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Human and Animal Physiology

Results of the Study of Arginine-Containing Molybdenum and Boron Chelates

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The aim of the work is the synthesis and research of molybdenum and boron chelating compounds containing arginine. Chelates of the general formula were obtained: $[H_2MoO_4(Arg)_x].yH_2O$ (where x=1 or 2 y=0 or 3); $[(H_3BO_3)x(Arg)_y]. zH_2O$ (where x=1÷3; y=1 or 2; z=2 or 4) using the diffractographic method of study there has been established that chelates are individual compounds. On the basis of spectrophotometric research, the bond character of arginine with molybdenum and boron atoms is shown. An experiment was conducted on rabbits by studying the productivity indicators it was found that all the data of the experimental group are higher than those of the control group.

molybdenum, boron, chelate, arginine, diffractographic study, rabbits

The role and importance of both microelements and organic substances (amino acids, oxy-acids, vitamins etc.) for the normal growth and development of agricultural animals and poultry is widely recognized. At that, the microelements are used in the chelate form in the poultry and animal feed, since in such case microelements have low toxicity, high digestibility and respectively, increased level of efficiency when used in small doses that in its turn provides ecological safety of chelate microelements application. This fact is confirmed both by foreign scientists [1-8], and by the results of our studies conducted for years [9-11]. Exactly these advantages explain the expansion (scale-up) of production of premixes containingchelate-form microelements, compared to non-chelate ones. As for today, a number of vital (essential) microelements, which can't be replaced by other microelements and in case of deficit or absence of which, organisms are unable to normally grow and develop and to end a life-cycle is far more than an amount of microelements (Mn, Zn, Fe, Co, Cu, I) entered in the premixes composition. Among these microelements we have focused our attention on molybdenum and boron, while among bioorganic ligands – on amino acid and arginine.

Molybdenum is characterized by less toxicity compared to other microelements. It enters into composition of khantine oxidase, sulfite oxidase and aldehyde oxidase enzymes, manifests clearly expressed antioxidant properties, provides toxic substances decomposition and release from organism, has a beneficial effect on colon microflora, takes an active part in synthesis of amino acids and vitamins (especially vitamin C). It has fluorine-holding ability that provides normal state maintenance for tooth tissue and gums and let us avoid a caries. Molybdenum facilitates iron assimilation by the organism that promotes anemia prevention. In its turn, molybdenum deficit stipulates anabolic process development, weakening of immune system [12-14].

Boron takes a part in the central nervous system regulation, has an impact on sexual and thyroid glands function. It acts an important role in bone tissue formation, promotes its strength, prevents osteoporosis development. It is assumed that boron improves calcium assimilation by bone tissue. There are some data on life extension under positive effect of boron. At that it is known that boron, in the form of boric acid and its compounds, for a long time has been used as antimicrobial and antiseptic agents [15,16].

Boron and molybdenum as microelements entering into anions composition are characterized by less tendency to formation of chelated compounds. Taking into account the contemporary views on microelements complex-formation ability, we have received the chelate compounds of molybdenum and boron.

Arginine selected by us in the capacity of chelate-forming organic ligand is a basic amino acid, in the living organism it transforms into nitrogen oxide, which promotes blood-vascular system elasticity that is very important in case of angina attack; it substantially increases blood flow to the brain and heart muscles, has a beneficial impact on genital system. Scientific studies confirm the positive effect of nitrogen oxide on arterial blood pressure control, immunity and central nervous system action [17-20].

Materials and Methods

- Diffractographic method of study – for identification of chelates individuality.
- Thermographic analysis method – for study of the-rmal stability and thermal decomposition nature.
- Spectrophotometric method of study – for establishment of bond character between organic ligand and central atom.
- Weighing method at the beginning and at the end of a test – for determination of rabbit live weight (gr) and absolute live weight gain (gr).

Results and Discussion

Following our studies of arginine-containing molybdic acid and boric acid chelate compounds [11], in order to establish their individuality X-ray-diffractometric study of thesynthesized compounds: $[H_3BO_3(Arg)]\cdot 2H_2O$, $[H_3BO_3(Arg)_2]\cdot 2H_2O$, $[(H_3BO_3)_2Arg]\cdot 4H_2O$ $[H_2MoO_4(Arg)]$, $[H_2MoO_4(Arg)_2]\cdot 3H_2O$ has been conducted using DRON-4with $Cu_{k\alpha}(\lambda=0.154184\,nm)$ irradiation. During exposition samples have rotated in their plain by means of special device – GP-13.

As is seen from the analysis of diffractograms (X-Ray diffraction patterns) given in Figs. 1 and 2, location and intensities of diffraction maximums for compounds differ markedly from each other, that points at the fact that in all cases formation of new individual compounds takes place.

In order to study the thermal stability and sequence of thermolysis process in the synthesized arginine-containing chelates $[H_3BO_3(Arg)_2]\cdot 2H_2O$, $[H_2MoO_4(Arg)]$, $[H_2MoO_4(Arg)_2]\cdot 3H_2O$, $[H_3BO_3Arg.]\cdot 2H_2O$, a thermographic study has been conducted using the device: NETZSCH STA2500, with sample heating rate 10 degree/min. As is seen from the thermographic study, all thermograms are

distinguished by several exo- and endo-effects (Table 1).

For boron chelate compound $[H_3BO_3(Arg)_2]\cdot 2H_2O$ the first endo-effect at 150°C corresponds with detachment of 2 moles of water (weight loss: practical 7.89%, theoretical 8.04%), at the ensuing endo-effect (410°C) oxidation of 0.5 moles of arginine takes place (practical 19.44%, theoretical 19.31%), at 530°C – 1 mole of arginine (practical 54.07%, theoretical 53.72%), while a strong exo-effect at 600°C corresponds with oxidation of the rest 0.5 moles of arginine molecule (weight loss: practical 57.45%, theoretical 58.08%), so the mixture of boron and coal is the end product of thermal decomposition.

A thermal dissociation of molybdenum and boron chelates containing water molecule runs in a similar fashion, stage-by-stage. In particular, water molecule detachment takes place at the exo-effect within the range of 110-150°C temperatures; a gradual oxidation of arginine molecules proceeds in the 310-600°C temperature range, also at the endo-

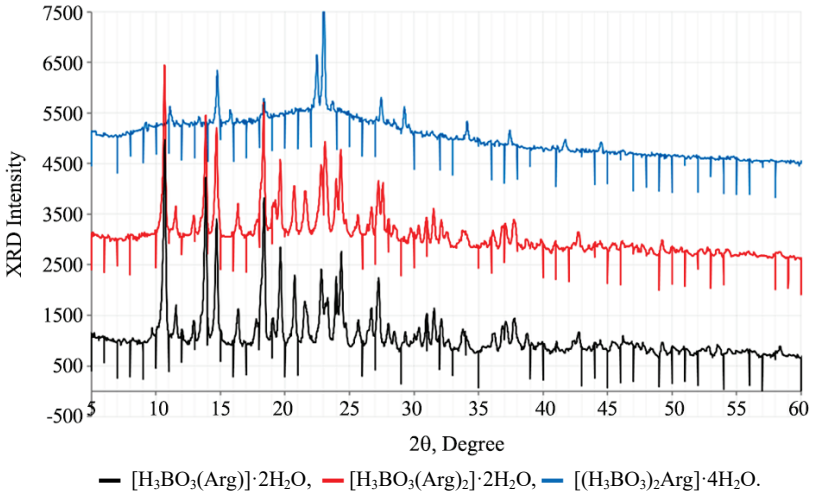


Fig. 1. Diffractogram of arginine-containing boron chelate.

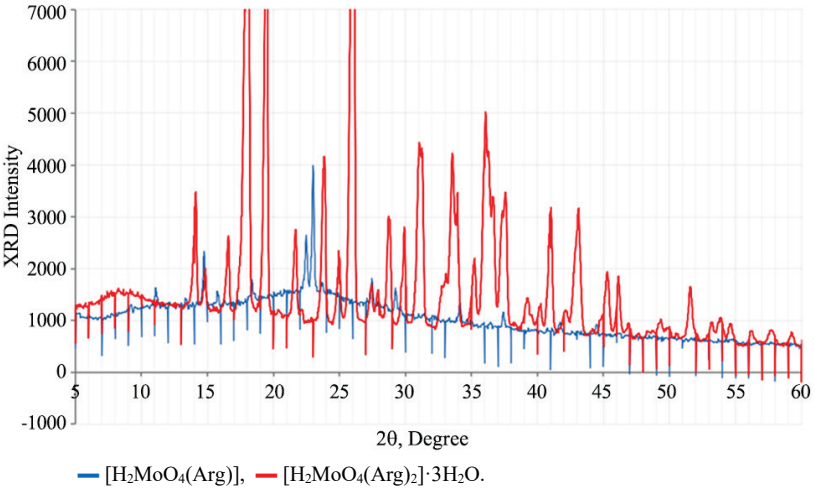


Fig. 2. Diffractogram of arginine containing molybdenum chelate.

Table 1. Results of a temographic study of molybdenum and boron chelates containing arginine

#	formula	T^0 C	mass loss %		detached molecule mole	solid product of decomposition
			Pract.	Theor.		
1	$[H_3BO_3(Arg)_2]\cdot 2H_2O$	150 410 530 600	7.89 19.44 54.07 57.45	8.04 19.31 53.72 58.08	$2H_2O$ 0.5 Arg Arg 0.5 Arg	H_3BO_3 Arg_2 H_3BO_3 $Arg_{1.5}$ H_3BO_3 $Arg_{0.5}$ B +3C
2	$[H_2MoO_4(Arg)]$	280 440 500	13.11 14.04 32.17	12.96 14.89 31.93	0.25 Arg 0.25 Arg 0.5 Arg	$[H_2MoO_4(Arg)_{0.75}]$ $[H_2MoO_4(Arg)_{0.5}]$ MoO_3
3	$[H_2MoO_4(Arg)_2]\cdot 3H_2O$	125 150 310 360 600	5.67 11.99 16.69 40.82 31.99	5.82 12.36 17.06 41.16 32.28	H_2O $2H_2O$ 0.5Arg Arg 0.5 Arg	$H_2MoO_4(Arg)_2]\cdot 2H_2O$ $[H_2MoO_4(Arg)_2$ $[H_2MoO_4(Arg)_{1.5}$ $[H_2MoO_4(Arg)_{0.5}$ Mo_3O_4
4	H_3BO_3 Arg. $\cdot 2H_2O$	110 320 410 480	12.89 18.05 21.79 43.87	13.22 18.45 22.62 44.23	2 H_2O 0.25 Arg 0.25 Arg 0.5Arg	H_3BO_3 Arg H_3BO_3 Arg.$_{0.75}$ H_3BO_3 Arg.$_{0.5}$ B+4 C

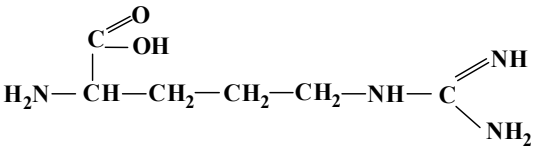
effect, while a strong exo-effect at 480-600°C corresponds with thermolysis process termination and the mixture of molybdenum (or boron) oxide and coal is the end product of thermolysis.

As for thermal decomposition of anhydrous chelate $[H_2MoO_4(Arg)]$ (2), analysis of its thermogravigram shows that the compound thermolysis starts with endo-effect at 280°C, with partial oxidation of arginine molecule. Its decomposition continues at the ensuing endo-effect (440°C) and the thermolysis process ends at the strong exo-effect (at 500°C), with formation of MoO_3.

Thus, based on the analysis of thermolysis process one may conclude that the thermal decomposition of arginine-containing chelate runs by stages: at the first stage a gradual oxidation of water molecules, while at the second – arginine molecules takes place, and the end products of decomposition are presented by the mixture of metallic boron and coal (1), molybdenum oxide (2, 3) or the mixture of boron and coal (4).

In order to establish the arginine nature in the arginine-containing compounds, and its bond character with central atom, IR absorption spectra of synthesized compounds have been studied. IR absorption spectra (400-40000 cm^{-1}) are recorded at spectrophotometer Agilent Cary630, in the form of powder pressed with KBr.

Arginine, which contains amino groups in α-state and guanidine groupsin δ-state, is a basic amino acid:



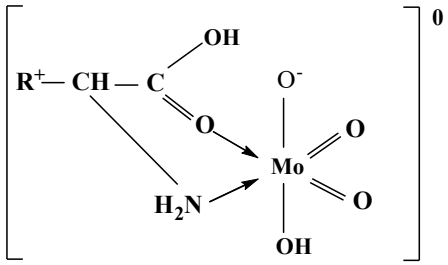
That is why it is capable to simultaneously play the role of monodentate (by means of COOH-group oxygen atoms or α-NH_2 group nitrogen atoms) and bidentate ligand(by means of the same oxygen atoms and α-NH_2 group nitrogen atoms). It is known, as well that according to reaction conditions, amino acids, while forming zwitterions (NH_3^+-RCH-COO^-), bond with central atoms via coordinate linkagesby deprotonated carboxyl-group oxygen atoms and α-NH_2 group nitrogen atoms [20, 21]. During protonization, δ-guanidine NH_2- group thanks to charge resonance delocalization is in a protonated cationic form and has a multiple hydrogen bonds formation ability.

Belonging of some vibration frequencies of IR-absorption spectra of investigated compounds is given in Table 2. Arginine spectral data are given for comparison, as well.

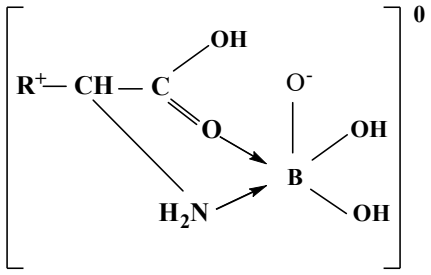
Table 2. Belonging of some vibration frequencies of IR-absorption spectra of compounds under study

Arg	$[H_3BO_3(Arg)]\cdot 2H_2O$	$[H_3BO_3(Arg)_2]\cdot 2H_2O$	$[H_2MoO_4(Arg)]$	$[H_2MoO_4(Arg)_2]\cdot 3H_2O$	Belonging
3350	3345	3360	3375	3355	ν_{as} (NH_2)
3100	3090	3080	3150	3175	ν_s (NH_2)
1680	1650	1690	1660	1675	$\delta(NH_2)$
1750	1745	1755	1735	1740	νCOOH
-	520	560	550	570	ν(M–N)
-	440	420	395	390	ν(M–O)

As is seen from the Table, in the spectrum of the chelate compounds of arginine with boric acid (molybdic acid) $[H_3BO_3(Arg)]\cdot 2H_2O$, $[H_3BO_3(Arg)_2]\cdot \cdot 2H_2O$, $[H_2MoO_4(Arg)]$, $[H_2MoO_4(Arg)_2]\cdot 3H_2O$ absorption bands are perceptible in the spectral region of 1735-1755cm^{-1}, which may be attached to stretching vibrations of non-dissociated carbonyl group (C=O), while the absorption bands in the regions of 3345–3375 cm^{-1} and 3080–3175 cm^{-1} may be referred to asymmetric and symmetric $\nu_{as}(NH_2)$ and $\nu_s(NH_2)$ vibrations of arginine α-amino group, respectively. Absorption bands in the region of 1650-1690 cm^{-1} belong to deformation vibration $\delta(NH_2)$ of arginine α-amino group. Absorption bands observed in 520–570 cm^{-1} and 395–440 cm^{-1} spectralregions, may be referred to stretching vibrations of boron (molybdenum) – nitrogen and boron (molybdenum) – oxygen bonds. Based on this fact, one may suppose that arginine plays the role of cyclic ligand in the compounds. It establishes a bond with a central atom by means ofnitrogen atom of α-amino group and oxygen atom of non-dissociated carbonyl group (C=O), with formation of five-membered me allacycles.



Scheme 1. The character of the bond of arginine with molybdenum.



Scheme 2. The character of the bond of arginine with boron.

We think that guanidylic protonated amino groups form multiple hydrogen bonds.

In order to study the biological activity an experiment has been conducted on rabbits. The research set a goal of study the effect of premixes prepared on the basis of chelate compounds on the productivity of meet breed rabbits.

According to the analogue principle we have selected 30-day young hares (all of them were of male sex and were born at the same day). Two groups have been selected for the experiment: I – test group and II – control group (10 animals in each group, 5 – Great Giants and 5 Californian rabbits). Tests have been lasted for three months. The first weighing has been conducted at the age of 45 days, 15 days later of livestock completing, while the rest stages of weighinghave been carried out at the ages of 60, 90, and 120 days.

The live weight at the beginning of a test was nearly equal and comprised 1.55 kg (control group) and 1.57 kg (test group). Technological parameters of upbringing (temperature, humidity, lightening etc.) were the same for both groups, and only feed was different: rabbits of the I – test group were

nourished throughout a test period by 0.5% premixes, which contained the mixture of molybdenum and boron chelates; II – control group rabbits during a test took a feed containing 1% of rabbit premixes available at the factory.

Impact of premixes prepared on the basis of chelate compounds on the rabbit productivity has been studied. Results have been entered in Table 3.

Table 3. Productivity indices for meat breed rabbits

Indices	Unit of meas.	Group	
		I test	II control
Livestock	piece	100	100
Test period	day	90	90
Survival during a test	%	99	97
Rabbit live weight at the beginning of a test	gr	1570	1550
Rabbit live weight at the end of a test	gr	3310	3150

Resulting from the experiment it has been established that during a test, which lasted 90 days, survival in the first test group has reached 99%, while in the second control group this index was 2% less (97%) (Table 1). It is seen from the Table that at the beginning of a test the live weight of both group rabbits was nearly the same and equaled 1550-1570 gr. At the age of 120 days, after fattening (putting on weight), rabbitsof the I test group had a greater live weight – 3310 gr compared to those of the control group and surpassed them by 160 gr that comprises 4.8%. It is obvious that both the absolute and daily weight gains were higher in the I test group compared to the control one. The absolute weight gain in the I test group was 8.75% higher than in the control group.

Feed consumption during a fattening period was 9.6 kg per animal among the rabbits of the I test group that is 6.25% less than the same index for the control group. During a fattening period, a feed consumption per 1 kg of weight gain was equal to 5.5 kg in the I test group that is 13.78% less than that of the control group.

Conclusion

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

- Arginine-containing molybdenum and boron chelate compounds are individual substances.
- Arginine molecule is bonded with chelate-forming central atom (boron or molybdenum) by means of nitrogen atom ofα-amino group and oxygen atom ofnon-dissociated carbonyl group (C=O), with formation of five-membered metallacycle.
- Using the study of biological activity it is established that entering of arginine-containing molybdenum and boron chelate compounds into compositions of combined feed premixes causes increase in rabbit productivity index, which is predetermined by the fact that when using microelements in the form of chelate compounds there takes place improvement of animals physiological state, inhibition of feed mass impaction through gastrointestinal tract (prolongation effect) and therefore, increase in feed digestibility, toxicity reduction, immune system stability improvement and feed consumption reduction.

ადამიანისა და ცხოველთა ფიზიოლოგია

არგინინის შემცველი მოლიბდენის და ბორის ხელატების კვლევის შედეგები

ი. ბეშკენაძე*, ნ. კლარჯეიშვილი*, მ. გოგალაძე**, ე. სალუქვაძე*, ლ. ჯაფარიძე*

*ივანე ჯავახიშვილის სახ. თბილისის სახელმწიფო უნივერსიტეტი, პეტრე მელიქიშვილის ფიზიკური და ორგანული ქიმიის ინსტიტუტი, თბილისი, საქართველო
**საქართველოს ტექნიკური უნივერსიტეტი, აგრარული მეცნიერებების და ბიოსისტემების ინჟინერინგის ფაკულტეტი, თბილისი საქართველო

(წარმოდგენილია აკადემიის წევრის ნ. მითაგვარიას მიერ)

სამუშაოს მიზანია არგინინის შემცველი მოლიბდენის და ბორის ხელატური ნაერთების სინთეზი და კვლევა. მიღებულია ხელატები ზოგადი ფორმულებით: $[H_2MoO_4(Arg)_x]\cdot yH_2O$ (სადაც x=1 ან 2 y=0 ან 3); $[(H_3BO_3)_x(Arg)_y]\cdot zH_2O$ (სადაც x=1÷3; y=1 ან 2; z=2 ან 4). დიფრაქტოგრაფიული კვლევის მეთოდით დადგენილია, რომ ხელატები წარმოადგენს ინდივიდუალურ ნაერთებს. სპექტროფოტომეტრული კვლევის საფუძველზე ნაჩვენებია არგინინის ბმის ხასიათი მოლიბდენის და ბორის ატომებთან. ჩატარებულია ექსპერიმენტი ბოცვერზე, რომელთა პროდუქტიულობის მაჩვენებლების შესწავლით დადგენილია, რომ საცდელი ჯგუფის ყველა მონაცემი აღემატება საკონტროლო ჯგუფისას.

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