Annals of Agrarian Science

Journal homepage: http://journals.org.ge/index.php

Cluster Chelates on the Basis of Natural Raw Materials

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Received: 15 December 2019; accepted: 25 February 2020

A B S T R A C T

The synthesis methods are shown and Mn, Zn and Cr cluster chelate compounds are synthesized on the basis of metal acetates and concentrate DAS (Ds) of vegetable origin. Different number of metals is bonded to each other in the synthesized compounds depending on synthesis conditions. The individuality of synthesized compounds is studied using trace element analysis and X-Ray radiography methods, as well as through melting temperature determination. Qualitative solubility of compounds in different solvents is defined. An experiment on earthworms was carried out in order to determine biological activity of synthesized cluster chelates. For this purpose three doses (maximum, minimum and normal) of mixtures of Mn, Zn and Cr compounds were prepared and their impact on earthworms' protein mass and degree of cocoon reproduction was studied. Effective and optimum doses of chelate compounds were established. Based on the results of conducted experiments it may be said that maximum mass change (115.66%) takes place in that test group, to which a minimum dose of chelate mixtures is added, while the degree of cocoon propagation reaches the maximum value (576.32%) in that test group, to which a normal dose of chelate mixtures is added. Thus, on the basis of carried out researches a conclusion is drawn that a balancing of earthworm substrate with different doses of Mn, Zn and Cr compound mixtures has a positive impact on both earthworm's protein mass gain and on the substantial increase of degree of cocoon reproduction, and 0.38g per 200 g of substrate is the optimum effective dose for cluster chelate mixtures. For the same chelate mixtures of Mn, Zn and Cr the preliminary check-up tests were conducted on broiler chickens. Based on analysis of obtained results (7.5% weight gain in the test group during upbringing period, and survival is 3.3% higher compared to control group) we deem reasonable to carry out experiments on a wider scale for determination of effective and optimum doses of chelate mixtures.

Keywords: Cluster, Chelate, Vermiculture, Worm, Cocoon, Concentrate.

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Introduction

Population provision with high-quality, ecologically safe agricultural products is very topical as of today. Their provision with definite quantitative and qualitative composition and optimum ratio of microelements is one of the most important prerequisites of solution of this problem. It can be attained through creation and use of premixes containing chelate microelements, as far as the digestibility of chelate microelements by living organisms roughly equals to 60-70%, while in case of simple inorganic forms it is equal to 7-10%. Today the synthesized biologically active organic substances (amino-acids, oxy-acids etc.) are mainly used for microelements' chelation [1-15]. However there are only scarce data on chelates' obtaining on the basis of natural raw materials and on their use in the feed of poultry (agricultural birds) and farm animals [16-18]. Taking into account a number of positive factors (diversity and, respectively, high biochemical activity of natural raw materials, their cheapness and accessibility) we selected DAS – the concentrate of vegetable origin obtained on the base of

Biorational Technology Research Center as a research subject. It is distinguished by the diversity of organic substances of different classes entering its composition, and, respectively, by high biological and chemical activity [19-22]. In order to establish the biological activity of chelates obtained on their and metals' (Manganese, Zinc and Chromium) basis their impact on earthworm mass and degree of reproduction is studied. Experiment was conducted on a new species of earthworms, called "Georgian New", which is cultivated at the biofarm of the company Macro-Prim LLC by selectionist Guram Gejadze [23]. Preliminary check-up tests on broilers were conducted as well, taking into account the obtained results.

Methods used

- Trace element analysis – for determination of metal percentage in synthesized compounds;
- Melting temperature determination and X-Ray radiography study – for establishment the individuality of chelates;
- Solubility – for determination of qualitative solubility of chelate compounds in different solvents.

We have conducted the experiment on the earthworm according to the methodology, developed by us [24,25] and we have used for this purpose:

- Weighting method – for determination of earthworm mass; this method was used for determination of broiler live weight, as well;
- Count method – for identification of cocoons number.

Results and discussion

Within the frameworks of memorandum concluded between the Agrarian Chemistry laboratory of P. Melikishvili Institute of Physical and Organic Chemistry at Iv. Javakhishvili Tbilisi State University and Biorational Technology Research Center there were conducted studies aimed to determination of synthesis conditions for chelate compounds on the basis of Mn, Zn and Cr and concentrate DAS, as well as to synthesis and study of their biological activity. With observance of proper synthesis conditions and based on acetates and DAS so-called homonuclear, cluster-type chelate compounds of Manganese, Zinc and Chromium are obtained, in which the different number of metal atoms are bound to each other according to synthesis conditions (Table 1).

As is seen from the Table, in case of 0.02-0.03 mole Mn(CH3COO)2.4H2O+20ml.Ds ratio of reacting components the percentage of Manganese in compounds obtained in water bath through evaporation varies within the limits of 7.9-18.52%, while for that obtained through separation – within the limits of 23.10-43.43%. In case of 0.03-0.05 mole Zn(CH3COO)2.2H2O+15ml.Ds ratio the percentage of Zinc in compounds obtained in water bath through evaporation varies within the limits of 5.37-21.05%%, while for that obtained through separation – within the limits of 44.10-57.92%. For 0.03-0.05mole Cr(CH3COO)3+20ml.Ds ratio the percentage of Chromium in compounds obtained in water bath through evaporation varies within the limits of 10.63–18.22%, while for that obtained through separation – within the limits of 20.33-28.57%.

Table 1 . *Metal percentage in cluster chelates*

Ratio of reacting components	Synthesis conditions	Metal %
0.02–0.03 mole $Mn(CH_3COO)_2.4H_2O$ +20ml.Ds	Evapor. in water bath	7.9–18.52
	Separation	23.10–43.43
0.03–0.05 mole $Zn(CH_3COO)_2.2H_2O$ +15ml. Ds	Evapor. in water bath	5.37–21.05
	Separation	44.10–57.92
0.03–0.05 mole $Cr(CH_3COO)_3$+20ml. Ds	Evapor. in water bath	10.63–18.22
	Separation	20.33–28.57

In Table 2 some physical-chemical characteristics of cluster chelate compounds are given. As is seen from the Table, melting temperature of Mn and Zn compounds obtained via separation method is more than 300°C, while for Cr-chelate equals to 150°C. As for compounds obtained through evaporation, they are adhesive ones and melt at lower temperature, roughly within the limits of 55-65°C. Definite regularity is observed regarding solubility of compounds obtained through separation and evaporation in different solvents. In particular, compounds obtained via evaporation method are characterized by good water solubility, and compounds generated through separation are virtually insoluble Compounds obtained via both methods, are characterized by low or poor solubility in organic solvents.

Table 2. *Some physical-chemical properties of cluster chelates*

Chelates	Color	Melting ^{0}C	Solubility			
			Water	Alc.	Acet.	DMS*
Mn·Ds (via evapor.)	White-pinky	65	+	Low sol.	Low sol.	Low sol.
Mn·Ds (via selection)	Dark-pinky	>300	–	Virt. insol.	Virt. insol.	Virt. insol.
Zn·Ds (via evapor.)	White	60	+	Low sol.	Low sol.	Low sol.
Zn·Ds (via selection)	White	>300	_	Virt. insol.	Virt. insol.	Virt. insol.
Cr·Ds (via evapor.)	Blue-violet	55	+	Low sol.	+t	Low sol.
Cr·Ds (via selection)	Dark-violet	>300	+t	Low sol.	Low sol.	Low sol.

DMS*- Dimethylsulfoxyde

In addition to melting temperature determination, the individuality is established using diffractometric method, as well. X-ray diffractometric study is conducted using "ДРОН-4" at Cukα (λ=0.154184nm.) radiation. During exposition, the samples were rotated in their own plane by means of special device "ГП-13". For comparison purposes, the X-ray patterns of initial salts were taken, as well. As is seen from X-ray pictures that reflect the dependence between angle of reflection 2θ (in degrees) and relative intensity towards the greatest peak I/Io, chelate compounds containing chromium and Das are amorphous solid substances [pictures 1and 2]. X-ray pictures of Zinc-DAS, Manga-

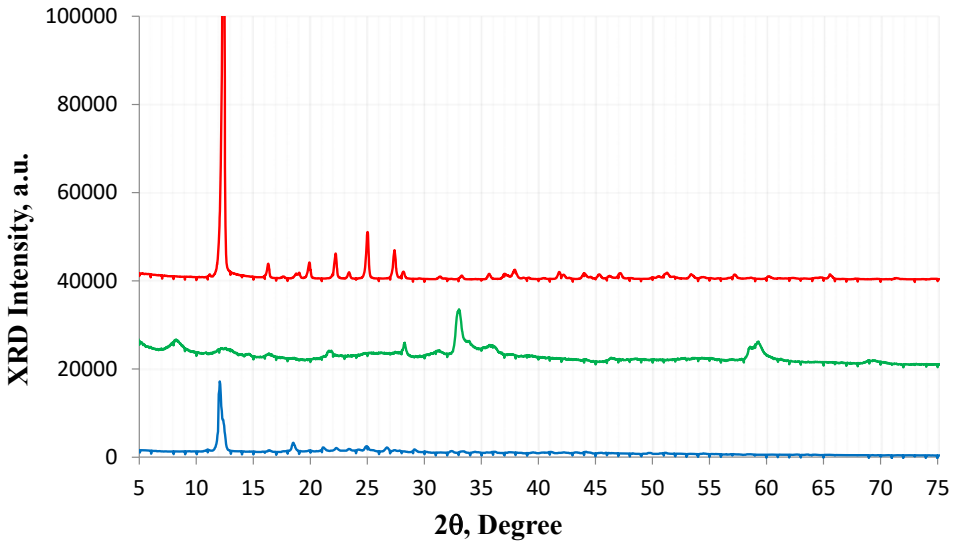

Fig. 1. *X-ray of Zinc-DAS chelate compounds*
Zn-acetate, ---- Zn·Ds (via evapor.), ---- Zn·Ds (via selection)

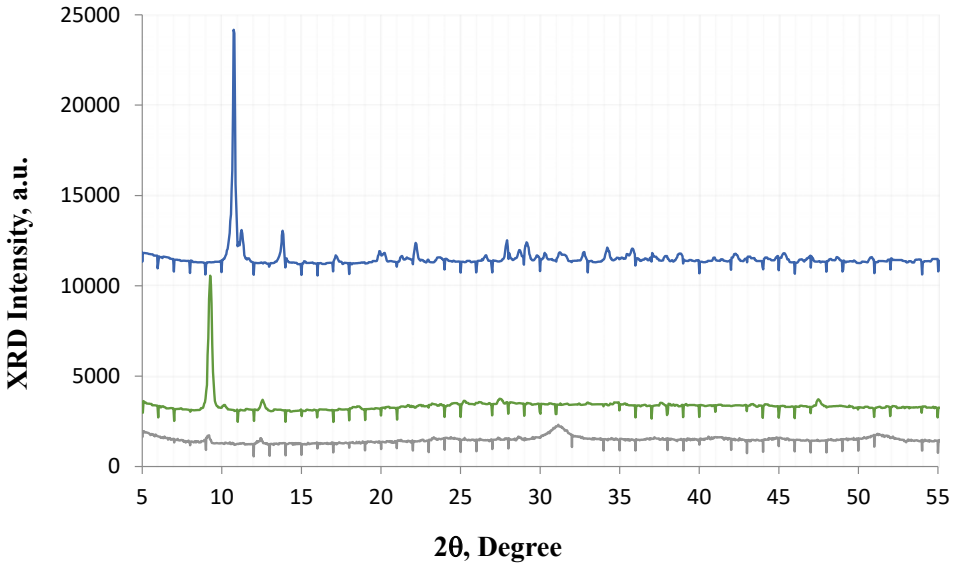

Fig. 2. *X-ray of Manganese -DAS chelate compounds*
---- Mn-acetate, ---- Mn·Ds (via evapor.), ---- Mn·Ds (via selection)

nese-DAS compounds, obtained via separation and evaporation, as well as of their initial salts differ markedly from each other.

X-ray pictures of new synthesized compounds don't contain diffraction maximums peculiar for initial compounds. So, based on X-ray patterns' analysis it may be said that Chromium-DAS, Zinc-DAS and Manganese-DAS clusters obtained via separation and evaporation are individual compounds.

In order to study biological activity, three doses of Mn, Zn and Cr-chelate mixtures (maximum, minimum and normal) were prepared based on concentrate DAS. When conducting tests on earthworms we set a goal to establish under the impact of chelate mixtures added to substrate: a) earthworm protein mass change; b) degree of cocoon reproduction and c) effective and optimal dose of chelate mixtures. In total, we tested four– one control and three test groups, each one with three repetitions: control group substrate of all three repetitions was dampened by ordinary water, I test group – by aqueous solution containing maximum (0.76g.) dose of chelate mixtures, II test group – by aqueous solution containing normal (0.38g.) dose of chelate mixtures, and III test group – by aqueous solution containing minimum (0.76g.) dose of chelate mixtures. 200g. of dry substrate and 5 earthworms of roughly equal mass were selected for all repetitions of both control and test groups. First weighing and cocoons calculation were made on the 21st day of experiment, while second and third – on 31st and 41st days, respectively. During all three weighing procedures, we calculated average mass and average number of cocoons for each group. After completion of the experiment an average mass change (in g. and percentage terms) and average number of cocoons (in pcs and percentage terms) overall for all stages was calculated for control and all three test groups (Table 3).

Table 3 . *Chelate mixture composition added to premix calculated for 50 kg of combined feed*

Mixture composition	Mass (g.)
Mn·Ds	18.2
Zn·Ds	11.54
Cr·Ds	2.11

As is seen from the Table, weight gain takes place at all three stages throughout the experiment. Compared to initial mass, the average mass change equals 1.98g. in control group and we conventionally took it as 100%. In parallel, the average mass change in I test group (Max.) is 99.49% (1.97g.), in II test group (Norm.) – 106.06% (2.1g.), while in III test group (Min.) – 115.66% (2.29g.). As the Table shows, the degree of cocoon reproduction is far more in all test groups, compared to control one. Particularly, if we conventionally take the average number (in pcs) of cocoon reproduction – 2.66 pieces as 100%, then it equals to 484.58% (12.89 pieces) in I test group, 576.32% (15.33 pieces) in II test group, while in III test group it is 559.65% (14.89 pieces).

Thus, based on the analysis of experimental results it may be said that maximum mass change takes place in III test group 115.66% (2.29 g.), when a minimal dose of chelate mixtures is added, while the degree of cocoon reproduction reaches its maximum value 576.32% (15.33 pieces) in II test group, to which a normal dose of chelate mixtures is added.

Preliminary check-up tests were conducted on broiler chickens for the same chelate mixtures. Two groups were composed for this purpose – test and control. 15 birds were included in each group, and experiment lasted for 35 days. First, the quantity of necessary combined feed was calculated (50kg.), and afterwards – the quantity of cluster chelate mixture added to premix intended for this quantity of feed (Table 4).

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Table 4 . *Main zootechnical indicators of broilers*

Indicators	Groups	
	Test	Control
Live weight (gr): 1-day	40	40
7-day	112	108
14-day	314	292
28-day		
Hen (female)	1026	965
Rooster (male)	1195	1039
Average	**1110.5**	**1039**
35-day		
Hen (female)	1715	1595
Rooster (male)	1845	1700
Average	**1780**	**1647.5**
Daily average weight gain in upbringing period, %	50.86	47.05
Bird survival in upbringing period, %	96.6	93.3

The basic experiment started on 1-day chickens, and combined feed was prepared and delivered in three stages: I stage – 1-7 days, II stage – 7-21 days, III stage – 21-35 days.

During the test there were studied:

- broiler growth and development – through individual weighing on 7, 14, 21, 28 and 35 days.
- average and daily gain;
- bird survival in upbringing period

Main zootechnical indicators of broilers are given in Table 5.

Table 5. *Main zootechnical indicators of broilers*

Groups (dose, gr.)	Container #	Test stages														Results			
		Initial		I weighing				II weighing				III weighing				Mass change		Aver. quant. of cocoons	
		mass (g)	mass (average)	mass (g)	mass (average)	quantity of cocoons	quantity of cocoons (ave.)	mass (g)	mass (average)	quantity of cocoons	quantity of cocoons (ave.)	mass (g)	mass (average)	quantity of cocoons	quantity of cocoons (ave.)	(g)	(%)	(piece)	(%)
Control 0.00	1	1.12	1.18	3.01	2.99	1	1.33	3.15	3.10	5	4.33	3.21	3.16	2	2.33	1.98	100	2.66	100
	2	1.23		2.94		1		3.01		4		3.11		2					
	3	1.20		3.01		2		3.15		4		3.16		3					
Max.(I) 0.76	4	1.34	1.21	3.80	3.43	4	3.67	3.14	3.21	20	19.33	3.12	3.18	17	15.67	1.97	99.49	12.89	484.58
	5	1.14		3.31		3		3.35		19		3.32		15					
	6	1.15		3.18		4		3.15		19		3.09		15					
Norm.(II) 0.38	7	1.24	1.19	3.31	3.22	7	7.67	3.47	3.31	19	19.67	3.49	3.29	18	18.67	2.1	106.06	15.33	576.32
	8	1.29		3.17		8		3.22		20		3.17		19					
	9	1.04		3.18		8		3.25		20		3.23		19					
Min.(III) 0.19	10	1.40	1.32	3.81	3.53	7	6	3.83	3.64	19	19.33	3.82	3.61	19	19.33	2.29	115.66	14.89	559.65
	11	1.41		3.46		5		3.71		19		3.66		19					
	12	1.16		3.32		6		3.37		20		3.35		20					

It is seen from the Table that in 14-day age the live weight of first group broilers prevails by 7.0% those of control group, by 6.4% at the age of 28 days. In the end of the upbringing period – in 35-day age the live weight of test group broiler is 1780g. in average that is 7.4% more than in control group. Daily weight gain in test group is 50.86g. throughout the upbringing period that is averagely 7.7% more compared to control group. Survival in test group was 96.6% in the upbringing period, and 93.3% - in control group.

On the basis of carried out experiments we deem necessary to conduct tests on a wider scale.

Conclusion

Based on carried-out researches the following conclusions can be drawn:

- So-called homonuclear cluster chelate compounds are obtained on the basis of microelements and concentrate DAS, where the atoms of the same metals, number of which depends on synthesis conditions, are bound to each other. Melting temperature and solubility in different solvents depend on number of atoms entering the composition of compounds, as well. Organic substances of different classes forming concentrate DAS are in coordinate bonds with metal atoms in chelate clusters. Under the proper synthesis conditions, in addition to homonuclear clusters, it is possible to obtain heteronuclear, similar- and different-ligand clusters. This fact makes it possible to obtain cluster chelate compounds with pre-planned desirable quantitative/qualitative composition and, respectively, with desirable biological activity.

Through study of biological activity of Mn, Zn and Cr-chelate mixtures prepared on the basis of concentrate DAS it was established that:

- Earthworm substrate balancing with different doses of mixtures has a positive impact on both earthworm protein mass increase and

on the substantial increase of degree of cocoon reproduction. 0.38g. per 200g. of substrate is considered as an optimum, effective dose of chelate mixtures. We suppose that the obtained effect is preconditioned by both Mn, Zn and Cr impact and by the diversity of biologically active compounds of different chemical classes entering into concentrate DAS composition. The obtained results are of great importance for that direction of vermiculture, in which a protein mass is used for balancing combined feed intended for poultry and farm animals as vitamin-protein, high-quality and concentrated additive.

• Based on the results of check-up tests (7.5% weight gain in the test group during upbringing period, and survival is 3.3% higher compared to control group) conducted on broilers for Mn, Zn and Cr-chelate mixtures we deem reasonable to carry out experiments on a wider scale for determination of effective and optimal dose of chelate mixtures.

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