Method for processing deer bones

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Abstract. The article presents the data on the assessment of a method for the production of biosubstance from the bones, of Siberian red deer including the removal of fat and protein fractions using infrared radiation, ultrasound and fermentation, as well as softening the bone by autoclaving, followed by grinding of the resulting product. The prerequisite for the study was the importance and necessity to create a biosubstance of natural origin with a high degree of digestibility for the prevention of the development of musculoskeletal system diseases due to the intensive spread among population. The research material was tubular bones of Siberian red deer with removed bone marrow. It was shown that the optimal mode during processing of deer bone is the phased exposure of infrared rays for 2-3 hours at a temperature of 80-85 °C, followed by removal of the protein fraction using ultrasound equipment at a temperature range of 75 to 80 °C and exposure time 4-5 hours, autoclaving at the temperature of 120 °C and pressure of 1.5 atm for 3 hours. This technological scheme allowed obtaining biosubstance from deer bones with a yield of mineral substances up to 98.6% and a maximum level of purification of the product from organic impurities.

1. Introduction
The diseases of musculoskeletal system (MSS) are one of the most common chronic diseases in the world. This is a whole group of diseases that differ in pathophysiology and are usually associated with pain and impaired physical function. These diseases cover a range of conditions, including osteoarthritis, rheumatoid arthritis, osteoporosis and lower back pain [1]. According to the statistics, diseases of spine and large joints affect up to 80% of the population of the Russian Federation [2]. According to British scientists, this group of diseases accounted for 22% of the total incidence in the UK [3]. In the countries of the European Union and the USA, from 17 to 27 million people with MSS diseases are registered annually. Doctors note that today the diseases of musculoskeletal system are increasingly observed in young people of working age and are often the cause of disability, causing deterioration in the quality of life and social adaptation [4].

Taking into account the increase in the number of pathologies of musculoskeletal system, there is growing need to prevent their development by means of preventive measures, including the use in the daily diet of functional food products of improved composition and specialized products. One of the key components in the prevention and treatment of MSS diseases are minerals, in particular calcium and phosphorus, which must be balanced when entering a human body. Foreign researchers in the experiments on laboratory mice found that feeding products with a high phosphorus content and low calcium level, or vice versa, leads to the decrease in bone tissue by up to 56% [5].
According to numerous biochemical studies, bones, including the bones of Siberian red deer, contain a significant amount of calcium phosphoric salts [6]. A group of Soviet scientists led by A. Sharpenak while studying the bones of food-producing animals determined that calcium from bone samples has a high digestibility, which can be compared to the digestibility of calcium from milk and dairy products [7].

Nowadays the method of processing farm animal bones is mainly used in the national industry. It includes its grinding, heat treatment, followed by polishing and drying. These technological operations allow extracting only a part of the bone mass and do not provide a sufficient degree of purification of the bone product, which is subsequently used only for feed purposes for farm animals in the form of meat and bone meal [8]. Therefore, there is a need to create a technology for processing bone, which will allow getting a product of a high degree of purification with the possibility of its use for food purposes.

In its turn, waste-free processing of deer bone can help processing enterprises to reduce labor and energy costs, make more efficient use of production facilities and increase the profitability of deer breeding industry in general.

According to the above-mentioned aspects, the purpose of our study was to develop a method for processing deer bones with a maximum yield of mineral substances with a high degree of purification of the final product from organic particles.

2. Materials and methods

The research material was tubular bones of deer with removed bone marrow. In order to obtain a mineral additive, the raw materials were crushed to particles no larger than 0.5 cm in size using a crusher and subjected to heat treatment in three stages.

At the first stage, various modes of fat extraction were tested by applying infrared radiation to bone mass at a temperature of 70, 75, 80, 85, 90 °C for 1, 2, 3 hours with subsequent weighing of the released fat.

At the second stage of the study, protein components, in particular, periosteum residues were removed from bone raw materials. For this purpose, we tested the ultrasonic equipment with a frequency of ultrasonic vibrations of 37 kHz, 1:5 hydraulic module at exposure temperature from 70 to 95 °C with the exposure time from 3 to 6 hours. The extract was added to the domestic proteolytic enzyme of microbial origin Protomix based on the fungus Penicillium canescens, introduced in the amount of 0.5% of the volume of raw materials with the activity of the enzyme agent, 20 UNITS of action per 1 g of extract, pH 3-5. The assessment of the efficiency of technological modes at various stages of processing deer bones was carried out by the amount of total protein and mineral elements in the hydrolyzate according to generally accepted methods (GOST 25011-2017).

The third stage of the study included the determination of the optimal mode for softening bone tissue. For this, autoclaving was carried out at a temperature of 120 °C and a pressure of 1.5 and 2.0 atm, the exposure time of bone mass varied from 3 to 5 hours. The assessment of the softening of bone substrate was carried out determining the degree of grinding after final drying. The grinding of mineral additives was carried out in a mill to a particle size of 20 μm, the remaining solid particles were weighed and compared to the best sample.

The effect of the obtained biosubstance from deer bone on not pedigree hypotrophic dogs in “Laska” shelter was assessed. The experimental group consisted of puppies (n = 10) weighing 3-5 kg, which received a biological substance from deer bone in the amount of 500 mg per animal per day for 30 days as an additive to the main diet. The control group consisted of puppies (n = 10) weighing 3-5 kg, which received the calcium gluconate in the amount of 500 mg per animal per day as an additive.

Before setting up the experiment and 30 days after the weighing of animals, clinical examination and blood sampling were performed before the first feeding.
3. Results and discussion

The important step in the processing of deer bone is fat extraction, since the quality of the finished product depends on this process. Deer fatty tissue is highly refractory. According to various literature sources, the melting point of deer fat is from 43 to 50 °C [9]. We have conducted the experiment to determine the optimal mode for the fat extraction from bone tissue. For maximum purification and better warming of the fat mass from the bone, temperature optimums from 70 to 90 °C were selected, since at 50 °C - 60 °C the fat melts, but not completely (table 1).

| Temperature, ° | Exposure time, hour | Amount of fat, % |
|---------------|---------------------|------------------|
| 70            | 1                   | 6.5              |
|               | 2                   | 10.1             |
|               | 3                   | 14.6             |
| 75            | 1                   | 16.8             |
|               | 2                   | 17.3             |
|               | 3                   | 17.5             |
| 80            | 1                   | 17.9             |
|               | 2                   | 18.4             |
|               | 3                   | 18.6             |
| 85            | 1                   | 18.0             |
|               | 2                   | 18.6             |
|               | 3                   | 18.6             |
| 90            | 1                   | 18.6             |
|               | 2                   | 18.6             |
|               | 3                   | 18.6             |

According to the results of the study, the temperature range from 70 to 75 °C allowed removing from 6.5 to 14.6% of fat from bone tissue during 1 to 3 hours of exposure to infrared rays. Based on the previous studies, native powder from antler deer bones contains 17.2-18.6% of fat [10]. Therefore, this technological mode did not ensure the complete extraction of fat components from bone mass. The exposure to bone tissue at the temperature range of 80-85 °C during exposure to infrared rays for 2-3 hours ensured the removal of 18.6% of fat component of bones. The increase in temperature to 90 °C did not cause a further increase in the extraction of fat from bone mass, but at the same time it contributed to the increase in the energy expenditure for fat extraction, which is economically inexpedient. Thus, the optimal mode of removal of fatty tissue is exposure to infrared rays for 2-3 hours at a temperature of 80-85 °C.

According to literature sources, the organic matrix of bone tissue consists of 90-95% of collagen and a small amount of proteoglycans [11]. At the second stage of the study, protein fractions were removed using ultrasonic equipment in combination with domestic enzyme agents. Enzymatic treatment causes the breakdown of hydrolytic collagen. As a result of this treatment, protein compounds, depending on the mode, were denatured and hydrolyzed, with the introduction of the resulting substances to the extract (table 2). The exposure to ultrasonic vibrations intensified the fermentation process. Due to ultrasonic vibrations, mass transfer processes are accelerated, which allows increasing the yield of finished products and improving their quality [12].
Table 2. Protein content in the extract

| Temperature, °C | Extraction time, hour | Mass content of protein, % | Loss of minerals with extract, % |
|----------------|----------------------|---------------------------|----------------------------------|
| 70             | 3                    | 6.5                       | 5.4                              |
|                | 4                    | 10.6                      | 6.0                              |
|                | 5                    | 12.7                      | 6.0                              |
| 75             | 3                    | 18.1                      | 6.6                              |
|                | 4                    | 23.4                      | 7.0                              |
|                | 5                    | 24.0                      | 7.4                              |
| 80             | 3                    | 24.1                      | 8.0                              |
|                | 4                    | 25.4                      | 8.5                              |
|                | 5                    | 25.8                      | 9.3                              |
| 85             | 3                    | 25.9                      | 16.1                             |
|                | 4                    | 25.5                      | 17.3                             |
|                | 5                    | 25.5                      | 20.4                             |
| 90             | 3                    | 25.7                      | 20.0                             |
|                | 4                    | 25.6                      | 21.1                             |
|                | 5                    | 25.7                      | 20.7                             |

Analyzing the results of table 2, it was found that the first experiment at a temperature of 70 °C and the extraction time of 3-5 hours removes a small amount of protein substances from 6.5 to 12.7% with minimal loss of mineral substances in the hydrolyzate (5.4-6.0%). In the second experiment, at a temperature of 75 °C and the exposure time of 4-5 hours, the amount of protein substances increased 2.2-1.8 times compared to the previous experiment and amounted to 18.1-24.0%, and the loss of mineral substances was 16.6%. In previous biochemical studies, the content of protein fractions in the bone material of deer was determined – it was 24-26% [13]. Therefore, the percentage of extraction of the protein fraction from bones corresponded to almost complete removal of protein from bone tissue. Temperature rise to 80 °C increased the yield of protein substances by 7.5% and reached complete removal, but the loss of mineral substances was 25.7%. A further increase in temperature to 85-90 °C led to a significant loss of mineral substances in the raw material during their transition to the extract, while the mass fraction of these components in the extract increased by 3.5 times in comparison with the previous experiment. Taking into account the above mentioned aspects, the temperature range from 75 to 80 °C with the extraction time is 4-5 hours should be considered as the optimal range of removal of protein fractions while preserving mineral substances of bone mass.

The final stage in the process of obtaining the mineral supplement was the softening of bone mass after fat extraction and removal of protein fractions. Thus, at a temperature of 120 °C and a pressure of 1.5 atm. after 3 hours of exposure, a mineral powder with a particle size of 20 μm in the amount of 94.3% was obtained. The increase in the processing time of bone raw materials up to 4 hours resulted in 98.5%, up to 5 hours - 98.6% of the mineral supplement. The changes in pressure mode by 2.0 atm. after 3 hours of exposure allowed obtaining 94.5% of the mineral additive, which was similar at a pressure of 1.5 atm., but at the same time led to significant energy consumption. The increase in time to 4 and 5 hours did not allow obtaining a higher yield of mineral powder.

According to a biochemical study, the level of mineral components of the obtained biosubstance was 380.0, including 171.0 of phosphorus, 5.6 of magnesium, 0.8 of chlorine, 1.6 g / kg of sulfur, 39.0 of iron and 298.3 mg / kg of zinc.

4. Assessment of the effect of mineral supplements on animals

The research was conducted in order to identify the effect of biosubstance from deer bone on the growth and biochemical blood parameters of hypotrophic dogs. One of the informative indicators of the treatment of animal hypotrophy is the increase in the mass of animals. According to the results of
the study, it was found that the main diet with a mineral supplement from deer bone led increased body weight by 37.2% in the experimental group and by 23.5% in the control group. The difference was 13.7% (Fig. 1).

![Figure 1. Weight dynamics of puppies](image)

According to the results of the experiments conducted, the changes in blood values of puppies were established. The results are presented in table 3.

**Table 3. Biochemical blood parameters of puppies in the experiment**

| Indicator                  | Before | Control (Calcium Gluconate) after 30 days | 1 group (Bioorganic calcium) after 30 days |
|----------------------------|--------|------------------------------------------|------------------------------------------|
| Hemoglobin, g / l         | 58.00  | 65.9                                     | 96.67                                    |
| Red blood cells, mln / μl | 4.43   | 4.51                                     | 5.26                                     |
| White blood cells, thousand / μl | 15.00 | 12.3                                     | 7.9                                      |
| Total protein, g / l      | 44.10  | 50.3                                     | 63.60                                    |
| Calcium, mmol / L         | 2.07   | 2.62                                     | 3.05                                     |
| Phosphorus, mmol / L      | 1.18   | 1.96                                     | 3.00                                     |
| Magnesium, mmol / L       | 1.23   | 1.30                                     | 1.52                                     |

The study of biochemical parameters allows assessing the physiological state of dogs, since the calcium content in the serum of puppies before the experiment was at the lower limit of standard values, calcium-phosphorus ratio was violated, which indicated hypotrophic changes in their bodies. At the end of the experiment, the most significant results were achieved in the content of calcium and phosphorus in the blood serum of dogs. The level of calcium in the blood of experimental dogs receiving the drug from bone increased by 47.3%, phosphorus by 2.5 times, magnesium by 19.0%, hemoglobin increased by 66.7%, and before the start of the experiment, this indicator was significantly lower than normal; the protein content increased by 44.2%. Red blood cells, white blood cells, total protein were within normal values. The data obtained convincingly indicate a good digestibility of biosubstance from deer bones.

5. **Conclusion**

During the course of the research, a method for processing deer bone was developed, which allows obtaining a biosubstance with a high content of minerals. The process of fat extraction contributed to the removal of 18.6% of fat with technological parameters: the exposure to infrared rays for 2-3 hours at a temperature of 80-85 °C. The full removal of the protein fraction - 25.8% was carried out using ultrasound equipment and domestic enzymes at a temperature of 75-80 °C, the extraction time was 4-5 hours. Softening of bone raw materials in order to obtain a mineral supplement in the amount of
94.3% of the total volume of raw materials was carried out under a pressure of 1.5 atm. at a temperature of 120 °C for 3 hours. Good assimilation of the bone product was shown, which was evidenced by the data on changes in the blood chemistry of dogs after the use of the bone supplement, in particular, the increase in the level of calcium by 47.3%, phosphorus by 2.5 times, magnesium by 19.0%, protein by 44.2% and hemoglobin by 66.7%.

6. Acknowledgments

According to the results of the studies, a patent RF No. 2677042 “Method for the integrated processing of deer bones” 01/15/2019 was granted.

References

[1] Thomas R 2016 Basic Concepts in Morbidity Analysis in Sickness and in Health, in: Disease and Disability in Contemporary America (Springer) pp 11-14
[2] Mironov S P and Rodionova T M 2009 Organizational aspects of the problem of osteoporosis in traumatology and orthopedics Bulletin of Traumatology and Orthopedics named after N. N. Priorov 1 3-6
[3] Hurley M, Dickson K, Hallett R, Grant R, Hauari H, Walsh N, Stansfield C and Oliver S 2018 Exercise interventions and patient beliefs for people with hip, knee or hip and knee osteoarthritis: a mixed methods review Cochrane Database of Systematic Reviews 4
[4] Ismagilov M F, Galiullin N I and Mangaleev D R 2005 Costs of Modern Practical Neurology Neurological Bulletin XXXVII(1-2)
[5] Begot L, Collombet J, Renault S, Butigieg X, Andre C, Zerath E and Holy X 2011 Effects of high-phosphorus and / or low-calcium diets on bone tissue in trained male rats Medicine and science in sports and exercise January 43(1) 54-63
[6] Kakimov A K, Kabulov V V, Esimbekov Zh S and Kuderinova N A 2016 The use of meat and bone paste as a protein supplement in the production of meat products Theory and practice of meat processing 2 42-50
[7] Kakimov A K, Teleuov E T and Kuderinova N A 2006 Processing of meat and bone raw materials for food purposes (Semipalatinsk: Tengri) 130 p
[8] Martynov O A, Fayvishevsky M L, Ivanov V E, Utkin A G and Nikiforov A P, RF patent No. 962293 The method of processing bone (30 September 1982)
[9] Martsekha E V, Batyrev O N and Shelepov V G 2010 Characterization of the fatty acid composition of wild reindeer meat Achievements of science and technology of agro-industrial complex 7 72-74
[10] Grishaeva I N and Krotova M G 2018 Methods of processing deer raw materials to obtain functional biosubstance Materials of the II Interregional Scientific-Practical Conference “Off-Products to Bioeconomics”
[11] Antipova L V and Stallions N A 1991 Biochemistry of meat and meat products (Voronezh) 182 p
[12] Khmelev V N, Kuzovnikov Yu M and Khmelev M V 2017 Ultrasonic devices for scientific research South Siberian Scientific Bulletin 1 5-13
[13] Grishaev I N 2018 Deer bones - new raw materials for the food industry Collection of materials of the VII World Reindeer Herder Congress