Biosafety of the application of biogenic nanometal powders in husbandry

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Abstract

Effects of iron and copper nanopowders (particle size of 20–40 nm) were investigated on rabbits of 1 month age and heifers of 6 months. For introduction of nanometals into the animal’s ration, the mixed fodder was treated with the nanometal powder suspension in such a way: 0.08 mg of nanoiron per kg of animal’s body weight and 0.04 mg kg\(^{-1}\) for nanocopper. The weight gain of the heifers who received nanoiron and nanocopper after 8 months was 22.4 and 10.7% higher than that of the control, respectively. For the rabbits who received nano Fe and Cu after 3 months, the weight gain was 11.7 and 7.3% compared to the control, respectively. Under the action of metal nanopowders morphological indices of blood were changed in comparison with the control: after 8 months the quantity of erythrocytes increased by 19.6%, hemoglobin by 17.1% and leukocytes by 7.6%. There was a realignment in leukocytic formula: the quantity of lymphocytes increased by 9% compared to the control. Biogenic metals in superdispersive state were able to stimulate immune, enzymatic and humoral systems of the animal’s organism, promoting metabolism. Adding Co and Cu metal nanopowders to the bull-calves’ fodder rations increased content of Ca by 31.8 and 0%, Fe by 38.8 and 37.5%, K by 19.2 and 15.3%, Mg by 17.6 and 23.5%, Mn by 9.8 and 45% and Na by 20.5 and 8.8%, respectively, compared to control. Metal nanopowders improved the quality indices and meat productivity of black–white bull-calves, expressed in intensive growth of muscle, tissue and more nutritious meat. The conducted veterinary–sanitary expertise showed that the supplements based on iron, cobalt and copper nanopowders can be used as safe bioactive supplements in animal husbandry.

Keywords: biosafety, husbandry, nanoiron, nanocopper, leukocyte

Classification numbers: 1.00, 2.03, 2.04, 4.02, 4, 04

1. Introduction

Among the factors appraising usefulness of growth and development of farming animals, bioactive substances (BAS), including those based on biogenic microelements, are of great interest. These elements are part of vitally important substances, participate in the processes of synthesis and disintegration, absorption and desorption of different biochemical products and form a favorable medium for normal function of enzymes, hormones and vitamins. The modern market of bioactive preparations used in agriculture is saturated by domestic as well as western products. However, on the whole, all well-known biostimulators for animals are based on the use of growth hormones. These products interacting with animals’ systems are able to embed into the process of metabolism and their permanent intake might result in adaptation. In consequence, their own hormonal systems would be inactivated. All the above-listed factors give rise...
to an irregular growth as well as strongest violation of the metabolism and, as a result, to the acquisition of dangerous products for human health. Modern agricultural production requires the development and introduction of new products, operation principles and composition which are different from traditional analogues.

Development of new safe and highly effective products is the research objective in the agroindustrial sector. The basis of these products may be metal nanopowders, in particular copper and iron [1, 2]. Distinctive features of copper and iron nanoparticles are their small size (20–30 nm) and extra-small doses used. Results of our research have demonstrated their effectiveness in plant growing and cattle breeding.

The aim of this study is to demonstrate the application safety of iron and copper nanopowders in animals feeding, as well as to study the effects of nanoparticles on biological properties of the tested objects and possibility of the further safe use in animal production for human nourishment (according to the regulations of SanPiN 2.3.2.1078-01 index 1.1.1).

2. Material and methods

Iron and copper nanopowders were received from Moscow Institute of Metallurgy and Materials Science named after A A Baikov, wherein the zerovalent metal nanopowders were produced by low-temperature hydrogen reduction method. Before preparation of fodder rations mixed with nanocrystalline metals (particles size of 20–30 nm), the nanopowder was dispersed in deionized water and sonicated for 30 min (800 W, 25 kHz) to obtain superdispersive suspension.

The research was carried out in 2006–2012 and consisted of two experimental sets—laboratory and scientific-economic ones. The objects of the study were rabbits of strain ‘Soviet shinshilla’ (92 head), heifers of strain ‘black–white’ (32 head) and black–white bull-calves (32 head). Animals used for experiment were kept in identical conditions of feeding and maintenance. Introduction of metal nanopowders into animals’ rations was carried out by spraying metal suspensions of different concentration on the fodder mixture during fodder preparation on the basis of 11 of suspension per 1 ton of mixed fodder [1]. Concentration of the prepared suspensions were calculated to obtain appropriate quantity of the nanometal in a live weight unit (0.04, 0.06, 0.08, 0.10 and 0.120 mg kg⁻¹ of animal’s live weight).

Blood parameters (quantity of erythrocytes, hemoglobin, trombocytes, leukocytes and leukocytic formula) were determined on a hematological analyzer ‘Abacus Junior Vet’. Total proteins, albumins, content of enzymes γ-glutamyl transpeptidase, alkaline phosphatase, α-amylase, uric acid, creatinine, total cholesterol, K, Na, Ca, P, Cl, Fe, etc were determined on an automatic immunoenzymatic analyzer ‘ChemWell’.

Slaughter of trial and control animals as well as tissues sampling were conducted in a specialized slaughter shop. For implementation of the study on biosafety of slaughter products three individuals of age 18 months from each group were taken, then the slaughter of the animals and tissue sampling were carried out. Laboratory studies of the slaughter products were conducted using the following analytical methods: content of peptides in meat was analyzed on a gas chromatograph ‘Crystal 2000 M’, heavy metals, on a voltamperometric analyzer TA-2. Amino acid content in muscles was determined with an automatic analyzer AAA-T-399.

3. Results and discussion

3.1. Determination of optimal doses of metal nanopowders for rabbits

Since nanomaterials have not been used in agriculture for a relatively long time, it was necessary to track the biosafety chain soil–plant–animal by successively studying the effects of metal nanoparticles on the soil and on the plants, which were used in animals rations, and then on the laboratory animals.

A concentration of 0.080 mg of iron nanopowder per kg live weight might be considered as the optimum dose. According to the experimental data, this dose was able to increase the rabbit’s weight up to 7.5% compared to the control on the 30th day after introduction of iron nanopowder, while the other nanoiron doses gave less effect. For copper nanopowder the optimum dose was found to be 0.04 mg kg⁻¹ live weight, which allowed increasing the weight of trial animals by 4.8% compared to the control. Stopping introduction of the metal nanopowder did not result in the decrease of animals’ bodyweight, on the contrary, it continued to increase even after 10 days of stoppage of metal nanopowder’s introduction. Morphological blood indices of the tested rabbits were within the physiological norm and at the same time content of erythrocytes, hemoglobin and leukocytes increased, witnessing a positive effect of nanometals on the animal’s general state.

3.2. Effects of metal nanopowders on physiological state of rabbits

Introduction of optimum doses into rabbits’ rations improved the animals’ live weight (table 1) and morpho-biochemical blood parameters (table 2). It can be seen that all rabbits in

Table 1. Live weight of rabbits after introducing metal nanopowders into fodder rations at the 30th day.

| Age (days) | Group 1 | Group 2 | Group 3 |
|-----------|---------|---------|---------|
| 30⁴       | 850 ± 10| 840 ± 20| 840 ± 15|
| 40        | 1030 ± 30| 1130 ± 40| 1090 ± 50|
| 50        | 1150 ± 50| 1250 ± 50| 1185 ± 50|
| 60        | 1250 ± 50| 1360 ± 50| 1290 ± 50|
| 70        | 1550 ± 50| 1700 ± 50| 1610 ± 50|
| 80        | 1850 ± 50| 2010 ± 25| 1930 ± 50|
| 90        | 2050 ± 90| 2290 ± 80| 2200 ± 50|

Notice p ⩽ 0.05.
⁴ The day of metal introduction.
Group 1 (Control): fodder without metal nanopowder.
Group 2: fodder with Fe nanopowder.
Group 3: fodder with Cu nanopowder.
three groups were active, regularly gathered their weight and possessed good appetite. A positive result of their introduction of superdispersive metals into animals’ rations became remarkable after 10 days from the introduction [3].

Introduction of iron metal nanopowder into rations at the 30th day reliably increased live weight of rabbits by 8.8% after 10 days and by 11.7% to the end of the experiment, while for copper metal nanopowder by 7.3% compared to the control (table 1). Investigating rabbit’s blood in all tested groups it was shown that there was a reliable increase of the content of erythrocytes, hemoglobin, leukocytes, total proteins and enzymes.

The best indices were found in the group that received iron nanopowder. In this case the quantity of erythrocytes increased by 9.3%, hemoglobin by 9.1% and leukocytes by 15.9%, while leukocytic formula changed with the increase of lymphocytes (by 8%) and the decrease in granulocytes (by 8%), and content of total proteins in blood serum was enhanced to 10.5%. The increase of the content of γ-globulins and lymphocytes (table 2) confirmed the increase of immunological reactivity. Similar changes were found in other experimental groups. The increase of the content of α1-, γ-globulins and β-globulins brought strengthening of the transport of carbohydrates and lipids to the tissues and activated the metabolism [4].

The experimental data presented in tables 1 and 2 showed that iron nanopowder gave a better live weight value than copper nanopowder, but morpho-biochemical blood parameters were indistinguishable.

3.3. Effect of metal nanopowders on physiological indices of heifers and bull-calves of ‘black–white’ strain

Black–white heifers and bull-calves with an age of 4 months and live weight of 95 or 120 kg were taken for the experiment. Live weight of the tested animals within the experimental period presented in tables 3 and 4 showed that during the whole period of experiment live weight of the tested animals always exceeded that of the control. In particular, with the addition of iron nanopowder to heifers’ rations after 8 months live weight increased by 22.4% and of copper nanopowder by 10.7% compared to the control. With the addition of nanocrystalline cobalt to bull-calves’ rations after 12 months, live weight increased by 13.4% and of copper by 11.1% compared to the control. Influence of metal nanopowders on the increase of live weight can be explained by the ability of the metal nanoparticles to catalyze many biochemical processes in animal organism, strengthening digestivity and assimilation of nutrient substances in the rations, as well as increase of the activity of red-ox processes and metabolism [5, 6].

3.4. Effect of metal nanopowders on morpho-biochemical indices of black–white heifers and bull-calves

Morpho-biochemical and mineral parameters in blood of nanometals-treated heifers are demonstrated in tables 5 and 6. These parameters similarly changed those of the experimental groups of rabbits. The increase of the content of lymphocytes in leukocytic formula corroborated the change of protective functions, while the increase of hemoglobin and erythrocytes improved hemopoietic functions. The presence of metal nanopowders in animal’s rations stimulated the growth and renovation of blood cells and enhanced oxidative processes. As a result, the animal’s physiological state was improved [7, 8].

Addition of iron nanopowder to the rations considerably increased the content of many enzymes in the animal’s blood. It may be linked with the increase of intensity of protein

### Table 2. Morpho-biochemical blood parameters of rabbits, which received metal nanopowders.

| Blood parameters | Group 1 | Group 2 | Group 3 |
|------------------|---------|---------|---------|
| Erythrocytes \( \times 10^{12} \text{l}^{-1} \) | 5.4 ± 0.3 | 5.9 ± 0.3 | 5.8 ± 0.3 |
| Hemoglobin (g l\(^{-1} \)) | 110 ± 2.0 | 120 ± 3.0 | 118 ± 2.0 |
| Trombocytes \( \times 10^{9} \text{l}^{-1} \) | 205 ± 6.0 | 200 ± 7.0 | 210 ± 5.0 |
| Leukocytes \( \times 10^9 \text{l}^{-1} \) | 4.1 ± 0.4 | 5.3 ± 0.5 | 5.1 ± 0.6 |

Leukocytic formula (%)

- **Lymphocytes** 43 ± 0.3
- **Monocytes** 6 ± 0.02
- **Granulocytes** 51 ± 0.5
- **Total serum proteins (g l\(^{-1} \))** 52.5 ± 1.6

Protein fraction (%)

- **α\(_1\)-globulins** 3.4 ± 0.1
- **α\(_2\)-globulins** 11.2 ± 0.3
- **β-globulins** 8.0 ± 0.04
- **γ-globulins** 10.0 ± 0.3
- **Albumins** 67.4 ± 0.8

### Table 3. Live weight of heifers after introducing metal nanopowders into fodder rations at the fourth month.

| Age (months) | Group 1 | Group 2 | Group 3 |
|-------------|---------|---------|---------|
| 4\(^a\) | 95.0 ± 1.5 | 94.4 ± 1.2 | 94.0 ± 1.3 |
| 5          | 112.8 ± 2.3 | 122.0 ± 2.1 | 116.5 ± 2.1 |
| 6          | 132.3 ± 2.8 | 146.8 ± 2.6 | 138.4 ± 2.9 |
| 7          | 147.8 ± 2.7 | 173.2 ± 2.9 | 155.0 ± 3.0 |
| 8          | 166.6 ± 3.1 | 199.6 ± 3.0 | 180.2 ± 2.9 |
| 9          | 187.8 ± 3.4 | 219.5 ± 3.8 | 200.0 ± 3.5 |
| 10         | 202.0 ± 4.0 | 238.5 ± 3.9 | 218.5 ± 4.3 |
| 11         | 216.5 ± 4.3 | 260.5 ± 4.2 | 237.5 ± 4.5 |
| 12         | 233.8 ± 4.5 | 286.1 ± 4.8 | 258.8 ± 4.5 |

\( p < 0.05 \).

\(^a\) The month of metal introduction.

Group 1 (Control): fodder without metal nanopowder.

Group 2: fodder with Fe nanopowder.

Group 3: fodder with Cu nanopowder.

### Table 4. Live weight of bull-calves after introducing metal powders into fodder rations at the fourth month.

| Age (months) | Group 1 | Group 2 | Group 3 |
|-------------|---------|---------|---------|
| 6          | 165.8 ± 4.4 | 183.2 ± 3.8 | 174.5 ± 5.6 |
| 12         | 280.8 ± 3.8 | 318.1 ± 4.8 | 311.8 ± 5.1 |
| 15         | 341.6 ± 3.0 | 389.0 ± 3.1 | 378.5 ± 1.2 |
| 18         | 402.6 ± 1.9 | 459.8 ± 8.3 | 448.3 ± 4.9 |

\( p < 0.05 \).

Group 1 (control): fodder without metal nanopowder.

Group 2: fodder with Fe nanopowder.

Group 3: fodder with Cu nanopowder.
the animal’s organism. That also linked with the increase of the protein exchange in the same time, quantity of uric acid and creatinine, respectively, increased on average by 5.1, 3.6, 15.5 and 3.9% compared to the control. Meanwhile, content of iron and copper in the animal’s blood increased on average by 7.9 ± 0.5 mmol l⁻¹. The data presented in table 5 showed that iron and copper nanopowders, in animal’s blood the content of K, Na, Ca and P increased on average by 16.7 and 6.8% compared to the control, that also linked with the increase of the protein exchange in the animal’s organism.

The data presented in table 6 showed that iron and copper metallic nanoparticles stimulated the process of mineral exchange. After 8 months of the experiment with nanopowders, in animal’s blood the content of K, Na, Ca and P increased on average by 5.1, 3.6, 15.5 and 3.9% compared to the control, respectively. The increase of Ca and P content promoted the formation of bone tissues, which is vitally important for the growth and development of young animals. Meanwhile, content of iron and copper in the animal’s blood

Table 5. Morphological and biochemical blood indices of heifers, which received iron and copper nanopowders.

| Blood indices | Group 1 | Group 2 | Group 3 |
|---------------|---------|---------|---------|
| Morphological indices |         |         |         |
| Erythrocytes × 10¹² l⁻¹ | 5.6 ± 0.2 | 6.7 ± 0.3 | 6.2 ± 0.2 |
| Hemoglobin (g l⁻¹) | 105 ± 5 | 123 ± 5 | 118 ± 4 |
| Trombocytes × 10⁹ l⁻¹ | 338 ± 21 | 310 ± 29 | 330 ± 25 |
| Leukocytes × 10⁹ l⁻¹ | 7.9 ± 0.5 | 8.5 ± 0.3 | 8.0 ± 0.6 |
| Leukocytic formula (%) |         |         |         |
| Lymphocytes | 61 ± 4 | 70 ± 2 | 62 ± 4 |
| Monocytes | 5 ± 1 | 4 ± 1 | 5 ± 2 |
| Granulocytes | 34 ± 4 | 26 ± 3 | 33 ± 3 |
| Stab neutrophil | 3 | 2 | 1 |
| Segmentonuclear neutrophil | 30 ± 2.0 | 22 ± 2 | 28 ± 4 |
| Basophils | – | 1 | 1 |
| Eosinophils | 1 | 1 | 3 |
| Biochemical indices |         |         |         |
| α-amylose (mg l⁻¹ l⁻¹) | 3.5 ± 0.1 | 4.5 ± 0.2 | 3.8 ± 0.3 |
| Alkaline phosphatase (µmol l⁻¹) | 310 ± 15 | 401 ± 10 | 325 ± 24 |
| γ-glutamnitranspeptidase (µmol l⁻¹) | 14.0 ± 3.2 | 16.2 ± 1.5 | 15.5 ± 1.2 |
| Uric acid (mmol l⁻¹) | 0.03 ± 0.004 | 0.035 ± 0.006 | 0.03 ± 0.001 |
| Creatinin (µmol l⁻¹) | 3.0 ± 0.3 | 2.5 ± 0.3 | 2.4 ± 0.3 |
| Cholesterol (mmol l⁻¹) | 8.5 ± 2.9 | 74.5 ± 2.4 | 69.6 ± 3.7 |
| Protein fractions |         |         |         |
| α₂- globulins (%) | 4 ± 1 | 4 ± 0.1 | 6 ± 0.5 |
| α₂- globulins (%) | 16 ± 0.8 | 5 ± 0.1 | 9 ± 0.5 |
| β- globulins (%) | 12 ± 1 | 13 ± 1.1 | 14 ± 0.9 |
| γ- globulins (%) | 30 ± 2 | 36 ± 2.7 | 31 ± 1.9 |
| Albumins (%) | 38 ± 4 | 42 ± 3.4 | 40 ± 2.6 |

p ≤ 0.05.
Group 1 (Control): fodder without metal nanopowder.
Group 2: fodder with Fe nanopowder.
Group 3: fodder with Cu nanopowder.

Table 6. Effect of metal nanopowders on the mineral content in heifer blood serum and bull-calf muscular tissue.

| Chemical elements | Heifers’ blood serum 8 months experiment | Bull-calves’ muscles 12 months experiment |
|-------------------|----------------------------------------|----------------------------------------|
|                   | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 | Group 6 | Group 7 | Group 8 |
| K | 4.53 ± 0.1 | 4.82 ± 0.1 | 4.71 ± 0.04 | 2.6 × 10³ | 3.1 × 10³ | 3.0 × 10³ |
| Na | 139.6 ± 1.4 | 144.2 ± 1.4 | 145.0 ± 1.0 | 340 ± 3 | 410 ± 5 | 370 ± 4 |
| Ca | 2.10 ± 0.1 | 2.50 ± 0.1 | 2.35 ± 0.04 | 220 ± 2 | 290 ± 2 | 220 ± 2 |
| P | 2.30 ± 0.1 | 2.40 ± 0.1 | 2.29 ± 0.04 | – | – | – |
| Fe | 16.9 ± 0.6 | 20.9 ± 0.3 | 19.3 ± 0.5 | 240 ± 2 | 314 ± 3 | 330 ± 4 |
| Cu | 21.6 ± 1.0 | 28.1 ± 0.5 | 26.8 ± 0.7 | 2.50 ± 0.02 | 2.80 ± 0.03 | 2.70 ± 0.04 |
| Co | – | – | – | 0.10 ± 0.03 | 0.12 ± 0.03 | 0.12 ± 0.04 |
| Cr | – | – | – | 1.40 ± 0.04 | 1.30 ± 0.06 | 1.30 ± 0.05 |
| Mg | – | – | – | 170 ± 1.5 | 200 ± 1.9 | 210 ± 1.4 |
| Mn | – | – | – | 5.10 ± 0.04 | 5.60 ± 0.06 | 7.40 ± 0.08 |
| Mo | – | – | – | 0.2 ± 0.005 | 0.2 ± 0.003 | 0.2 ± 0.002 |

p ≤ 0.05.
Group 1 (control): fodder without metal nanopowder, mmol l⁻¹.
Group 2: fodder with Fe nanopowder, mmol l⁻¹.
Group 3: fodder with Cu nanopowder, mmol l⁻¹.
Group 4 (control): fodder without metal nanopowder, mg kg⁻¹.
Group 5: fodder with Co nanopowder, mg kg⁻¹.
Group 6: fodder with Cu nanopowder, mg kg⁻¹.
The fact that the de Rittis index for both control and trial groups was within the physiological range when content of aminotransferases increased, indicates the normal development of the bull-calves. Simultaneously, the reduction of urea and creatinine in blood indicated the increase of nitrogen compounds’ fixation, which was proved by free amino acids presence in animals’ blood and muscles. The experimental data (table 8) indicated that by the end of the experiment (12 months) essential amino acids in the blood of the bull-calves, which received cobalt and copper nanopowders, increased by 8.2 and 8.8%, respectively, while with nanocopper by 4.7 and 2.4%, respectively.

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content of toxic elements Pb, Cd, As and Hg in slaughtered products of metal nanopowder-treated heifers and bull-calves were much smaller than the MPC (maximum permissible concentration) and even smaller than that of the control. These results proved that iron and copper nanopowders digested by animals did not favor the assimilation of toxic elements from fodder rations.

Results of veterinary–sanitary examination of the slaughtered products of the animals which received metal nanopowders from fodder proved that content of toxic metals and pesticides was much less than the permissible values, while antibiotics were not detected and microbiological indices were in compliance with the existing standards.

4. Conclusions

It was proved that nanocrystalline metals can be used to stimulate the metabolic processes, enhancing livestock yield and improving total physiological state of animals. Optimum doses of metal nanopowders added to fodder rations were determined: 0.08 mg of iron nanopowder for 1 kg of live weight in 1 day, for copper nanopowder 0.04 mg kg\(^{-1}\) live weight.

Inclusion of nanocrystalline metals in rabbits’ rations improved their physiological state. Live weight gain by using Fe nanopowders accounted for 11.7% and by using Cu nanopowders 7.3%. Metal nanopowders stimulated hemopoiesis function, increasing the quantity of erythrocytes by 9.3%, hemoglobin 9.1% and the per cent composition of leukocytic formula was changed toward the increase of lymphocyte quantity by 8% compared to the control. Total proteins in rabbits’ blood increased by 10.5%, indicating the stabilization of protein exchange and \(\gamma\)-globulin by 2.5%, indicating the improvement of immunobiochemical reactions.

Introduction into farming animals’ rations of nanocrystalline metals at optimum doses furthered the enhancement of live mass: for the case of heifers, by using iron nanopowder the live weight increased by 22.4%, quantity of erythrocytes by 19.6%, of hemoglobin by 17.1%, leukocytes up 7.6%, lymphocytes up 9%, total protein up 8.8%, \(\gamma\)-globulins up 6%, \(\alpha\)-amylase by 28.6%, alkaline phosphatase by 29.3% and \(\gamma\)-glutamyltranspeptidase by 26.9%, indicating the increase of intensity of protein and carbohydrate metabolism.

Analytical results of morphobiochemical and mineral indices of bull-calves’ blood and muscles showed that metal nanopowders introduced into fodder rations favored the activation of digestive enzymes, improving digestion and assimilation of nutrient substances by animals, stabilizing metabolic processes and function of enzymatic systems.

The conducted veterinary–sanitary examination allowed us to conclude that nanocrystalline metals can be used as BAS in fodder rations for farming animals. The slaughtered products of animals grown with nanodispersive biogenic metals were ecologically and biologically safe and can be used in food for humans.

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