indirect impact on endocrine gland function
- Act on the gastrointestinal tract symbiotic microflora
- Form the part of cell genetic apparatus and biologically active compounds.

Strong influence of microelements on physiological processes is caused by the fact that they form part of enzymes, coenzymes and hormones, participate in vital processes regulation. Thus, metabolism is relied on concerted separate

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biochemical reactions, which are caused by catalytic action of microelement-containing enzymes, coenzymes and hormones.

Still a pair decade ago microelements were used in inorganic form in poultry premixes. However, salts being in inorganic form are characterized by high toxicity, low digestibility and low indices of efficiency that is caused by formation in the animal and poultry gastrointestinal tract of poorly soluble and not-easily assimilable compounds. On the contrary, microelements being in chelate form have very low toxicity, high digestibility and, as a consequence, increased efficiency degree when used in small doses that, in its turn provides environmental safety of microelements' use in this form. This fact is evidenced by the results of studies carried out both by foreign scientists [1-16], and by our multi-year explorations [17-27]. Exactly this advantage explains the scaling-up of manufacturing the premixes containing microelements being in chelate form compared to non-chelate ones and this trend has permanently increasing character.

Among microelements we selected iron and cobalt as a research subject. The role of iron in living organisms were established as far back as in the XVIII century. Main function of iron in organism lies in oxygen transfer and participation in oxidation processes (by means of dozen iron-containing enzymes). Iron enters into composition of hemoglobin, myoglobin and cytochromes. In organism, the major part of iron is presented in the composition of erythrocytes and brain cells. Iron plays very important role in energy release processes, enzymatic reactions, immune function maintenance, cholesterol metabolism. Cells and tissues are saturated with iron by means of protein transferrin, which has an ability of trivalent iron ions transfer.

Cobalt is an indispensable element both for humans and animals. Blood formation process in humans and animals can be implemented only in case of proper interaction of three bioelements – cobalt, copper and iron. Cobalt entry into bone tissue increases formation of young erythrocytes (normocytes) and hemoglobin. Vitamin B_{12} in addition to its impact on blood coagulation process, has a quite high effect on metabolism, first of all, on protein synthesis, and has an ability of – S – S groups' restoration, which participate in toxic elements disposal and blocking processes.

Among organic substances, amino acids are most commonly used as chelating agents, since they perform one of the most important functions in living organisms. Taking these factors into account, we have selected arginin as an organic ligand. It is a basic amino acid which transforms into nitrogen oxide in living organisms and promotes blood vascular system elasticity that is so important during angina attack, substantially increases blood flow to the brain and heart muscles, has a positive impact on genital system. Scientific investigations have confirmed the beneficial effect of nitrogen oxide on arterial blood pressure adjustment, immunity and central nervous system performance [28-32].

Thus, based on the above cited, we have set a goal of synthesis, physical-and-chemical study of arginin-containing iron and cobalt chelate compounds and their biological activity.

2. Computational Method

- Trace element analysis – for establishment of chelate compounds composition;
- Melting temperature determination – for identification of chelate individuality;
- Solubility – for study of qualitative solubility of chelate compounds in different solvents;
- Conductometric study – for determination of dissociation constant of solutions containing chelate compounds;
- Weighing method – for determination of poultry weight gain;
- Count method – for establishment of poultry survival rate.

3. Results and Discussion

In order to synthesize arginin (Arg) containing cobalt chelates $Co(Arg)_n(CH_3COO)_2{\cdot}2H_2O$, where n = 1÷3; they have taken cobalt acetate $Co(CH_3COO)_2{\cdot}4H_2O$ and arginin mixtures at a molar ratio of 1:1; 1:2 or 1:3, then dissolved them in minimal volume of water under conditions of warming and vigorous mixing. Obtained true solutions for concentration purposes have been exposed at water steam bath, formed compounds have been treated several times by water. Finally, the obtained chelates have been washed up with small quantity of water, ether and dried at room temperature.

Hydrolysis, oxidation of chelates and formation of iron trivalent hydroxide takes place during Fe chelates receipt via the same method during multiple water treatment. That is why, for obtaining iron chelates with general formula: $[Fe(Arg)_n](CH_3COO)_2{\cdot}mH_2O$, where n = 1÷3; three sample weights of iron acetate $Fe(CH_3COO)_2{\cdot}4H_2O$ have been taken, then dissolved in solution acidified by acetic acid (solution pH = 3.0-4.0), and arginin has been added at a molar ratio of

1:1, 1:2 and 1:3 under conditions of vigorous mixing and heating. Solutions have been filtered and boiled out at the bath. Obtained chelates without further water treatment have been washed by water and ether.

The individuality of synthesized chelates has been identified through melting temperature measurement at the melting point /SMP10/ device. Qualitative solubility of compounds in different solvents has been also established, according to which they are characterized with high solubility in water, and poor solubility in alcohol, acetone and dimethylformamide (Table 1).

Table 1 Some physical characteristics of chelate compounds

#	Formula Compound	Molar mass g/mol.	Melting t^0c	Solubility				Conductometric study results	
				water	ethanol	acetone	ДМФА*	R^2	pKa
1	$[Fe (Arg)](CH_3COO)_2{\cdot}H_2O$	348.49	143	+	slig. sol.	slig. sol.	+t	0.89	1.91
2	$[Fe (Arg)_2](CH_3COO)_2{\cdot}4H_2O$	576.74	125	+	slig. sol.	slig. sol.	+t	0.87	1.41
3	$[Fe(Arg)_3](CH_3COO)_2{\cdot}3H_2O$	817.44	135	+	slig. sol.	slig. sol.	slig. sol.	0.88	4.40
4	$[Co(Arg)](CH_3COO)_2{\cdot}2H_2O$	387.41	131	+	-	-	-	0.94	1.26
5	$[Co(Arg)_2](CH_3COO)_2{\cdot}2H_2O$	561.89	122	+	-	-	-	0.87	1.28
6	$[Co(Arg)_3](CH_3COO)_2{\cdot}H_2O$	736.37	110	+	-	-	-	0.81	1.40

DMFA* - dimethylformamide, R^2 regression evaluation indicator,+ soluble,- insoluble, slig. sol.- slightly soluble, +t- t soluble by heating,

In order to determine the dissociation constant of arginin-containing chelate compounds there has been conducted a conductometric study using the pH and Conductivity Sensor LE703 device. For this purpose, there have been prepared solutions of these compounds with concentration in the range from 0.025N to 0.0006503N. Experiment has been conducted in the thermostat at 25°C. Experimental results are given in Table 1.

R^2 – regression assessment indicator, which shows how close are the experimental data to the function corresponding to graph; it is quite high and varies within the limits of 0,94-0,81. Dissociation constants of initial salts have been measured under the same conditions, in particular pKa $[Fe(CH_3COO)_2{\cdot}4H_2O]$ = 1.28 and pKa$[Co(CH_3COO)_2{\cdot}4H_2O]$ = 1.33. As is seen from the Table, dissociation constants of chelates and its initial salts are of the same order (withing same limits). Based on this fact, one may presume that under conditions of the carried-out experiment chelate dissociation proceeds with detachment of the second (external) sphere (acetate-ions) only, while there is no dissociation in the first (internal) sphere. It may be explained by formation of stable heterocyclic five-membered cycles between metal and organic ligand, with Me-Arg bond strength.

In addition to melting temperature determination, the individuality of the compounds: $[Fe(Arg)](CH_3COO)_2{\cdot}H_2O$(1), $[Fe(Arg)_2](CH_3COO)_24H_2O$(2), $[Co(Arg)](CH_3COO)_2{\cdot}2H_2O$ (3) has been also identified via diffractographic method. X-ray-diffractographic study has been conducted using ДРОН-4.07 at $Cu_{k\alpha}$(λ=0.154184 nm) irradiation. During exposition, samples were rotated in their own plane by means of special device – ГП-13. Initial compounds' diffractograms have been recorded, as well for comparison purposes (Fig.1-3).

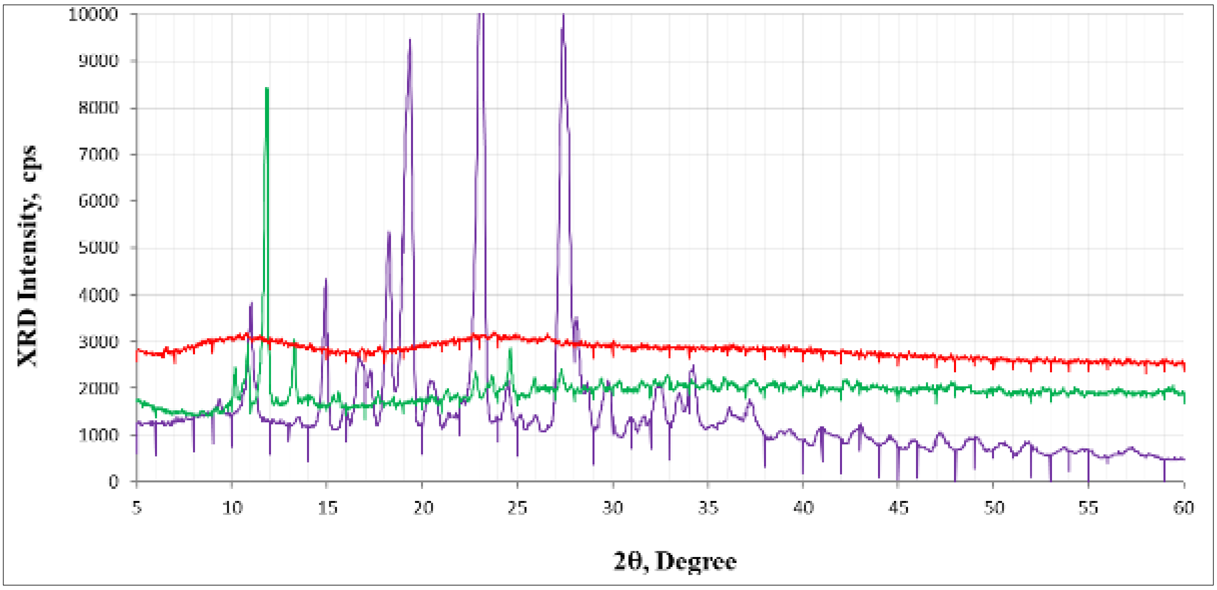

Figure 1 ■ [Fe (Arg)]($(CH_3COO)_2 \cdot H_2O$; ■ $Fe(CH_3COO)_2 \cdot 4H_2O$; ■ Arg

As is seen from diffractograms analysis, diffractograms of reacting substances are characterized by location and intensity of diffraction maximums peculiar for them, while the obtained chelate compound [Fe (Arg)]$(CH_3COO)_2 \cdot H_2O$ (Fig. 1) is in amorphous state that points at the fact that formation of new individual compounds takes place.

In case of $[Fe(Arg)_2](CH_3COO)_2 4H_2O$ (Fig. 2), diffractogram of the obtained compound differs from location and intensity of diffraction maximums of initial reacting substances (arginin and iron acetate) that also points at formation of a new individual compound.

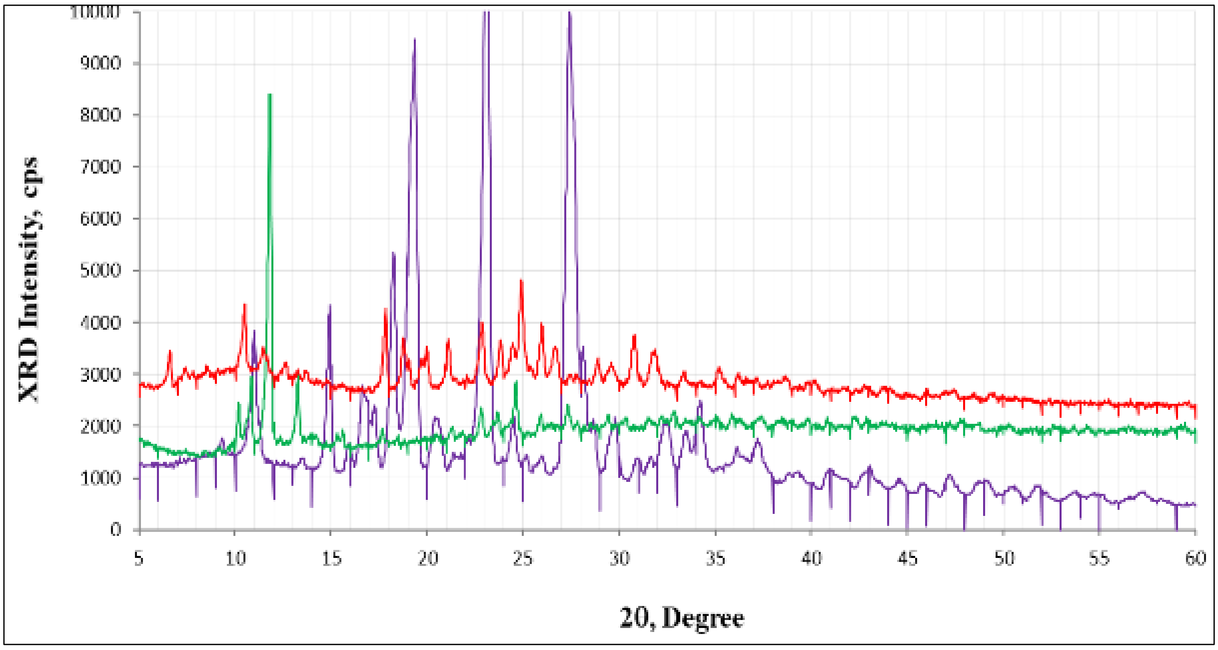

Figure 2 ■ $[Fe(Arg)_2](CH_3COO)_2 4H_2O$ ■ $Fe(CH_3COO) \cdot 4H_2O$, ■ Arg

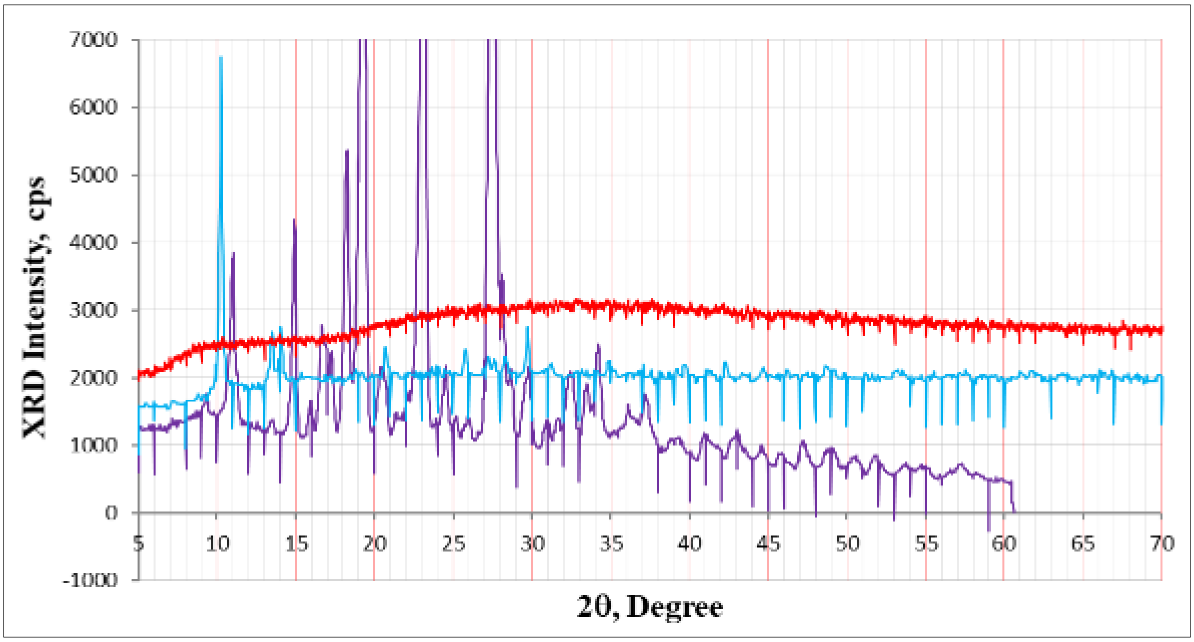

Figure 3 ■ [Co(Arg)]$(CH_3COO)_2 \cdot 2H_2O$; ■ Arg; ■ $Co(CH_3COO)_2 \cdot 4H_2O$

As one can see from Fig. 3, chelate compound [Co(Arg)]$(CH_3COO) \cdot 2H_2O$, as well as (1) is obtained in amorphous state. It doesn't contain in the mixture form the initial substances – cobalt acetate and arginin (which are featured by diffraction maximums and intensity peculiar for them) that points at formation of the new individual compound, as well.

In order to study biological activity of synthesized compounds, a trial test on broilers has been conducted. Chelate mixtures for 100 kg broilers' combined feed premix have been prepared (Table 2).

Table 2 Mixture composition

X mixture	**X norm(gram)**	**X max gram (gram)**
[Fe $(Arg)_2$]$(CH_3COO)_2 \cdot 4H_2O$	51.49	59.21
[Co$(Arg)_2$]$(CH_3COO)_2 \cdot 2H_2O$	1.911	2.19

20 birds each, for two experimental and one control group have been selected for a test according to zootechnical analogue principle. The experiment has lasted for a month. During a test period we have studied poultry live weight in the beginning and in the end of a test, and the results are given in Table 3

Table 3 Change in live weight during the trial period

Group	**Live weight dynamics (gram)**					
	Start experiment			Completing the experiment		
	Mini	Max	M+m	Mini	Max	M+m
Control group	1400	1590	1495±95	1430	1620	1525±95
X norm	1490	1640	1565±75	1535	1680	1607±73
X max	1500	1680	1590±90	1550	1700	1625±75

Poultry survival rate has been studied as well (Table 4), according to which birds have been maximally survived in the experimental groups.

Table 4 Keeping poultry during the trial period %

Group	Number of birds, (wing)		Keeping %
	Beginning of the experiment	End of the experiment	
Control group	20	17	85
X norm	20	19	95
X max	20	20	100

Based on the preliminary trial tests one may presume that entry of arginin-containing iron and cobalt chelates into composition of broilers' combined feed premixes has had a positive impact on live weight gain and poultry survival rate.

4. Conclusion

Based on the conducted studies the following conclusions can be made:

- Synthesis conditions are established and arginin-containing iron and cobalt chelate compounds are synthesized;
- Chelates are individual compounds, which are characterized by high solubility in water, but poor solubility in organic solvents;
- Individuality of synthesized compounds is identified via melting temperature measurement and diffractographic study;
- Dissociation constants of compounds are calculated through conductometric study method;
- Based on preliminary trial tests carried out with a purpose of biological activity study one may presume that entry of arginin-containing iron and cobalt chelates into composition of broilers' combined feed premixes has had a beneficial impact on live weight gain and poultry survival rate;
- There is expressed an opinion on the reasonability of basic tests conduction in order to determine doses of arginin-containing iron and cobalt chelate compounds optimal for broilers.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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