Perspectives of Using Ultra-Fine Metals as Universal Safe Bio-Stimulators to Get Cattle Breeding Quality Products

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Abstract. We have conducted investigations of ultra-fine metals biological activity with lab non-pedigree white rats, rabbits breed “Soviet chinchilla” and cattle young stock of the black and white breed as the most widely spread in the central part of Russia. One can see the possibility of using microelements of ultra-fine iron, cobalt and copper as cheap, non-toxic and highly effective biological catalyst of biochemical processes in the organism that improve physiological state, morphological and biochemical blood parameters increasing activity of the experimental animals’ ferment systems and their productivity and meat biological value. We have proved the ultra-fine powders safety when adding them to the animals’ diet.

1. Introduction
Modern vet and livestock technologies are closely connected with nano-technologies providing potential vector of solving many actual problems of mankind, mainly food industry. The aim of agriculture and livestock breeding in particular is providing people with high quality full value food products. Healthy animals’ products consumption is the basis for human diseases prevention, improving lifetime and its quality.

As far as transferring the livestock sector to the industrial ground and increasing production it is necessary to pay more attention to full value, balanced animal nutrition and increasing the index of fodder efficiency. Being the necessary component of many biologically active compounds like proteins, ferments, hormones, vitamins and pigments or influencing their functions microelements participate in different processes of animals’ life activity and metabolism. Bio-drugs of new generation, i.e. microelements in the form of metals ultra-fine powders (MUFP) arouse great interest. Ultra-fine powders of iron, cobalt, copper, manganese, selenium and others have higher biological activity. Investigations conducted in last years have shown their efficiency in crop breeding, fodder production and livestock raising [1, 2, 3, 11].

2. Methods
We have considered the multi-factorial variant of using nanomaterials, i.e. ultra-fine metals as highly active bio-stimulators of animals’ physiological processes. This optimizes the hormone, immune and bio-chemical status of animals and increases the young stock livability.

We have studied these bio-drugs in different regions of Russia (Moscow, Ryazan, Tula and other oblasts, Krasnodar and Stavropol territories). We have been studying ultra-fine powders at Ryazan State
Agrotechnological University Named after P.A. Kostychev since 1997. We had experiments in 2001-2014 and they included lab and farm tests.

3. Results and Discussion

3.1. Complex influence of iron, copper and cobalt ultra-fine powders in the system Plant – Animal

We have shown the possibility of their use in agriculture as cheap and non-toxic drugs partially solving the problem of expensive micro-fertilizers deficit for feed crops. In a case of adding these drugs to the animals’ diet there is improvement of rabbits’ physiological state (live weight, survival and offspring livability and reproduction) and morpho-biochemical parameters of their blood.

Based on the experimental data connected with metallic nano-powders use for vetch seeds cultivar Lygovskaya-28 pre-plant treatment we have estimated that the optimal concentration of iron and cobalt ultra-fine powders is 0.03 g per 12 kg of seeds. At that the vetch green mass yield has increased by 25.5% and 32.1 % correspondingly. Ascorbic acid in vetch green mass has 1.5 times increased when seeds pre-plant treatment with iron nano-powder and 3-4 times when using cobalt nano-powder as compared with the control. Iron and cobalt nano-powders have increased carotene in vetch green mass 1.5 and 4-5 times correspondingly as compared with the control. When treating the seeds with nanoparticles there has been increase of water-miscible polysaccharides by 68 %, protein by 45 % and lectin has declined by 24 % with iron nano-powder and by 59 % with cobalt nano-powder that has increased the vetch fodder value. Adding the vetch grass grown with iron and cobalt nano-powders to the rabbits’ diet has increased live weight gain on the average by 9 % and 18 % correspondingly as compared with the control due to the increase of polysaccharides and decline of lectin fraction.

Adding vetch grass grown with iron and cobalt nano-powders has stimulated the hematogenesis function that manifested in erythrocytes and hemoglobin increase. There has also been a change of leucocytes formula percentage to lymphocytes increase. One could also see some true increase of crude protein in blood serum at the expense of globulin-rich fraction that has testified the increase of immune-biological reaction (Table 1). The increase of LDG has presupposed intensively going glycolysis, i.e. carbohydrate metabolism strengthening and ALT increase has shown protein metabolism stimulation.

| Table 1. Morpho-Biochemical Blood Parameters of Rabbits that Received Metal Nanopowders |
|---------------------------------------------|---------------------------------------------|---------------------------------------|
| Blood Parameters                           | Control                                    | Fe Nanopowder                          | Cu Nanopowder                          |
| Erythrocytes, *1012/l                      | 5.4±0.03                                  | 5.9±0.02                               | 5.8±0.04                               |
| Hemoglobins, g/l                           | 110±2                                     | 120±3                                  | 118±0.5                                |
| Trombocytes, n.103/l                       | 205±6                                     | 200±7                                  | 210±3                                  |
| Leukocytes, *109/l                         | 4.1±0.04                                  | 5.3±0.05                               | 5.1±0.06                               |
| Leukocytic formula, %                      |                                           |                                       |                                        |
| Lymphocytes                                | 43.0±0.3                                  | 51.0±0.4                               | 50.0±0.4                               |
| Monocytes                                  | 6.0±0.2                                   | 6.0±0.3                                | 6.1±0.01                               |
| Granulocytes                               | 51.0±0.5                                  | 43.0±0.4                               | 44.0±0.4                               |
| Total serum proteins, g/l                  | 52.5±0.06                                 | 58.0±0.07                              | 56.5±0.05                              |
| Protein fraction, %                        |                                           |                                       |                                        |
| α1 - globulins                             | 3.4±0.05                                  | 4.0±0.5                                | 4.2±0.06                               |
| α2 - globulins                             | 11.2±0.03                                 | 6.5±0.04                               | 9.5±0.02                               |
| β - globulins                              | 8.0±0.4                                   | 10.6±0.05                              | 13.0±0.1                               |
| γ - globulins                              | 10.0±0.3                                  | 12.5±0.04                              | 12.0±0.2                               |
| Abumins                                    | 67.4±0.08                                 | 66.4±0.07                              | 61.3±0.08                              |

Note: - P ≤ 0.05
The degustation commission has discovered that organoleptic parameters (taste, aroma, delicacy, juiciness and others) of broth, fried and boiled meat of the experimental and control groups have not differed greatly and can be eaten without any restrictions.

3.2. Influence of nano-crystal metals on physiological state of lab and agricultural animals and safety of livestock products when adding them to the diet

Nano-powders are in meta-stable state and therefore have higher chemical activity subjected both dimensioned and structural factors and changed mechanical, electrical, optical and other properties of substances. Nano-particles possess more energy than traditional powders. So they have got a number of unique physical and chemical properties. Nano-particles are digested and produce physiological action with the organism several times stronger than familiar natural mineral substances or their chemical analogues. Biological activity of nano-powders is due to small sizes of particles and their possibility to penetrate into the organism. As opposed to metal ions nano-particles before their penetration into the plant and animals cells do not have any charge therefore they do not form large complexes with transmitting protein and can easily pass through pores of plasmodesma in a bi-lipid layer of cell membranes sized 50 nm that is comparable with the size of nano-particles (25-35 nm) and get into cytoplasm. One can see the nano-powders effect on the organism in nano-particles on the cell and micro-molecular levels [4, 5].

For full development and safe implementation of biologically active drugs based on metals nano-powders sized 25-45 nm in livestock raising we have developed their veterinary and toxicological characteristic. As a result of the conducted experiments we have estimated that nano-powders of iron, cobalt, copper and copper oxide do not possess any cumulative properties and differ by lower toxicity than inorganic salt of these metals. This allows using them in premixes as a source of micro-elements [6, 7]. We have tested their efficiency for prophylaxis of cows' postnatal complications and newborn calves’ enteric infections. The primary evaluation of safety and efficiency has shown high preventive properties of the drug and its safety.

As a result of some preventive measures the cows’ lochial period has passed without any complications. The cows have been hogging timely that has proved the normal revival of the reproductive system after calving. 80 % of animals getting the drug have not got any postnatal complications (endometritis). 20 % of the experiment cows have had postnatal endometritis in a benignant catarrhal form. More than 60 % of cows have been inseminated in one hog without being stimulated by hormones. The newborn calves’ prophylaxis has left out young stock mortality from enteric infections. The use of nano-materials having high biological activity can make a considerable contribution to solving this problem and offer an efficient system of diseases prophylaxis by activating own protective strength of the organism.

We have studied the influence of metals ultra-fine powders on immune, bio-chemical and hormone parameters of the organism so that physiologically justify the use of metals nano-particles as non-medicinal drugs of animals' prophylaxis and correction.

We have chosen animals to the groups for the experiment according to the principle of the balance group-analogues considering gender, age, breed, live weight and the same feeding and housing conditions. We have taken blood from the side ear vein four- timely at interval of 10 days before morning feeding. We have determined all blood parameters (hemoglobin, erythrocytes, leucocytes, leucocytic formula and blood sedimentation rate) according to unitized methods of clinical diagnostics [9]. We have analyzed the biochemical parameters of blood with spectrophotometer “Spectrum” firm “Abbot” according to unitized methods of clinical lab investigations.

3.3. Determining optimal doses of metals nano-powders when adding them to the diet of lab animals

We have had experiments with rabbits breed “Soviet chinchilla” aged 30 days. We have got 10 experiment groups 16 rabbits weighing 800±0.5 g each. We have used the mixed fodder treated with the drugs suspension to add metals nano-powders to the diet. We kept the rabbits in recommended
housing and 2 times feeding conditions. The influence of different doses of nano-crystal metals on live weight in the control and experiment groups of animals is shown in figure 1.

![Figure 1. Live Weight of Lab Rabbits, g](image)

One can consider 0.08 mg/kg of live weight as the optimal dose of iron nano-powder. The increase of the animals’ weight has been 25% after 30 days of giving the drug. The optimal cobalt nano-powder concentration has been 0.02 mg/kg that has allowed increasing the live weight by 27 %. The corresponding data for copper have been 0.04 mg/kg and 26 %. The cancellation of MUFP has not led to decline of the experiment animals’ weight. Moreover the tendency of mass increase has remained even after 10 days after the cancellation of metals ultra-fine powders (Figure 1). We have studied the influence of these concentrations nano-powders on rabbits’ physiological parameters and morpho-biochemical parameters of blood. The experiment lasted for 60 days and the age of the animals at the beginning of the experiment was 30 days. The animals of all 4 groups have regularly gained the weight. They have been active and got a good appetite. Adding iron nano-powder to the diet has increased the rabbits’ live weight by 11.7 % by the end of the experiment as compared with that of the control, cobalt nano-powder by 17.8 % and copper by 16.3 %. We have also investigated morphological and biochemical blood parameters of the experiment animals.

All parameters of blood serum when adding nano-crystal metals have been within the limits of the physiological norm. But there have been authentic increase of erythrocytes and hemoglobin, crude protein at the expense of the globulin fraction and α1- and β-globulins in particular. Increasing these fractions leads to strengthening the active transfer of carbohydrates and lipids to tissues that influences the globulins number and activity that activates metabolism. One can see the same character of changes with γ-globulins. At the expense of γ-globulins increase as compared with the control there has been an increase of immune-biological reactivity as they are defensive antibodies (immunoglobulins) and are responsible for specific immune reply. The immune system that is structurally and functionally organized complex of lymphoid cells interacting with each other and accessorial cell elements on some stages of immunogenesis can serve an indicator reflecting the nano-materials affect on the organism. If consider that microelements in ultra-fine state activate the immune, ferment and humoral systems of the organism promoting the increase of metabolism and better digestion and nutrition then one can use them as bio-additives [10, 11].

3.4. The influence of nano-crystal iron, cobalt and copper on the physiological state of the young stock (heifers)

We have had experiments with black and white cattle as most widely spread in Ryazan oblast at farm JSC “Starozhilovo stud farm”.

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We have treated the mixed fodder with water powders suspension before feeding the animals. Suspension consumption has been 1 l per 1 t of mixed fodder. They have added the treated mixed fodder to the diet of the control and experiment animals on the basis of 2 kg of mixed fodder per 1 animal a day during the whole experiment. The age of experiment and control animals at the beginning of the experiment has been approximately 4 months. The experiment lasted for 8 months. We weighed all animals once a month. The first group (control) did not get ultra-fine powders, the second one (experiment) got nano-crystal iron (0.08 mg/kg of live weight a day), the third experiment group has got nano-crystal cobalt (0.02 mg/kg), the forth experiment group has got nano-crystal copper (0.04 mg/kg).

The experiment animals during the whole experiment have regularly exceeded the control animals in live weight beginning from the 1st month of the experiment. At that, every next month the weight gain of the live weight of the experiment heifers has increased. Adding nano-crystal iron to the heifers’ diet has promoted the live weight increase in 8 months by 22.4 % as compared with the control, cobalt by 13.7 % and copper by 10.7 % as compared with the control. Daily average gain has also increased (Figure 2).

![Figure 2. Influence of Iron, Cobalt and Copper Nano-Powders on Gross Increase of Experimental Animals, kg](image)

By the 8th month of the experiment the gross gain in the group getting iron nano-powder has exceeded the control by 53.8 %, the group getting cobalt nano-powder by 25.4 % and the group with copper nano-powder by 23.1 %.

The increase of live weight, daily average and gross gain in a case of adding nano-crystal metals proves catalytical action of these bio-drugs that manifests in metabolism strengthening on the whole and protein metabolism in particular.

3.5. Influence of nano-crystal iron, cobalt and copper on morphological parameters of heifers’ blood

| Blood Parameters       | Control | Fe Nano-Powder | Co Nano-Powder | Cu Nano-Powder |
|------------------------|---------|----------------|----------------|---------------|
| Erythrocytes, *10^12/l | 5.60±0.08| 6.70±0.07      | 6.60±0.02      | 6.20±0.03     |
| Hemoglobins, g/l       | 105.0±0.5| 123.0±0.5      | 120.0±0.4      | 118.0±0.6     |
| Trombocytes, 10^9/l    | 338±0.9  | 310±0.8        | 315±0.4        | 330±0.5       |
| Leucocytes, 10^9/l     | 7.9±0.05 | 8.5±0.03       | 8.3±0.04       | 8.0±0.06      |
| Leucocytic formula, %  |         |                |                |               |
Copper nano-powder influence on blood morphological content (Table 2) has manifested in little rise of erythrocytes (by 10.7 %) and hemoglobin (by 12.4 %) as compared with the control. All other blood parameters have remained within the norm and differed inconsiderably from the control.

One could see leucocytes increase in the 8th month of the experiment by 7.6 % (iron) and 5.1 % (cobalt) and the change of the leucogram, i.e. lymphocytes increase for iron cobalt and copper has been 11.4 %, 8.2 % and 5 % higher than the control. These changes prove the increase of the immune system of animals as the main function of B-lymphotcytes is producing antibodies or protective immunoglobulins. The lymphocytes increase has caused the significant decline of granulocytes and neutrophils lower than the control in particular.

The lymphocytes increase in the leucogram has proved strengthening protective functions, whereas the increase of hemoglobin and erythrocytes has proved the improvement of the hemopoietic system of the organism. With nano-powders there is growth and renewal of blood cells and strengthening of oxidation processes that is one of the criteria in diagnosing and evaluating the nano-materials safety.

### 3.6. Influence of nano-crystal iron, cobalt and copper on bio-chemical blood parameters of the experiment animals

Bio-chemical blood parameters reflect metabolism of protein, fat, carbohydrates vitamins, hormones and water-mineral characteristics of the organism. They let interpret the growth and development of the organism, help to discover latent diseases.

Using ultra-fine iron has considerably activated much ferment. So by the end of the experiment ALT content in blood serum has increased by 12.5 % and AST by 7.6 % as compared with the control. At that de Rittis coefficient (correlation of AST to ALT) has changed from 1.65 in the control to 1.40 in the first experiment group of animals that got nano-iron and up to 1.55 in the 2nd and 3rd experiment groups that got nano-cobalt and nano-copper correspondingly. This parameter in the experiment groups has reached the physiological norm (1.2 – 1.4) that proves stabilization of aniamals’ metabolisms. These ferment activation in the blood of the experimental animals is connected with strengthening the protein metabolism that, in its turn, leads to intensive gain of the live weight (Table 5). This is also connected with the increase of γ-glutamyltransferase in blood serum by 14.2 - 21.4 % when using nano-metals as compared with the control. This ferment participates in amino acids metabolism and its activity also reflects protein metabolism intensity. The uric acid has increased by 13.3 - 23.3 %, urea by 13.6 - 18.2 %, creatinine by 5.5 - 9.5 % higher than the control one. These substances are products of protein metabolism and the fall under residual nitrogen and the increase of their amount are connected with strengthening protein metabolism intensity in animals.
Table 3. Biochemical Parameters of Heifers Blood when Adding Iron, Cobalt and Copper Nano-Powders to Their Diet

| Blood Parameters                        | Control             | Fe Nano-Powder | Co Nano-Powder | Cu Nano-Powder |
|-----------------------------------------|---------------------|----------------|----------------|---------------|
| 1. Alanine Aminotransferase (AAT), mmol/l | 0.8±0.01            | 1.0±0.06       | 0.9±0.04       | 0.9±0.03      |
| 2. Aspartate Aminotransferase (AST), mmol/l | 1.30±0.01           | 1.4±0.02       | 1.6±0.01       | 1.4±0.05      |
| 3. α-amylase, mg/sec*1                   | 3.5±0.01            | 4.5±0.02       | 4.2±0.03       | 3.8±0.05      |
| 4. Alkaline Phosphatase, u/l             | 310±1               | 401±1          | 345±2          | 325±3         |
| 5. γ-glutamitranspeptidase, u/l          | 14.0±0.3            | 16.0±0.5       | 17.0±0.2       | 16.0±0.2      |
| 6. Uric Acid, mmol/l                    | 30.0±0.4            | 35.0±0.6       | 37.0±0.2       | 34.0±0.1      |
| 7. Urea, mmol/l                         | 2.2±0.02            | 2.5±0.04       | 2.6±0.03       | 2.4±0.04      |
| 8. Creatinine, mmol/l                   | 73.0±1              | 78.0±0.5       | 77.0±0.2       | 80±0.4        |
| 9. Cholesterol, mmol/l                  | 3.4±0.03            | 2.5±0.02       | 2.2±0.04       | 2.4±0.03      |
| 10. Crude Protein, g/l                  | 68.5±0.09           | 74.5±0.04      | 66.8±0.01      | 69.6±0.03     |

11. Protein Fractions:

| Abumins, % | 38.0±0.4 | 42.0±0.03 | 40±0.8 | 40±0.6 |
| α1-globulins, % | 4.0±0.4 | 5.0±0.1 | 5.0±0.3 | 6.0±0.5 |
| α2-globulins, % | 16.0±0.8 | 8.0±0.1 | 10±0.6 | 9.0±0.5 |
| β-globulins, % | 12.0±0.7 | 13.0±0.4 | 13.0±0.2 | 14.0±0.9 |
| γ-globulins, % | 30.0±0.2 | 38.0±0.7 | 33.0±0.6 | 31.0±0.7 |

Note: - P ≤ 0.05

Cholesterol in blood of the experimental animals as compared with the control has declined by 30% that positively influences the animals' physiological state. This is proved by the increase of α-amylase by 17.1 – 28.6% as compared with the control. The intensive growth of the organism also proves the activity of alkaline phosphatase. Its content is 24.2 - 29.3% higher than the control. This ferment participates in regulating the processes of bone tissue formation and catalyzes phosphorus metabolism in animals and the increase of alkaline phosphatase activity is particularly important for the growing organism.

Such a considerable change of ferment system activity is connected with using some inconsiderable amount of nanocrystal metals that has presupposed some additional increase of micro-elements at the expense of these elements better digestion in animals' digestive duct.

3.7. Nano-metals influence on mineral substances in animals' blood, muscle tissue and liver
The results of our investigations have proved that nano-metals stimulate accumulation of other macro- and micro-elements (Table 6). That has been proved by the analysis of blood, muscles and liver mineral composition.

Table 4. Mineral Substances Content in Heifers Blood Serum when Adding Iron, Cobalt and Copper Nano-Powders to Their Diet

| Blood Parameters | Control   | Fe Nano-Powder | Co Nano-Powder | Cu Nano-Powder |
|------------------|-----------|----------------|----------------|---------------|
| K (Kalium), mmol/l | 4.5±0.08  | 4.8±0.06       | 5.0±0.1        | 4.8±0.04      |
| Na (Natrium), mmol/l | 138.0±0.4 | 144.0±0.3      | 142.0±0.7      | 145.0±0.1     |
| Ca (Calcium), mmol/l | 2.1±0.05  | 2.5±0.06       | 2.4±0.06       | 2.4±0.04      |

Note: - P ≤ 0.05
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After 8 months of the experiment there has been an authentic increase of kalium by 6.6-11.0 %, natrium by 3.5 -5.1%, calcium by 14-19 % and phosphorus by 4.4-13.0 % in blood of animals that have got ferum nano-powder. The content of chlorine ions has increased by 5.1-8.8 %. There has also been 6.0 % increase of iron and 9.0 % of copper as compared with the control.

In this case the iron increase has promoted copper accumulation. This proves that we have selected correct doses as iron surplus in the organism could have led to copper and calcium deficit.

Table 5. Influence of Nano-Powders in the Diet on Mineral Substances in Muscle Tissue and Liver of Heifers, mg/kg

| Substance      | Muscle Tissue |Liver  |
|----------------|--------------|------|
|                | Control      | Fe Nano-Powder | Co Nano-Powder | Cu Nano-Powder | Control | Cu Nano-Powder | Co Nano-Powder | Cu Nano-Powder |
| Barium (B)     | 27±0.1       | 29±0.1       | 26±0.1        | 27±0.1         | 25±0.1   | 30±0.1        | 27±0.1         | 27±0.1         |
| Calcium (Ca)   | 210±0.2      | 370±0.3      | 280±0.8       | 210±0.6        | 40±0.9   | 80±0.3        | 20±0.4         | 0±0.2          |
| Cobalt (Co)    | 0.10±0.03    | 0.13±0.005   | 0.12±0.002    | 0.12±0.005     | 1.1±0.03 | 1.3±0.05      | 1.2±0.02       | 1.4±0.05       |
| Chrome (Cr)    | 1.5±0.04     | 1.7±0.03     | 1.2±0.06      | 1.4±0.05       | <0.1     | <0.1          | <0.1           | <0.1           |
| Copper (Cu)    | 2.5±0.07     | 3.0±0.1      | 2.9±0.03      | 2.7±0.04       | <0.1     | <0.1          | <0.1           | <0.1           |
| Iron (Fe)      | 250±0.2      | 255±0.3      | 264±0.3       | 282±0.4        | 190±0.2  | 200±0.3       | 204±0.6        | 200±0.1        |
| Kalium (K)     | 2.7*10^3     | 3.4*10^3     | 3.0*10^3      | 2.6*10^3       | 3.8*10^3 | 2.8*10^3      | 3.0*10^3       | 3.0*10^3       |
| Magnesium      | 170±0.5      | 190±0.7      | 210±0.9       | 200±0.4        | 140±0.5  | 170±0.7       | 150±0.9        | 150±0.4        |
| Manganese      | 5.3±0.04     | 6.9±0.06     | 5.4±0.06      | 7.1±0.08       | 8.8±0.04 | 9.3±0.06      | 9.4±0.06       | 8.1±0.08       |
| Molybdenum     | 0.2±0.05     | 0.3±0.02     | 0.2±0.03      | 0.2±0.02       | 0.7±0.05 | 0.9±0.02      | 0.8±0.03       | 0.6±0.02       |
| Natrium (Na)   | 350±0.3      | 360±0.4      | 430±0.5       | 380±0.4        | 530±0.3  | 600±0.4       | 680±0.5        | 700±0.4        |
| Nickel (Ni)    | <0.1         | <0.1         | <0.1          | <0.1           | <0.1     | <0.1          | <0.1           | <0.1           |
| Plumb (Pb)     | <0.1         | <0.1         | <0.1          | <0.1           | <0.1     | <0.1          | <0.1           | <0.1           |
| Selenium (Se)  | 0.5±0.03     | 0.6±0.04     | 0.4±0.02      | 0.5±0.03       | 0.7±0.03 | 0.9±0.04      | 0.5±0.02       | 0.8±0.03       |
| Strontium (Sr) | <0.1         | <0.1         | <0.1          | <0.1           | <0.1     | <0.1          | <0.1           | <0.1           |

Note: - P ≤ 0.05

Ultra-fine cobalt and copper have also contributed to kalium, calcium and phosphor increase in blood. Other substances content has not changed (Table 5).

Iron nano-powder in the diet has increased the content of calcium, copper, kalium, magnesium and manganese in muscles 20%-30 % higher than those of the control. Iron in muscle tissue has not exceeded the control one that shows the absence of cumulative properties of the drug and its ecological safety. Strengthening digestion and metabolism processes has not led to heavy metals accumulation in muscles that has influenced positively the general physiological state of the animals. Cobalt and copper nano-powders have also changed the content of mineral substances in animals’ muscles but the level of cobalt and copper themselves has not exceeded the control one. At that heavy metals have not accumulated. One can find similar results when analyzing the mineral composition of the control and experiment animals’ liver. We have not seen any metals including iron, copper and cobalt accumulation in blood, liver and muscles of the animals.
Table 6. Nano-powders Influence on Vitamins in Heifers Tissues, mg/100 g

| Substance | Muscle Tissue | Liver | | | |
|---|---|---|---|---|---|
| | Control | Fe Nano-Powder | Co Nano-Powder | Cu Nano-Powder | Control | Fe Nano-Powder | Co Nano-Powder | Cu Nano-Powder |
| Vitamin A | 1.2± | 1.4± | .5± | 1.7± | 3.5± | 4.8± | 3.9± | 4.5± |
| | 0.02 | 0.03 | 0.04 | 0.03 | 0.02 | 0.04 | 0.03 | 0.04 |
| Vitamin C | 8.4±0.03 | 9.7±0.05 | 8.8±0.07 | 9.3±0.06 | 17.7±0.1 | 20.9±0.2 | 19.5±0.1 | 21.3±0.02 |
| | 0.27± | 0.28± | 0.30± | 0.31± | 0.21± | 0.22± | 0.28± | 0.26± |
| E | 0.004 | 0.005 | 0.003 | 0.006 | 0.003 | 0.004 | 0.005 | 0.003 |

Note: - P ≤ 0.05

3.8. Biochemical investigations of internal tissues and organs

We have determined the protein value of meat as for the correlation between essential and non-essential amino acids. The number of essential amino acids in meat protein of animals that have got iron nano-powder has increased by 3.9 %, in case with cobalt by 4.2 % and copper by 4.8 % that means the increase of the meat food value of the experiment heifers. There has also been some increase of glycine, histidine, methionine and serine, in particular stimulating anti-oxidants accumulation.

The content of fatty acids in subcutis of the experiment animals has not differed from that of the control one that proves the absence of pathological biochemical processes in the organism and the normal development of lipid metabolism. There has been an increase of vitamin A up to 11-35%, vitamin C up to 10-20 % and E (up to 33 %) in the liver and in muscles (A 17-42 %; C 8-16 %; E to15 %) of the experiment animals that has also increased meat value as a food product.

If at the beginning of the experiment the amino acid composition has been approximately the same in the control and experiment groups, by the end of the experiment there has been a considerable common increase of amino acids content in blood of the animals getting cobalt nanopowder by 26.8 % and copper by 21.9 % as compared with the control (Figure3,4).

![Figure 3. Essential Amino Acids in Blood after Feeding Cobalt and Copper Nano-Powders](image)
We have got similar conclusions when studying the influence of cobalt and copper nano-particles on physiological and productive parameters of black and white bull calves (2008-2010). We have used the suspension on the basis of 1 liter per 1 ton of the mixed fodder. The metals nano-powders activity has remained during 1 month. The animals getting metals nano-powders have had higher digestive activity than the control ones. The dry matter digestion index has been 3.9 % and 2.5 % higher than the control for cobalt and copper nano-powders. The organic matter content has been 6.8 % and 3.5 % higher, protein 5.6 % and 3.8 % higher, fat 4.4 % and 3.8 % higher, fiber 3.6 % and 4.0 % higher and azotic extractive matters 3.8 % and 4.0 % higher than those of the control for cobalt and copper nano-powders correspondingly. Adding cobalt and copper nano-powders has caused the increase of nitrogen and calcium balance in the organism and the decline of their fecal and urinary excretion.

There have been some changes of the experiment animals’ meat productivity parameters. As a result of the slaughter the cows having nano-cobalt in the diet have had the hot carcass mass increased by 18.7 %, the dead weight by 16.9 % and the slaughter yield by 4.0 % as compared with the control. For copper the hot carcass mass has increased by 14.0 % and the slaughter yield by 3.5 %. At that the meat energetic value has increased by 10.1 – 12.5 %.

3.9. Immunity increase under the influence of iron, copper and cobalt nano-particles
We have conducted investigations during the postpartum period characterized by the organism immune depression. In many instances one can consider it to be the reason for most cows having complications as a result of necessary protective strengths absence. In comparison with ill animals the cows getting metals nano-particles safely escaped complications of the postpartum period. At that they have had T indexes, total lymphocytes, considerably lower, only 38.3 % as opposed to 53.1 % of ill animals, whereas healthy cows have had this index equal to 44 %. This shows the absence of total lymphocytosis and low specific immunity in the experiment group. T-killers of the tested cows have been considerably higher (24.7 %), especially under the influence of nano-particles as compared with sick animals having 3.7 %. This fraction provides activation of the specific cell immunity. As the animals having such high exponents under nano-particles have not fallen ill we can consider it a favorable factor. B-lymphocytes have been vice versa lower in the experiment group that also shows the absence of complications development threat. In this situation the organism of the animal with the help of nano-particles copes with the activation of only the cell link not including the humoral one that is, as you know, increases when acute bacterial infections. One can also connect with this T-helpers lowering of cows having got nano-particles and stable high level of T- suppressors. We suppose
stimulating the immunity is connected with the immune protective action of metals nano-particles. Copper and iron are important bio-metals participating in immune reactions. Their deficit weakens the function of the immune system: the decline of antimicrobial activity of macrophages, tissue saturation with granulocytes, cell eating suppression and anti-bodies formation. Production of lactoferrin that is an immune response modifier and an important component of supporting homeostase of the organism systems, participating in transporting iron and possessing antimicrobial, antitoxic and antiphlogistic activity is also connected with iron. All these facts prove the immune stimulating action of iron, copper and cobalt nano-particles in cows’ bodies.

3.10. Milk productivity
We have studied milk productivity and milk quality of the first calf black and white heifers having iron ultra-fine powder in winter housing. We have formed 2 groups of animals numbering 20 animals in each of them. While doing that we have considered the animals’ age, live weight and the cows’ pregnancy period. All the experiment animals have weighed 470... 475 kg being 5...6 months pregnant and been kept in the same housing and feeding conditions. As for feeding difference the experiment first calf heifers got not only the balanced diet (according to norms) but the mixed fodder with 0.009 mg of iron per 1 kg of the mixed fodder a day.

Table 7. Milk Productivity of First Calf Cows in the First Phase of Lactation

| Exponent | Control | Experimental I | Experimental II |
|----------|---------|----------------|----------------|
| Milk Yield, kg: |       |                |                |
| Daily Average Milk Yield, kg | 14.0 | 15.9 | 15.8 |
| Natural Milk within 100 Days of Lactation | 1402 | 1599 | 1508 |
| Fat Content, % | 3.90±0.06 | 4.2±0.01 | 4.3±0.02 |
| Milk Fat within 100 Days of Lactation, kg | 58.0 | 63.6 | 63.5 |
| Protein, % | 3.40±0.06 | 3.90±0.05 | 3.56±0.05 |
| Density, °A | 29.1±0.01 | 29.5±0.01 | 30.0±0.01 |
| Acidity, °T | 18.5±0.02 | 18.3±0.01 | 18.0±0.05 |
| Energy Value of 100 g of milk, kJ | 290.0±0.28 | 292.0±0.49 | 296.0±0.51 |
| Calcium in Milk, mg | 98.2±0.08 | 106.0±0.2 | 108.0±0.5 |
| Phosphorus in Milk, mg | 83.7±0.06 | 86.8±0.08 | 89.1±0.04 |

Note: P ≤0,05;

Adding iron ultra-fine powder do the diet of the experiment animals has increased the daily average milk yield of the first calf heifers by 13.3 % as compared with the control (Table 7). For the first 100 days of the experiment group first calf heifers’ lactation we have got 147 kg more milk as compared with the control group. Fat in the milk of the experiment animals has been 4.25 % that is 9.0 % higher than that of the control group. Milk fat in the milk for 100 days of lactation in the experiment group has been considerably higher (5.6 kg) than that in the control group as they have got more milk from the experiment cows and fat mass percentage in it has been a little higher.

Organoleptic estimation of the experiment first calf heifers’ milk has not shown any differences. It has met the requirements of GOST R 52054-2003 in taste, color and smell.

All parameters under study of the experiment group have to some extent exceeded the analogues of the control group. Calcium and phosphorus in milk of the experiment group first calf heifers has been 9.0% higher than those of the control group (Table 7).

Physical-chemical properties of milk determined by concentration and the dispersiveness degree of the compounds are of great importance for the processing industry. To physical-chemical properties of milk one can attribute density and acidity. Milk density of the experiment first calf heifers has been...
0.1-0.9° A higher that that of the control. The base-titratable acidity of all experiment animals’ milk has not practically differed and corresponded to the norm. Thus, adding ultra-fine iron to the diet has increased milk yield, milk fat and improved milk chemical composition.

4. Conclusions
Ultra-fine microelements affect the organism mainly indirectly changing activity of ferments, hormones, protein, vitamins and other biologically active substances including metals or sensitive to their concentration change in the environment. Adding nano-crystal metals especially iron and cobalt promotes intensive accumulation of the animals’ live weight due to the positive influence of protein, carbohydrates and mineral substances digestion. This supposition has been proved by morphological and biochemical investigations of blood and the content of mineral substances in blood serum. Ultra-fine microelements activate immune, enzymatic and humoral systems of the organism promoting metabolism increase and better digestion and fixation of the diet.

The increase of protein, carbohydrates and mineral metabolism leads to considerable increase of the experiment bull calves’ and black and white heifers’ meat productivity and the quality parameters of meat (chemical and biochemical composition, vitamins, essential amino acids, macro- and micro-elements) and milk.

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