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Synthesis and study of Arginin-containing copper and chrome chelates

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Abstract

Synthesis conditions have been established and arginin (Arg)-containing copper and chrome chelate compounds with the following general formulas: $CuArg_n(CH_3COO)_2 \cdot mH_2O$ and $CrArg_n(CH_3COO)_3 \cdot mH_2O$ (where n =1÷3; m=2 or 4) have been synthesized. Synthesized chelates have been studied using a number of physical and chemical research methods. In particular, their composition has been established via trace element analysis, while the individuality has been identified through melting temperature measurement and diffractographic method. Through study of qualitative solubility in different solvents there has been established that chelates are highly soluble in water and poorly soluble in organic solvents. Using the conductometric research method there has been calculated a dissociation constant of chelate compounds-containing solutions. Based on the preliminary trial tests carried out in order to study the biological activity, there is expressed an opinion that entry of arginin-containing copper and chrome chelates into composition of combined feed premixes of egg-laying (oviparous) poultry has had a positive impact on egg-laying intensity and poultry survival rate. There is given an opinion on the expediency of basic test conduct in order to determine the optimum doses of arginin-containing copper and chrome chelate compounds for egg-laying birds.

Keywords: Copper; Chrome; Chelate; Arginin; Laying Bird; Premix; Combined Feed.

1. Introduction

Improvement of environmental state and population health is a global problem nowadays and it is so relevant as never before. The necessary condition of this problem solution is population provision with cheap, high-quality, ecologically safe agricultural production (including agricultural poultry and animals). Deficit of microelements is the primary reason determining low quantitative and qualitative indicators of these food products. It is known that microelements along with other substances act important role in the metabolism, which is a unity of assimilation and dissimilation processes (synthesis and decay) and implies permanent self-renewal and self-restoration of living organisms. Their content in living organisms varies within limits of 10^{-3}-10^{-5} %. In physiological and biological processes running in the organism (including animal and poultry organism), microelements mainly perform their functions in the form of bio-coordinate (chelate) compounds. They participate in metabolism, substances transportation and synthesis, protein, carbohydrate and fat metabolism, and while being in optimum dose, they have an ability to actively influence on living organisms' growth and development, multiplication, productivity, and disease resistance. They enter into composition of metalloenzymes, metalloproteins and secure normal functioning of the majority of biochemical processes. All this predetermined the fact that indispensable microelements are entered into composition of poultry and animal feed premixes in chelate form only [1-14].

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Among vitally important (essential) microelements we have selected copper and chrome as the research subject. Copper is an important part of metalloproteins, which regulates (controls) oxidation-reduction reactions of cell respiration.

Different tyrosinases catalyze pigments (melanins) formation in animal and bird cells. Cu^{2+}-ion plays the activator role for a number of enzymes (sulfide oxidase, tyrosine kinase etc.). It is fixed in bone marrow, salivary glands, and pancreatic gland. Liver is a main source of copper and its concentration is an indicator of copper assimilation. Copper is necessary for blood formation in organism, for organism protective function enhancement, for down hair and feather keratinization and pigmentation. Lack of copper causes anemia development, change in blood-vessel elastin properties and structure that frequently leads to aortic wall tear. Copper shortage causes brain development disorder, as well. Tumors formation leads to pigmentation disorder that causes grayness of the hair [15-18].

The essential biological role of chrome lies in control of blood glucose level and regulation of carbohydrate metabolism, in general. Lack of chrome may become a reason of diabetes mellitus, arterial hypertension, and atherosclerosis may develop as a result of dyslipidemia.

Population survey in different geographic regions (endemic zones) has shown that where chrome consumption is high, the probability of cardiovascular diseases and diabetes mellitus distribution is far below.

So, chrome is one of the most important and demanded microelements. Though, despite such a high importance of chrome, according to detailed nutritional standards, it still is not entered into composition of poultry and animal premixes [19-27].

Amino acids are most frequently used as chelating agents during a synthesis. This may be explained by the fact that amino acids play important role in metabolism, are indispensable for formation of enzymes, hormones, antibodies, antitoxins etc. In general, amino acids are nutraceuticals adjusting organism resistance, that is why amino acid-containing chelates may be successfully used not only in premixes 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, which promotes relaxation and elasticity of blood vascular system. This fact is of great importance in the process of treatment of number of diseases (cardio-vascular system, brain, immune and nervous system, atherosclerosis, genital system diseases etc.). According to new studies arginin consumption by immune cells (which protect brain) in oversize amount is a reason of Alzheimer disease contraction [28-32].

Thus, proceeding from a brief literature analysis, we have set a goal of physical-chemical study of arginin-containing copper and chrome chelate compounds and investigation of their biological activity.

2. Computational Method

- Trace element analysis – for determination of chelate compounds' composition.
- Melting temperature determination – for establishment of chelates individuality.
- Solubility – for study of qualititative solubility of compounds in different solvents.
- Conductometric study – for determination of dissociation constant of chelate compound-containing solutions.
- Weighing method – for determination of poultry weight gain.
- Counting technique – for establishment of poultry survival rate.

3. Results and analysis

With the purpose of synthesis arginin (Arg)-containing chelate compounds with the following general formulas: $CuArg_n(CH_3COO)_2 \cdot mH_2O$ and $CrArg_n(CH_3COO)_3 \cdot mH_2O$ (where n =1÷3; m=2 or 4), mixtures of copper acetate (or chrome acetate) and arginin at the molar ratio of 1:1; 1:2 or 1:3 have been dissolved in the minimum amount of water under conditions of warming and vigorous mixing. Obtained solutions have been filtered and for concentration purposes have been exposed at the water steam bath for a short time. Compounds received via exposure at room temperature have been washed out by alcohol and water. Composition of synthesized compounds has been established through trace element analysis (Table 1). Some physical and chemical indicators of compounds are given in the same table, as well. In particular, via melting temperature measurement using the melting temperature determining device – Melting Point /SMP10/, there is established that compounds are individual substances and their melting temperatures are within a limit of 155-118^{0}C.

Table 1 Some physical characteristics of chelate compounds

#	Formula Compound	Molar mass g/mol	Melting t^0c	Solubility				Conductometric study results	
				water	ethanol	acetone	DMFA*	R^2	pKa
1	$[Cu(Arg)](CH_3COO)_2 \cdot 2H_2O$	391.98	155	+	-	-	-	0,8850	2.92
2	$[Cu(Arg)_2](CH_3COO)_2 \cdot 2H_2O$	566.46	129	+	-	-	-	0.8514	1.30
3	$[Cu(Arg)_3](CH_3COO)_2 \cdot 2H_2O$	735.53	121	+	-	-	-	0.9133	1.40
4	$[Cr(Arg)](CH_3COO)_3 \cdot 2H_2O$	439.63	138	+	+t	+t	-	0.9852	1.52
5	$[Cr(Arg)_2](CH_3COO)_3 \cdot 4H_2O$	650.11	127	+	-	-	-	0,9522	1.02
6	$[Cr(Arg)_3](CH_3COO)_3 \cdot 4H_2O$	824.59	118	+	-	-	-	0,9553	1.22

DMFA* - Dimethylformamide, + soluble. – insoluble, +t soluble by heating

Through study of their solubility in different solvents there is established that all chelates are highly soluble in water, while in other solvents these compounds are practically insoluble.

With the purpose of determination of arginin-containing chelate compounds' dissociation constant a conductometric study has been conducted at the device pH and Conductivity Sensor LE703. To this end the solutions have been prepared for these compounds with concentration limits from 0.025N to 0.0006503N. Experiment has been carried out in the thermostat at 25°C. Experimental results are given in Table 1.

R^2 – regression assessment indicator showing how close the experimental data are with the function corresponding to the graph; it is quite high and varies within 0,9812-0,8514 limits. Dissociation constants of the initial salts are measured under the same conditions, in particular pKa $Cu(CH_3COO)_2H_2O$ = 1.88, while pKa $Cr(CH_3COO)_3]$= 1.75. As is seen from the Table, the dissociation constants of chelates and their initial salts are of one and the same order (within the same limits). Based on this, one may assume that under conditions of the conducted experiment chelate dissociation proceeds with detachment of the second sphere (acetate-ions), while there is no dissociation in the first sphere. This fact may be explained by formation of stable heterocyclic five-membered cycles between metal and organic ligand.

In addition to melting temperature measurement, the individuality of the chelate compounds $[Cu(Arg)_2](CH_3COO)_2 \cdot 2H_2O$ (1), $[Cr(Arg)_3(CH_3COO)_3 \cdot 4H_2O$ (2) has been established via diffractographic method, as well. X-ray-diffractometric study has been conducted using ДРОН-4.07 at the $Cu_{k\alpha}$(λ=0.154184 nm) irradiation. During exposition, samples have been rotated in their own plane by means of special device – ГП-13. For comparison purposes, the diffractograms of initial reacting substances are recorded, as well (Fig. 1, 2). As is seen from diffractograms analysis, the location and intensities of diffraction maximums peculiar for diffractograms of the obtained chelate compounds $[Cu(Arg)_2](CH_3COO)_2 \cdot 2H_2O$ and $[Cr(Arg)_3](CH_3COO)_3 \cdot 4H_2O$ differ from location and intensities of diffraction maximums of reacting substances (Arg and metal acetates) that points at the fact that formation of new individual compounds ($[Cu(Arg)_2](CH_3COO)_2 \cdot 2H_2O$ and $[Cr(Arg)_3(CH_3COO)_3 \cdot 4H_2O$) takes place in both cases (Fig. 1, Fig. 2).

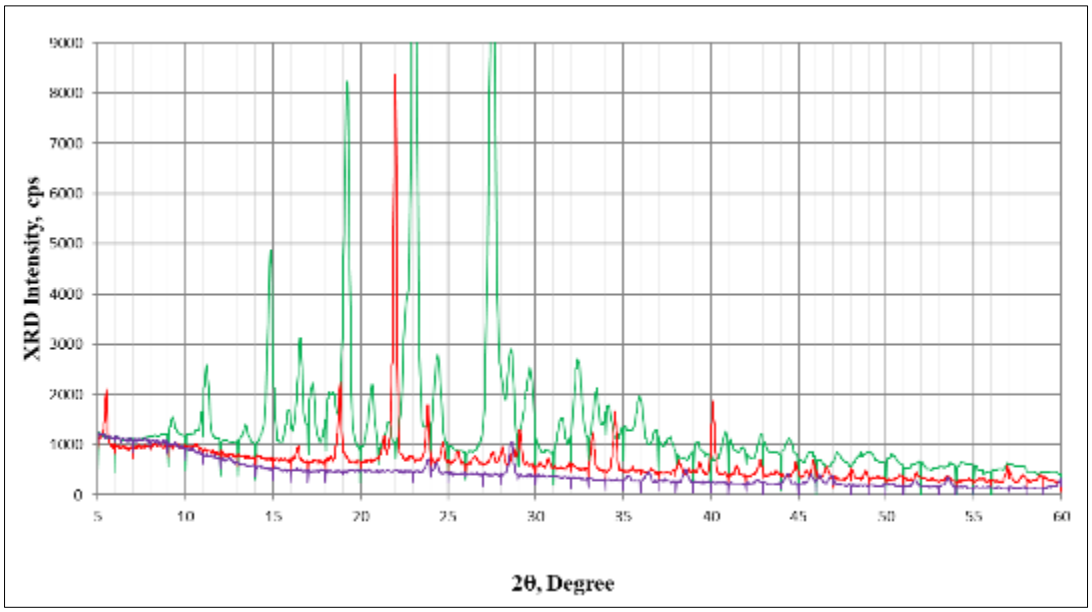

Figure 1 ■ $[Cu(Arg)_2](CH_3COO)_2 \cdot 2H_2O$; ■ $Cu(CH_3COO)_2 \cdot H_2O$; ■ Arg

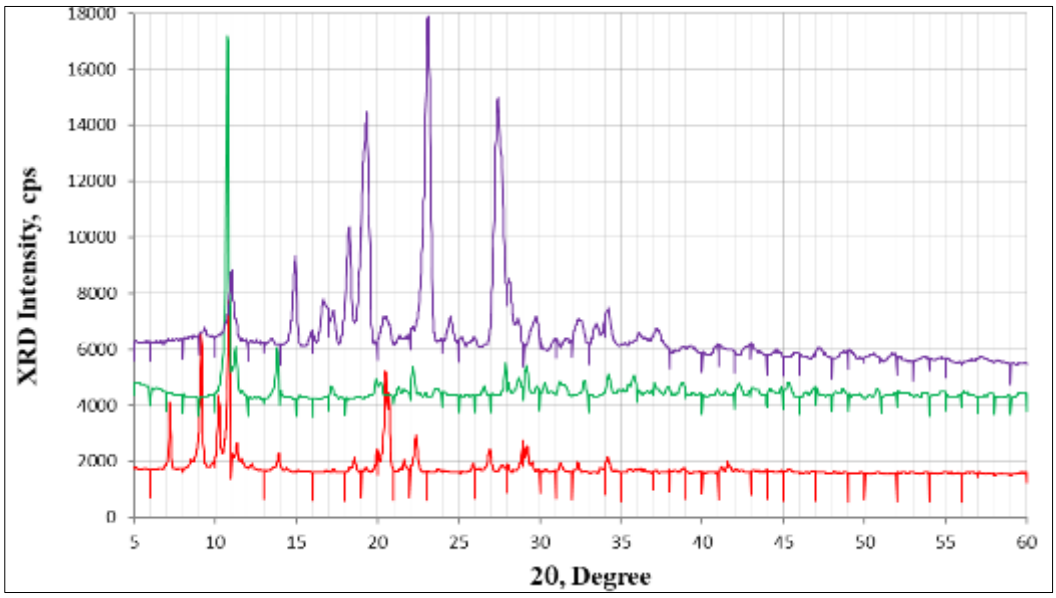

Figure 2 ■ $[Cr(Arg)_3](CH_3COO)_3 \cdot 4H_2O$, ■ $Cr(CH_3COO)_3$, ■ Arg

In order to study biological activity of synthesized compounds, a trial test has been conducted on egg-laying birds. There have been prepared chelate mixtures for 100 kg combined feed premixes intended for egg-laying birds (Table 2).

Table 2 Mixture composition for 100 kg of combined feed

X mixture	Xnorm(gram)	Xmax(gram)
$[Cu(Arg)_2](CH_3COO)_2 \cdot 2H_2O$	7.16	8.24
$[Cr(Arg)_2](CH_3COO)_3 \cdot 4H_2O$	1.14	1.32

20 birds each, for two test and one control groups, have been selected according to zootechnical analogue principle. The experiment has lasted for two months, during which we have studied poultry survival rate (Table 3).

Table 3 Poultry survival rate during a test

Group	Number of birds, (wing)		Survival %
	Beginning of test	End of test	
Control group	20	18	90
Znorm	20	20	100
Zmax	20	20	100

As is seen from the Table, there is no mortality or poisoning of poultry during the experimental period (60 days).Egg-laying intensity and egg shell quality have been studied, as well (Table 4).

Table 4 Poultry egg-laying rate and egg-laying intensity during a test

Indicators	Control group		
	Control	Znorm	Zmax
Start test			
Eggs laid per 1 bird (pieces) in 16 days	11.9	13.2	13.5
Egg-laying intensity %	80.61	82.50	81.92
the test period (60 Day)			
Eggs laid per 1 bird (pieces) in 16 days	47,4	51,5	52.8
Egg-laying intensity	79.03	85.83	87.16

Based on preliminary experiments carried out in order to study the biological activity one may assume that arginin-containing copper and chrome chelates have a positive effect on egg-laying birds survival rate, egg shell quality and egg-laying intensity.

4. Conclusion

The following conclusions may be drawn based on the carried-out studies:

- The synthesis conditions are established and arginin-containing copper and chrome chelate compounds are synthesized;
- Chelates are the individual substances, which are characterized by high solubility in water and poor solubility in organic solvents; the individuality of synthesized compounds is established through melting temperature measurement and diffractographic study method;
- Dissociation constants of compounds are calculated via conductometric study method;
- Based on the preliminary trial tests conducted with a purpose of study of biological activity, one may assume that entry of arginin-containing copper and chrome chelates into composition of combined feed premixes has had a positive effect on egg-laying bird survival rate, egg-laying intensity and egg shell quality;
- There is expressed an opinion on the reasonability of basic tests conduct on egg-laying birds in order to determine the doses of arginin-containing copper and chrome chelate compounds optimal for egg-laying birds.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this document.

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