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Study of Arginine-containing chelates

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Abstract

Our synthesized arginine-containing manganese, zinc, iron, cobalt, copper and chromium chelates have been investigated using spectrophotometric and thermographic methods. It has been established through spectrophotometric method that arginine molecule acts in the role of neutral, bidentate ligand in chelates and establishes a bond with the central atom by means of oxygen atom of carbonyl group and nitrogen atom of amino group, with formation of five-membered (pentatomic) cycles. Using thermographic method, it has been established that thermal decomposition runs stage-by-stage: oxidation of water molecule occurs at the first stage, arginine – at the second, acetate-ions – at the third, and final products are metal oxides or mixtures of metal oxides and coal.

Study of biological activity of premixes prepared with simultaneous content of all six microelements has shown that premixes prepared on the basis of chelates had a positive effect on all zootechnical parameters of broilers; optimum mixture dose has been established, as well and it comprises 150 gr per 100 kg of combined feed.

Keywords: Chelate; Arginine; Biological Activity; Premix; Broiler

1. Introduction

Improvement of environmental conditions and population health is a global problem nowadays and is so relevant as never before. Indispensable condition of this problem solution is population provision with cheap, high-quality, ecologically safe agricultural productions (including agricultural poultry and animals). Microelements' deficiency is one of the main reasons determining low qualitative and quantitative indicators of these food products.

Metabolism disorder underlies all pathological processes taking place in the organisms of agricultural poultry and animals. In its turn, metabolic disturbance is mainly reasoned by malnutrition (poor nutrition) of poultry and animals, in particular by insufficient supply of proteins, fats, carbohydrates, vitamins, macro- and microelements along with feed. At that it is known that in living organisms (including poultry and animals) the essential microelements generally perform their functions in the form of coordination (chelate) compounds, concentration of which is strictly controlled and their deviance from a norm causes disturbance of physiological processes running in living organisms and number of pathologies. Namely this factor preconditioned the necessity of inclusion of microelements being in chelate form into premixes. This fact is confirmed by the results of experiments carried out over the years by foreign and local scientists [1-10]. According to detailed nutritional standards for agricultural poultry and animals, as of today the following microelements are entered into composition of premixes: manganese, zinc, iron, cobalt, copper, iodine and selenium. Organic acids, oxy-acids, poly- and oligosaccharides, vitamins and amino acids are used in poultry and animal nutrition in the role of chelating agents (ligands). Among them, amino acids are of special importance when used in poultry and animal feeding [11-14].

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We have synthesized manganese, zinc, iron, cobalt, copper and chromium chelates containing basis amino acid – arginine, and they have been explored using number of methods of physical and chemical study. Experiments aimed to study of biological activity have been conducted on broilers [15-17].

This article deals with a) spectrophotometric and thermographic studies of arginine- containing manganese, zinc, iron, cobalt, copper and chromium chelates [18-19]; b) premix preparation with simultaneous content of chelates of all six microelements in order to establish the optimum doses and carrying-out of experiments on broilers.

2. Materials used and research methods

Manganese carbonate, Zinc, Cobalt, Iron, Chromium and Copper acetates and amino acid Arginine have been used for synthesis of chelate compounds. The mentioned reagents have been purchased under „Sigma-Aldrich'' trademark.

Chelates, vitamin-mineral premixes (NUTRI ENTRUM animal feeding,SEN TARIM SANAYIAS Turkey),broilers have been used for study of biological activity.

- Spectrophotometric research method is used for establishment of bond character between amino acid and metal.
- Thermographic research method – for establishment of thermal stability and nature of thermal decomposition of synthesized chelates.
- Weighing method – for determination of poultry weight gain.

3. Results and analysis

Infrared absorption spectra of synthesized compounds have been studied in order to establish arginin nature, its bond character with central ion in the arginine-containing compounds. Infrared absorption spectra (400-4000 cm^{-1}) have been recorded at the spectrophotometer Agilent Cary630, along with KBr, in the form of compacted powder.

Arginine, while containing amino groups in α-state and guanidine groups in δ-state, is a basic amino acid.

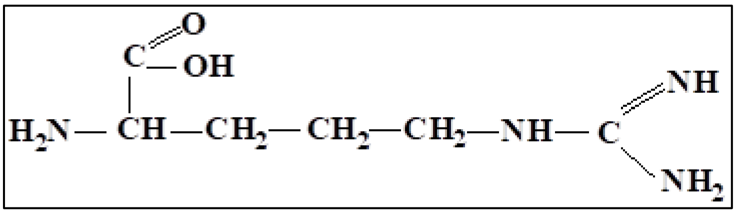


Figure 1 Arginine

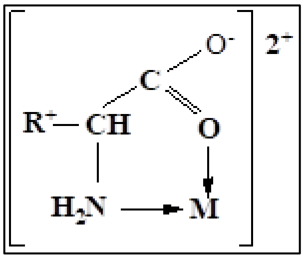
That is why in coordination compounds it can simultaneously perform the role of both monodentate (with oxygen atom of COOH group or nitrogen atom of α-NH_2 group) and bidentate ligand (with oxygen atom of COOH group and nitrogen atom of α-NH_2 group). It is also known that amino acids, while forming zwitter-ions (NH_3^+-RCH-COO^-) according to reaction conditions, establish coordination bonds with central atoms (by means of oxygen atom of deprotonated carboxyl group and nitrogen atoms of α-NH_2 group) [18, 19]. δ-guanidine NH_2-group, at the expense of resonance charge delocalization during protonation is in protonated cationic form and is unable to form numerous hydrogen bonds.

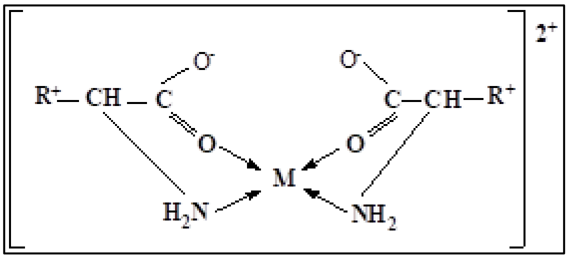
Affiliation of some vibration frequencies of IR absorption spectra of compounds under investigation is given in Table 1. Arginine spectral data are given for comparison purposes, as well.

Vibration frequencies (cm-1) of arginine amino acid NH2 and COO- groups found in infrared absorption spectra of compounds

Arg	[Cu(Arg)] $(CH_3COO)_2{\cdot}2H_2O$	[Cr(Arg)] $(CH_3COO)_3{\cdot}2H_2O$	[Mn(Arg)$_2$] $(CH_3COO){\cdot}4H_2O$	[Zn(Arg)$_2$] $(CH_3COO)_24H_2O$	atribution
3350	3340	3360	3375	3340	ν_{as} (NH_2)
3100	2880	3300	3250	3200	ν_s (NH_2)
1680	1690	1650	1690	1695	δ(NH_2)
1560	1570	1580	1550	1575	ν_{as} (COO^-)
1420	1425	1440	1440	1410	ν_s (COO^-)
1330	1345	1335	1350	1360	δ(C=O)
-	540	520	570	560	ν(M–N)
-	450	440	450	480	ν(M–O)

As is seen from the Table, a distinct absorption band in the spectral region 1750–1720 cm^{-1} peculiar for non-dissociated ν(COOH) carboxyl group is not visible (perceptible) in the infrared absorption spectrum of arginine and compounds synthesized on its basis that points at the fact that arginine is in form of zwitter both in compounds and independently. This is confirmed by appearance of absorption bands in arginine spectrum in 1550-1580cm^{-1} and 1410-1440cm^{-1} regions, while in compounds' spectra – in 1550-1580cm^{-1} and 1410-1440cm^{-1} regions, which may be affiliated to asymmetric and symmetric ν_{as} (NH_2) and ν_s (NH_2) vibrations of α-amino groups, respectively. Absorption bands in 1650-1695cm^{-1} region belong to deformation vibration δ(NH_2) of arginine α-amino group. Absorption bands in 1335-1360cm^{-1} region belong to deformation vibration δ(C=O) of carbonyl group. Absorption bands located in 520_570 cm^{-1} and 440-480 cm^{-1} regions may be affiliated to stretching vibrations of metal-nitrogen and metal-oxygen bonds. Based on this fact, one can assume that arginine acts in the role of cyclic ligand. It forms a bond with central atom by means of nitrogen atom of α-amino group and oxygen atom of carbonyl group (C=O), with formation of five-membered metallacycles, both in case of M:Arg = 1:1 molar ratio (Figure1), and M:Arg=1:2 molar ratio (Figure 2).





We suppose that protonated guanidine amino groups form multiple hydrogen bonds.

In order to study thermal stability and sequence of thermolysis process in chelates containing synthesized arginine, a thermographic study has been conducted at the device: NETZSCH STA2500, with sample heating rate 10 grade/min (Table 2).

Table 2 Results of thermographic study of arginine-containing

Compound	T^0 C	Weight loss % Practical	Weight loss % Theoretical	Detachment of water molecule mole	Solid decomposition product
$[Mn(Arg)](CH_3COO)_2.4HO$	120	11.21	10.74	$2.5H_2O$	$[Mn(Arg)](CH_3COO)_2.1.5H_2O$
	150	7.96	7.22	$1.5H_2O$	$[Mn(Arg)](CH_3COO)_2$
	300	20.54	20.09	0.4 Arg	$[Mn(Arg)_{0.6}](CH_3COO)_2$
	380	30.91	31.42	0.6 Arg	$Mn(CH_3COO)_2$
	500	64.13	63.98	$2CH_3COO$	Mn_3O_4 + 2C
$[Mn(Arg)_2](CH_3COO)_2.4HO$	125	5.87	6.07	2.0 H_2O	$[Mn(Arg)_2](CH_3COO)_2.2H_2O$
	140	6.14	6.45	2.0 H_2O	$[Mn(Arg)_2](CH_3COO)_2$
	330	49.79	50.15	1.5Arg	$[Mn(Arg)_{0.5}](CH_3COO)_2$
	490	42.34	42.63	0.5Arg	$Mn(CH_3COO)_2$
	520	50.93	51.31	$2CH_3COO$	Mn_3O_4 + C
$[Zn(Arg)_2](CH_3COO)_2{\cdot}4H_2O$	120	6.12	5.96	2 H_2O	$[Zn\ (Arg)_2](CH_3COO)_2{\cdot}2H_2O$
	140	5.97	6.34	2 H_2O	$[Zn\ (Arg)_2(CH_3COO)_2$
	280	16.59	16.39	0.5Arg	$[Zn\ (Arg)_{1.5}(CH_3COO)_2$
	420	59.05	58.84	1.5Arg	$Zn\ (CH_3COO)_2$
	650	48.09	47.76	$2(CH_3COO$	ZnO+3C
$[Cr\ Arg](CH_3COO)_3{\cdot}2H_2O$	120	8.07	8.19	2 H_2O	$[\ Cr\ Arg](CH_3COO)_3$
	270	10.41	10.81	0.25 Arg	$[\ Cr\ Arg_{0.75}](CH_3COO)_3$
	300	12.38	12.12	0.25 Arg	$[Cr\ Arg_{0.\ 5}](CH_3COO)$
	350	27.89	27.58	0.5 Arg	$Cr\ (CH_3COO)_3$
	500	52.06	51.50	$3CH_3COO^-$	$0.5Cr\ _2O_3$

As is seen from thermographic study, all thermograms are characterized by several endo- and exoeffects and respective effects on the curve .

Analysis of chelate compound $[Mn(Arg)](CH_3COO_2){\cdot}4H_20$ thermogravigram showed that the first endoeffect (120°C) corresponds with detachment of 2.5 mole of water molecule (weight loss: practical 11.21%, theoretical 10.74%), at the next endoeffect (150°C) takes place detachment of 1.5 H_2O molecule (weight loss: practical 7.96%, theoretical 7.22%). According to analysis, at the next endoeffect (300°C) arginin oxidation begins (0.4 mole Arg) (weight loss: practical 20.54%, theoretical 20.09%), which ends at 380°C (oxidation of 0.6 mole Arg) (weight loss: practical 30.91%, theoretical 31.42%). At strong exoeffect (500°C), complete oxidation of acetate-ions occurs and final products of thermolysis are represented by a mixture of Mn_3O_4 and coal.

Thermal dissociation of the rest chelates $[Mn(Arg)_2](CH_3COO)_2.4H_2O$, $[Zn(Arg)_2](CH_3COO)_2{\cdot}4H_2O$ and $[Cr(Arg)](CH_3COO)_3{\cdot}2H_2O$ is of the same character. In particular, in the 120-140°C temperature limits, thermal decomposition begins with water molecule detachment; in the chelate containing one mole of arginine $[Cr(Arg)](CH_3COO)_3{\cdot}2H_2O$ at the ensuing stage, within 270-350°C temperature limits takes place oxidation of arginine molecule, while in the compounds containing two moles of arginine, oxidation of arginine occurs at the endoeffect within 280-490°C temperature limits. In all cases, a thermal decomposition ends at strong endoeffect within temperature limits of 500-600°C with oxidation of acetate-ions, and final products of decomposition are metal oxides or mixtures of oxide and carbon.

Based on the analysis of thermolysis process, one may conclude that the thermal decomposition of arginine-containing chelates proceeds in stages: oxidation of water molecule occurs at the first stage, arginine – at the second, acetate-ions – at the third one and final products of decomposition are metal oxides or mixtures of metal oxide and carbon.

In order to study biological activity of synthesized compounds a trial test has been conducted on broilers. Chelate mixtures have been prepared for 100 kg premixes of broiler's combined feed (Table 3).

Table 3 Chelates mixture (X) for 100 kg of combined feed

Composition of the chelates mixture (X)	Norm (g)	Max (g)
$[Mn(Arg)](CH_3COO)_2{\cdot}4H_2O$	45.76	52.624
$[Zn(Arg)](CH_3COO)_2{\cdot}2H_2O$	33.03	37.98
$[Fe(Arg)](CH_3COO)_2{\cdot}H2O$	43.56	50.094
$[Co(Arg)](CH_3COO)_2{\cdot}H_2O$	0.263	0.303
$[Cu(Arg)](CH_3COO)_2{\cdot}H_2O$	7.14	8.211
$[Cr(Arg)](CH_3COO)_2{\cdot}H_2O$	1.16	1.334

Groups of twenty birds (two sets for test group and one – for control one) have been selected according to zootechnical analogy principle for a test. Experiment lasted for a month. During a test, poultry live weight in the beginning and in the end of the test has been studied, and results are given in Table 4.

Table 4 Live weight change during a test

Group	Live weight change (g/ poultry)					
	Start the test			End of test		
	Mini	Max	M+m	Mini	Max	M+m
Control	1420	1590	1505±85	1435	1625	1530±95
X norm	1490	1650	1570±80	1535	1690	1613±77.5
X max	1500	1690	1595±95	1550	1760	1655±105

Poultry survival rate has been studied as well (Table 5), and according to study maximal survival rate is obtained in the test groups.

Table 5 Poultry viability during the trial period

Group	Quality poultry (wing)		Viability %
	Start the test	End of test	
control	20	15	75
X norm	20	18	90
X max	20	20	100

4. Conclusion

Based on the carried-out experiments, the following conclusions may be drawn:

- According to spectrophotometric studies, in chelate compounds arginin acts in the role of cyclic ligand. It establishes bond with a central atom by means of nitrogen atom of α-amino group and oxygen atom of carbonyl group (C=O), with formation of five-membered metallacycles.

- Based on thermographic studies it has been established that chelate decomposition runs stage-by-stage: detachment of water molecules occurs at the first stage, gradual oxidation of arginine molecules – at the second, and thermal decomposition ends with formation of metal oxides or mixtures of oxide and carbon.
- Entry of mixture of microelement chelates into composition of combined feed premixes has had a positive effect on live weight growth and poultry survival rate.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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