ABSTRACT
Research goal was obtaining of ant stress, ecologically clean preparation with high bio-accessibility (bio-digestibility) and low toxicity intended for oral administration, and determination of prospects of its use in the area of live-stock farming (namely pig breeding). Manufacturing method of mentioned preparation is elaborated, which foresees the use of freshly-prepared iron carbonate paste synthesized via interaction of FeCl₂·4H₂O and NaHCO₃ as a source of main active component – Iron (II); interaction of iron carbonate and cobalt chloride with complex formation with monosaccharide D-Fructose having hemo-stimulating properties; concentrating of complex solutions up to syrup consistency; its extraction from reaction area in the free state using alcohol-ether mixture, its treatment with acetone, ether, and drying in vacuum conditions; infusion of complex mixtures containing certain quantities of Fe(II) Fructose and Co(II) Fructose with aqueous Askan-clay (through ultrasonic material dispersion); preparation of water suspension, its drying, grinding, manufacturing of solid form of preparation for oral administration. The preparation manufactured by mentioned method contains (in mass %): Fe(II)- Fructose 15.75-31.6, [Fe(II)- 3.75-7.50], Co(II)-Fructose 0.28, [Co(II)-0.07], natural Askan-clay 68.2-36.5. The offered method provides getting of highly digesstible, functional targeted product with maximum content of Fe(II). Therapeutic and preventive efficiency of manufactured preparation was tested on animals under study, namely on store pigs (toxicity of preparation was preliminary tested on laboratory white rats). Experiment result was expressed in getting rid of complications (iron deficiency anemia, diarrhea, dyspepsia) caused by stress factors related to termination of breast feeding of store pigs and food change, as well as in their normal growth and development, normal blood chemistry values and live weight gain.

Key words: Antianemic Fe(II)-complex, Bentonite, Application in pig breeding

INTRODUCTION
Metal deficiency anemia in animals is caused by deficit of indispensable microelement of vital importance – iron. Iron deficiency anemia in newly-born pigs is predetermined by significant difference between growth rates of newly-born pigs and quantity of microelements delivered with mother’s milk. Due to iron deficiency takes place functional disturbance of hematogenic (blood-forming) organs, there is observed the low level of hemoglobin and erythrocytes, vulnerability towards different diseases (diarrhea, body dehydration etc.). Against the background of anemia diseases pigs experience development of immunodeficiency, suppression of erythropoiesis, takes place development of secondary diseases of digestive, breathing and other organs. Based on the above mentioned it can be said that anemia is a general pathological process of many body organs and system as a whole. As a result, pig-breeding branch experiences significant economic damage. In the major part of large pig-farming enterprises pig anemia reaches 100%, while mortality index attains 10-15% (Bushev, 2011; Misik, 2006; Nikoladze, 2003; Biryukov, 2013).

Based on the above mentioned it is topical to offer a preparation for pig-breeding branch, which will supply animals with additions containing biologically active microelements, aminoacids, hydrocarbons, vitamins etc. under conditions of intensive growth and metabolic processes developing with speed-limit in organism that is characteristic for pigs (Kopteva, 2011; Shulaev and Dobry, 2003; Kokorev, 2013).

Use of non-traditional natural mineral additions, as the cheap source of calcium, phosphorus, silicium, magnesium and other indispensable micro- and macromolecules easily

digestible for animals is especially important. Various adsorbents, including natural minerals—zeolites (Ivanov and Grigorieva, 1998), glauconites, bentonites, marls etc., are added to animal ration with a mentioned purpose. Priority is always given to food additions prepared on the basis of cheap, local natural raw materials (Japparov et al., 2011).

Research goal was the manufacturing of antianemic preparation containing biogenic microelements Fe(II) and Co(II), hemo-stimulating organic compound D-fructose (Fru) and natural mineral, bentonite-Askan-clay used as an adsorbent, as well as its testing in live-stock farming (pig-breeding) for treatment and prevention of stress conditions related to termination of breast feeding of store (young) pigs (Gabela et al., 2014., Japaridze et al., 2017; Salukvadze et al., 2017).

Separation from mother pig is the most critical moment of piggies’ life— from the birth till a killing. In case of early termination of breast breeding period (26th day from the birth) two-stress-factors— separation from mother breast and food change have significant negative impact on animals (Avilov, 2006, Bekenev et al., 2015, Martinez-Miro et al., 2016). The mentioned negative development may cause recurrence of iron deficiency anemia (that was no more registered after two times injection of iron-containing injection solution to piglets).

There are frequent manifestations of complications in functioning of gastrointestinal tract (dyspepsia-diarrhea). As a result, takes place deterioration of general physiological and blood chemistry values of animals (Kairov, 2010, Barannikov, 2012).

**MATERIALS AND METHODS**

At the first stage Fe(II),Co(II)-D-Fru complexes are synthesized separately, under conditions of constant stirring, as a result of interaction between corresponding salts and monosaccharide D-fructose. Fe(II),Co(II)-D-Fru complexes are obtained in the alkaline medium (PH = 10-11). It should be noted that the yield of the complex Fe (II) with fructose, obtained resulting from interaction of iron source FeCl₂·4H₂O and D-fructose is low and equals to 74.34%. 25.66% of total iron quantity is in oxidized Fe(III) condition (Gabela et al., 2009). It is known that iron (III) ions, in contradistinction from iron (II) ions are characterized by low bi-accessibility (Stuklov and Semyonova, 2013). In order to manufacture the targeted product with maximum content of Fe(II) the method is developed that envisages the use of freshly prepared FeCO₃ as an iron source.

FeCO₃ was obtained through the interaction of hot saturated solutions of NaHCO₃ and FeCl₂·4H₂O·, under conditions of constant stirring. Reaction runs with emission of gas (CO₂). By means of decantation water is removed from the deposit formed after sedimentation. Afterwards it is washed thoroughly first with flowing water, and then by distilled water up to removal of chlorine ions. As a result, green-colored iron(II) carbonate is obtained, which is kept under water layer (in order to get rid of oxidation of bivalent iron ions into trivalent ones), (Japaridze et al., 2018).

Solutions of synthesized complexes are concentrated up to sirup consistency in the vacuum-evaporator at 60°C. Extraction of the complexes in the pure form from the reaction site is made by the mixture of alcohol-ether solutions. Complexes are washed out by acetone, ether and finally are dried using the lyophilisation method.

The second stage implies the bentonite-Askan clay drying at 90-100°C, down to a constant weight, its swelling by the distilled water (ratio clay : water = 1:5), paste preparation (moisture 25%). After 24 hours 20% of hydrochloric acid is added to a swelled, paste consistency Askan-clay (alkaline bentonite, pH of which is 9.15) in order to equate its acidity to 3.5 (according to preliminary researches conducted under conditions of different pHs it is established that a maximum adsorption at the Askan-clay surface is reached in the range of pH = 3.5-5.0). So, the mixtures of iron (II) and cobalt (II) complexes are entered into the prepared paste (pH = 3.5), which is treated for 1 hour via ultrasound dispersion method at 22 kHz. Then the distilled water is added. Water suspension is obtained. Suspension is dried at 60-70°C, is grinded in the ball mill and is sieved in 6400 hole/cm² sieve. Finally, the solid form of antianemic medical and preventive preparation for oral administration is obtained. The composition of the preparation is given in Table 1.

Manufacturing of medical and preventive preparation against animal anemia foresaw synthesis of Fe(II) and Co(II)-fructose complexes separately, under different temperature conditions, as a result of interaction of corresponding salts and D-fructose; infliction of mixture containing certain quantities of obtained complexes (through ultrasonic material dispersion) with Askan-clay water suspension paste, drying of obtained product, grinding, and manufacturing of solid form of preparation for oral administration. It should be noted that in case of FeCl₂·4H₂O use as Fe(II) source and resulting from D-fructose interaction Fe(II) content in the synthesized preparation is 74.32% of total iron content, while 25.66% is in oxidized Fe(III) condition It is noteworthy that the yield of Fe(II) fructose obtained as a result of interaction of FeCl₂·4H₂O (used as iron source) and D-fructose is low and equals to 74.34%. 25.66% of total iron quantity is in oxidized Fe(III) condition (Gabela et al., 2009). It is known that Fe(III) ions in contradistinction from Fe(II) ions are characterized by low bio-accessibility (Stuklov and Semyonova, 2013). With the purpose of preparation of Fe(II) targeted product with a maximum content there is elaborated a method, which foresees the use of newly prepared FeCO₃ as the iron source.

FeCO₃ was obtained through interaction of hot saturated solutions of NaHCO₃ and FeCl₂·4H₂O-, under conditions of constant stirring. Reaction runs with emission of gas (CO₂). By means of decantation water is removed from the deposit formed after sedimentation. Afterwards it is washed thoroughly first with flowing water, and then by distilled water up to removal of chlorine ions. As a result, green-colored iron(II) carbonate is obtained, which is kept under water layer (in order to get rid of oxidation of bivalent iron ions into trivalent ones). (Japaridze et al., 2018).

Studies related to determination of medical and preventive efficiency of preparation manufactured according to abovementioned method and in regard to weight change of animals being investigated was conducted in private pig-breeding farm located in Zahesi Village (farmer G. Tsiklauri), on 29 store pigs of three nests. On the basis of the agreement biochemical studies of blood samples were carried out in the laboratory of the Institute of Veterinary Medicine at Georgian Agrarian University (here should be noted that the preparation was...
primarily tested on toxicity in laboratory conditions, on 20 white rats. It was established that preparation with 3.75-7.5% content of iron (II) is non-toxic.

Experimental pigs were divided into three groups according to analogue principle for testing of manufactured preparation. With the purpose of preparation selection 20-day pigs were fed (along with mother’s milk) once a two days by 0.25-0.5 grams of preparation dissolved in water (along with 250 grams of liquid food). Starting with 26th day they were nourished everyday with 0.5-1.0 gram per 450 grams of food in the form of solid addition. Food addition for I group of pigs (12 piggies) contained 7.5-9.0% of elementary iron, II group (12 piggies) – 3.75-7.5%, while the food for III group of animals (5 piggies) didn’t contain any additions.

In order to determine the effect of antianemic solid preparation on the organism a control slaughter of 2-month pigs (n = 3) after 1, 6 hours from preparation administration and in the end of study – on the 40th day was conducted. The test material for pathanatomical and histological analyses was taken from subdermal tissue, muscles, differently-located lymphatic glands and parenchymal organs. Initial fixation of material was made in 10% formaldehyde solution (formalin), with its further transfer to 15% solution. Material was formed in paraffin. Hematoxylineozine solution was used slice taking. In case of necessity Sudan-3 was used for study of fatty degeneration and lipids.

RESULTS AND DISCUSSION

As a result of systematic observations over store pigs there were established that antianemic preparation has a positive impact on animals, and there was no negative development, in particular, no expected deterioration of general physical-physiological condition and blood chemistry values caused by stress-factors related to termination of breast breeding period (Table 2).

According to pathanatomical and histological studies in an hour after preparation intake the micro-morphological structure of pig body was preserved, slightly expressed serous fluid, single leucocytic infiltration and slightly expressed vascular response in the form of hyperemia were registered in venous capillaries. Micro- and macro-morphological indicators in the heart, kidneys and lungs were within the norm, and no abnormalities were registered in them.

In the end of the research, at the 40th day, the morphological structure of lymphatic nodes and organs were within a norm. According to micro- and macro-morphological researches it was established that the preparation use don’t cause any changes in dermal, subdermal tissue and internal organs and organism response to the preparation is within the norm.

It should be noted that slightly expressed capillary hyperemia registered in the derma itself and in regional lymphatic nodes, and perivascular infiltration that was not observed in 6 hours after preparation intake, cannot be the result of manifestation of preparation toxicity, its dermal accumulation and other adverse effects. The mentioned fact is most likely the indicator of stress reactions caused by manipulations on pigs (blood taking for analysis, food change).

**Table 1**: Content of antianemic preparation for oral administration, mass %.

<table>
<thead>
<tr>
<th>Preparative form</th>
<th>Component</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fe(II)-Fructose</td>
<td>15.75-31.6</td>
</tr>
<tr>
<td></td>
<td>D-Fructose</td>
<td>12.0-24.1</td>
</tr>
<tr>
<td>Preparation for oral administration</td>
<td>Fe(II)</td>
<td>3.75-7.5</td>
</tr>
<tr>
<td></td>
<td>Co(II)- Fructose</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td>D-Fructose</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>Co(II)</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>Natural Askan-clay</td>
<td>68.2-36.5</td>
</tr>
</tbody>
</table>

**Table 2**: Relative efficiency of antianemic preparation on biochemical indices of young pigs: I – preparation with 7.5-9.0%; content of Fe(II), n=12; II – preparation with 3.75-7.5%; content of Fe(II), n=12; III – control group, n=5.

<table>
<thead>
<tr>
<th>Group</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, days</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>96.5</td>
<td>101.3</td>
<td>109.6</td>
<td>112.2</td>
</tr>
<tr>
<td>II</td>
<td>102.0</td>
<td>109.1</td>
<td>118.0</td>
<td>120.5</td>
</tr>
<tr>
<td>III</td>
<td>82.3</td>
<td>84.3</td>
<td>86.1</td>
<td>88.3</td>
</tr>
<tr>
<td>Erythrocytes in the blood, 10³/l</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>7.2</td>
<td>7.4</td>
<td>7.7</td>
<td>7.9</td>
</tr>
<tr>
<td>II</td>
<td>7.2</td>
<td>7.3</td>
<td>8.1</td>
<td>8.5</td>
</tr>
<tr>
<td>III</td>
<td>5.3</td>
<td>5.4</td>
<td>5.4</td>
<td>5.6</td>
</tr>
<tr>
<td>Iron(II) in the blood, mmol/l</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>25.8</td>
<td>26.0</td>
<td>26.5</td>
<td>26.8</td>
</tr>
<tr>
<td>II</td>
<td>27.4</td>
<td>28.5</td>
<td>27.9</td>
<td>28.5</td>
</tr>
<tr>
<td>III</td>
<td>19.5</td>
<td>19.6</td>
<td>20.3</td>
<td>22.6</td>
</tr>
</tbody>
</table>

High therapeutic and preventive effect of targeted product is predetermined by:
- Chelate complex with high content of indispensable microelement – Fe(II) with hemo-stimulating compound D-fructose (Gabelia et al., 2008).
- Complex of cobalt – ultra component promoting iron accessibility (digestibility) with D-fructose (Gabelia et al., 2014).
- Adsorbent – local, ecologically clean, non-toxic, natural Askan-clay, thanks to which the preparation acquires the ability of double, parallel action – hemo-stimulating metal complexes fill a deficit of microelements in the organism, while Askan-clay, at the expense of high adsorbing, catalytic and prolonging properties will adjust functioning of gastrointestinal tract. At the same time, it will provide gradual, planned release of microelement complexes from clay surface (which is naturally released from organism), and eliminates or reduces to the minimum a side effect permanently accompanying anaemia (Japaridze et al., 2017).
- Bioaccessibility of preparation is also improved by treatment of Askan-clay and etalcomplex-containing system via ultrasonic material dispersion method, with dispersion of preparation particles sized up to nanosize under conditions of 22 kHz (the mentioned method is used for improvement of pharmacological and therapeutic properties of medicinal products and is considered as a new prospective direction of medicine (Laurence, 2012; Coffey, 2012; Japaridze et al., 2018).
- Manufactured preparation is kept in a dark, hermetically sealed vessel. Medical and preventive composite preparation for metal deficit has to contain 7.5-9.0% of iron (II). In case of different iron concentration takes place the correction of mentioned content.
According to studies carried out using adsorption-desorption processes, infrared-spectrophotometric, thermographic, complexometric titration, photometric analysis (Beshkenadze et al., 2018, Japaridze et al., 2018) and other physical and chemical methods there was shown that synthesized iron-fructose complex contains maximum quantity of bivalent iron. Adsorption of metal complexes on the clay surface is of physical nature that predetermines prolonging properties of preparation. According to studies carried out on rotation viscosimeter Reotest-2 in the pH=2.5-3.0 range (acidity of gastric juice of store pigs) the offered preparation meets the requirements applied to reological and structure-forming parameters of medicinal products (Gabelia et al., 2009).

Conclusions

So, the anemiaemic medical and preventive preparation with 3.75-9.0% content of iron(II) is not toxic, it is characterized by hemo-stimulating-antistress properties that provides stable live weight gain of store pigs in comparison with control analogues (Table 2, 3). As is seen from the Table, the preparation with 3.75-7.5% content of Fe(II) is the optimal one.

Acknowledgements

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Table 3: Impact of preparation manufactured according to offered way on live weight of young pigs (20-120 days): I – preparation with 7.5-9.0%; content of Fe(II), n=12; II preparation with 3.75-7.5%; content of Fe(II), n=12; III – control group, n=5.

<table>
<thead>
<tr>
<th>Group</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>16.85±2.29</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>16.85±2.29</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>16.85±2.29</td>
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