Scientific and production substantiation of the effectiveness of the product for dry hoof baths

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Abstract. This paper presents the results of a study of the adsorption activity of a developed complex product for the treatment and prevention of diseases of the distal extremities of cattle and its effectiveness with individual and group use using the method of dry hoof baths in a number of agricultural enterprises with tethered and freestall housing of cattle. It was shown that the developed product has a porous structure and a wide range of adsorption. The main cause of infection causing mass lesions of the fingers and hooves of cattle in the surveyed agricultural enterprises was an increase in the threshold number of the diversity of saprophytic and opportunistic microorganisms transmitted directly from one animal to another. Associations of bacteria and micromycetes determined the cause of the occurrence and spread of infectious diseases of the fingers and hooves. The test results of the developed complex product, which includes inorganic adsorbents and active substances with pronounced fungicidal, bactericidal and anti-inflammatory properties indicate the possibility of its introduction into the system of control measures with diseases of fingers and hooves in disadvantaged agricultural enterprises with both tethered and free-stall livestock keeping.

1 Introduction

Massive morbidity in ungulates, manifested by damage to the distal limbs, is the cause of significant economic damage caused to livestock [1-5]. Infectious diseases of fingers and hooves cause especially great damage to dairy cattle breeding [6-9]. Unsatisfactory organization of prevention and treatment of cattle with limb diseases leads to loss of productivity and premature culling of up to 70.0% of animals, which reduces the profitability of milk and meat production [10-14].

In the absence of prevention and timely assistance to animals at the onset of the disease, surgical and contagious infections of the fingers and hooves develop, affecting deep-lying tissues with severe complications in the form of purulent pododermatitis, necrosis of the claw bone, arthritis of the claw joint, podotrochleitis, pulp phlegmon and interclaw phlegmon, etc., requiring lengthy and expensive treatment [14-17].

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The listed clinical signs of the disease, related to lesions of moderate and severe severity, require a lot of time associated with labor-intensive manipulations of fixing animals, thorough cleaning, trimming and surgical removal of necrotic tissue, followed by a long course of treatment and the use of expensive medications, mainly antibiotics broad spectrum of action [18-23]. During such treatment, milk is rejected, the live weight of productive animals decreases, turnover is disrupted and the gene pool of the herd is lost. Predisposing factors for an uncontrolled increase in morbidity are high humidity and untimely sanitation of the distal extremities in mild to moderate cases of the disease [24-29]. One of the effective methods to prevent the widespread spread of diseases of the fingers and hooves is the use of group prevention using wet hoof baths [30, 31], which are widely and successfully used in many agricultural enterprises, which allows for less use of antibiotics. However, the process of carrying out wet baths is labor-intensive; when contaminated with animal feces, the activity of the disinfectant solution decreases. In addition, with constant use of such baths, toxic products accumulate, humidity increases and the microclimate of the premises is disrupted, and floor coverings become slippery. These problems are solved by the complex product we have developed in the form of a powder called “Dry Bath” (hereinafter “DB”), which in the future will expand the range of therapeutic and prophylactic agents, will increase the effectiveness of group nonspecific prevention and treatment of ungulates, and will prevent violations of the integrity of the skin in areas of the distal limbs. The use of dry baths will significantly reduce labor costs and humidity in livestock buildings. In addition, individual use of the product in the form of an application is possible. The development of new effective means for group prevention and treatment of diseases of the distal limbs of cattle using dry hoof baths is an urgent task for veterinary practice. “DB” will become one of the means of comprehensive prevention and treatment, increasing the period of economic use of cows and the profitability of the industry without the use of antibiotics.

One of the main properties of the developed complex product, in addition to the pronounced antimicrobial and anti-inflammatory effect, will be adsorption activity, which is closely related to the properties of functional groups and the morphology of the surface of the drug in contact with the skin and claw horn.

In this regard, the aim of the research was to study the adsorption activity of a new complex product for dry hoof baths and evaluate its effectiveness in group and individual use in production conditions.

When selecting components to create a product in powder form for the group prevention and treatment of infectious diseases of the distal limbs of cattle using the dry foot bath method, the need to meet the following requirements was taken into account: the presence of adsorption, bactericidal, fungicidal and anti-inflammatory properties; strengthening the hoof horn; low toxicity; maintaining flowability; optimal pH; no risk of slipping; corrosive action; the need to maintain temperature conditions; precise dosage; special disposal procedure; possibility of use without special baths, as well as in a mixture with various bedding materials used in livestock farms; the ability to add fresh powder as it is consumed and used using spray devices. Therefore, the new complex product “DB” for dry hoof baths includes inorganic adsorbents and active substances with pronounced fungicidal, bactericidal and anti-inflammatory properties. The promise of the combination of components included in the drug “DB” (copper and zinc salts, polyvinyl alcohol, sodium benzene sulfochloramide, etc.) is due not only to the synergism of their mechanisms of action (increasing antimicrobial, fungicidal, anti-inflammatory activity), but also to the presence of sorption properties in the product properties. The average powder consumption is 120-160 g per cow (40-50 kg per 300 animals).
2 Materials and methods

The morphology and capacity of the sorbent is determined by the presence of hollow structures (pores), forming a developed surface area available for adsorption. Pores of different sizes make it possible to remove toxins of different natures (low and medium molecular substances, protein substances), which is very important for diseases of the distal extremities. The porosity of the developed product was assessed in this work using model sorbates (methylene blue (MB) dye, iodine and gelatin), which are considered as “molecular probes” for determining micro- and mesopores [32-36].

When determining adsorption activity using the MS indicator, an aqueous solution of pharmacopoeial MS was prepared with a mass fraction of the main substance of at least 80.0%. To construct a calibration graph, 0.15 g of MS indicator was placed in a volumetric flask with a capacity of 1000 cm³, then dissolved in 200 cm³ of hot distilled water, then it was cooled, brought to the mark with distilled water (a working solution with a mass concentration of 150 mg/dm³ was obtained). The working solution was stored in a hermetically sealed dark glass container for no more than two weeks. To construct a calibration curve, reference solutions were prepared. In ten volumetric flasks with a capacity of 50 cm³ each, 0.5 was introduced; 1.0; 1.5; 2.0; 3.0; 4.0; 5.0; 6.0; 7.0; 8.0 cm³ of indicator solution, after which the volumes were adjusted to the mark with water. The resulting solutions contained 1 dm³, respectively, 15; thirty; 45; 60; 90; 120; 180; 210; 240 mg/dm³ indicator. The optical density of the prepared solutions was measured on a KFK-2MP photoelectrocolorimeter using a blue filter with a wavelength of 400 nm. Distilled water was used as a control solution. Based on the data obtained, a calibration graph of the dependence of optical density on the mass concentration of the reference solution was constructed.

We weighed 0.1 g of “DB”. For two repetitions, two samples were placed in separate conical flasks with a capacity of 50 cm³, 25 cm³ of indicator solution was added, capped and shaken for 20 minutes. After shaking, the suspensions were transferred into centrifugation tubes and centrifuged for 15 minutes, then 5 cm³ of the clarified solution were carefully pipetted and its optical density was determined.

Based on the obtained optical density value, using a calibration graph, the residual mass concentration of the indicator in the clarified solution was determined. Adsorption activity (A) by indicator in mg per 1 g of product was calculated using formula (1)

\[
A = \frac{m}{(C_1 - C_2) \times K \times 0.025} \quad (1)
\]

where \(C_1\) – the mass concentration of the initial indicator solution, mg/dm³;
\(C_2\) – mass concentration of the solution after contact with the carrier, mg/dm³;
\(K\) – the dilution factor of the solution taken for analysis after contact with the test substance (in our case \(K = 1\));
\(m\) – the mass of a sample of the active substance, g;
0.025 – volume of indicator solution taken for clarification, dm³.

The result of the analysis was taken as the arithmetic mean of two parallel determinations, the absolute discrepancy between which did not exceed the permissible discrepancy equal to 10 mg per 1 g of the mixture under study.

To determine the adsorption activity of “DB” for iodine in a volumetric flask, 25 g of potassium iodide was dissolved in 50-100 cm³ of distilled water, 12.7 g of iodine was added and the contents were mixed until the iodine was completely dissolved. Then the volume of the solution was adjusted to the mark with distilled water. 1 g of “DB” was weighed, placed in a conical flask with a capacity of 250 cm³, 100 cm³ of a solution of iodine in potassium iodide was added, capped and shaken continuously for 15 minutes. Then the solutions were allowed to settle and from the flasks, using a pipette, carefully so that sample particles did not get in, 10 cm³ of solutions were taken, placed in a conical flask with a capacity of 50 cm³
and titrated with 0.1 N sodium thiosulfate solution. At the end of the titration, 1 cm³ of 0.5% starch solution was added and titrated until the blue color disappeared. At the same time, the initial content of iodine in the solution was determined; for this purpose, 10 cm³ of a solution of iodine in potassium iodide was taken and titrated with a solution of sodium thiosulfate, adding a solution of starch at the end of the titration.

The adsorption activity of samples for iodine (X) as a percentage was calculated using formula (2)

\[ X = \frac{(V_1 - V_2) \times 0.0127 \times 100 \times 100}{m} \]  

(2)

where \( V_1 \) – the volume of sodium thiosulfate solution with a concentration of exactly 0.1 mol/dm³ (0.1 N), consumed for the titration of 10 cm³ of iodine solution in iodide porridge, cm³;

\( V_2 \) – volume of sodium thiosulfate solution with a concentration of exactly 0.1 mol/dm³ (0.1 N), used for titration of 10 cm³ of iodine solution in potassium iodide, after treatment with samples, cm³;

0.0127 – mass of iodine corresponding to 1 cm³ of sodium thiosulfate solution with a concentration of exactly 0.1 mol/dm³ (0.1 N), g;

100 – volume of iodine solution in potassium iodide taken to clarify coal, cm³;

\( m \) – the mass of the sample, g.

The result of the analysis was taken as the arithmetic mean of two parallel determinations, the absolute discrepancy between which did not exceed the permissible discrepancy of 3.0%.

To determine the adsorption activity of gelatin: 0.2 g of sample “DB” was placed in a conical flask with a capacity of 100 cm³, 25 cm³ of a 0.6% gelatin solution (solution A) was added, and shaken on a shaking apparatus for 30 minutes. Next, the suspension was transferred into centrifugation tubes and centrifuged for 20 minutes at 3000 rpm. 5 cm³ of supernatant liquid was pipetted, transferred into volumetric flasks with a capacity of 25 cm³, the volume of solutions was adjusted to the mark with biuret reagent, and mixed.

After 30 minutes, the optical density (D1) of the test solution was determined on a spectrophotometer at a wavelength of 560 nm in a cuvette with an absorbing layer thickness of 10 mm.

In parallel, the optical density (D0) of the gelatin solution (solution B) was determined. Biuret reagent diluted with distilled water in a ratio of 4:1 was used as a reference solution. The biuret reagent was prepared as follows: 4.5 g of Rochelle salt was dissolved in 40 ml of 0.2 mol/l sodium hydroxide, 1.5 g of copper sulfate and 0.5 g of potassium iodide were added and dissolved. Add 0.2 mol/l sodium hydroxide solution to 100 ml.

Adsorption activity in mg per 1 g of sample (X) was calculated using formula (3)

\[ X = \frac{(D_1 - D_0) \times m_1 \times 1 \times 25 \times 100 \times 1000}{D_1 \times 1 \times 100 \times m_0 \times 1} \]  

(3)

where \( D_0 \) – the optical density of the gelatin solution (solution B);

\( D_1 \) – optical density of the tested solutions;

\( m_0 \) – mass of a sample of gelatin for preparing solution A, g;

\( m_1 \) – mass of sample samples, g.

Statistical processing of the results was carried out using MS Excel using descriptive statistics methods. The arithmetic mean (M) and the error of the mean (m) were calculated.

After studying the adsorption activity of the developed product, its production tests were carried out in the conditions of agricultural enterprises that are unfavorable for diseases of the distal limbs of cattle. To work, a machine for fixing animals was installed in a technologically convenient place in the livestock building, where they examined, trimmed, trimmed hooves, diagnosed, sanitized and treated diseases of the fingers and hooves.

In each disadvantaged agricultural enterprise, before the start of the experiment, during and after its completion, the number of cows with hoof diseases was taken into account and the degree of damage to the limbs was assessed as mild, moderate and severe.
The study of the therapeutic effectiveness of the developed product when used individually was carried out in a strictly controlled experiment on 23 cows. Animals with mild and moderate manifestations of diseases of the fingers and hooves were divided into two groups according to the principle of analogues.

The studies were carried out while professional orthopedists were working to trim and trim the hooves. To determine the microflora of the affected tissues, samples of biomaterial were taken after clearing and trimming the cows' hooves strictly according to inventory numbers, by swabbing the tissue in the area of the most common soft tissue lesions (the arch of the intercliff cleft, the crumb and the corolla) with sterile cotton swabs, which were placed in labeled tubes for transportation.

After sanitation of the wound (cleaning, trimming the hooves and excision of necrotic tissue), the affected fingers and hooves of the cows of the first group (13 heads) were sprinkled with the “DB” product, covered with a light gauze bandage and a bandage was applied. After sanitation of the wound, claw lesions in cows of the second (control) group (10 heads) were treated with “Chemi Spray” (Industrial Veterinaria S.A. Invesa, Spain), and a gauze bandage and bandage were applied. On the 3rd day of treatment, the bandages were removed. If necessary, in animals with moderate damage to the fingers and hooves, the wound surface was re-sanitized and medicinal agents were applied.

To evaluate the preventive effectiveness of the developed product, in the conditions of a dairy complex with free-stall housing, when leaving robotic milking in barns for 200 heads, four foot baths were installed in pairs (15 cm deep, 2.8 m long, 0.9 m wide) at a distance 3 meters apart. The first 2 baths were filled with water, the second two baths were filled with “DB” (barn No. 1) or a 10.0% solution of copper sulfate (barn No. 2).

The experiment lasted for 20 days. The animals were driven through the baths every day, twice a day, after milking. In barn No. 1 - through “DB”, in barn No. 2 - copper sulfate solution, in barn No. 3 - hoof baths were not used.

During the experiment, as the animals were driven through, the consumed amount of powder was poured daily into the dry baths. The level of powder in the bath was adjusted to 15 cm so that it covered all the animal’s hooves. After all the cows in the barn had passed through, the dry baths were cleared of manure and, accordingly, “DB” was added or the copper sulfate solution was replaced. Water baths were changed as they became dirty.

To prevent hoof diseases in cattle when milking herds were kept in tethers, bathtubs were installed at the exit from the barn to the walking yards.

For the first experimental group (barn No. 1 with a livestock of 195 animals, of which 33 were lame (16.9%)), the first bath was filled with clean water, and “DB” was poured into the second bath. For the second experimental group - barn No. 2 (189 heads, 29 lame (15.3%)), a 10% solution of copper sulfate was used instead of the stated product. In the control group (barn No. 3 with a livestock of 179 animals, of which 28 were lame (15.6%)) baths were not used. The cows of the experimental groups were put through foot baths for 20 days.

### 3 Results and discussion

The study of the adsorption activity of the developed product in relation to markers of various natures (MS dye, iodine and gelatin) made it possible to determine one of the main technological properties of “DB”, characterizing its effectiveness (table 1).

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Adsorption activity by markers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gelatin, mg/g</td>
</tr>
<tr>
<td>&quot;DB&quot; product</td>
<td>9.75±0.07</td>
</tr>
</tbody>
</table>
Based on the results of studying the adsorption capacity for methylene blue, gelatin and iodine, it was established that the developed product has a developed porous structure and a wide range of adsorption, which determines certain values of sorption capacity for selected model sorbates.

In the surveyed agricultural enterprises, various diseases of the fingers and hooves of cattle were identified (injuries; ulcers of the soft tissues of the hooves in the area of the arch of the interclaw fissure, corolla and crumb; Rusterholz ulcer; white line disease; limax; laminitis; pododeratitis; digital dermatitis, etc.) with varying severity of the lesion. In many animals, severe maceration of the soft tissues of the claws and various injuries were noted.

In each disadvantaged agricultural enterprise, before the start of the experiment, during and after its completion, the number of cows with hoof diseases was taken into account and the severity of damage to the limbs was assessed. When conducting bacteriological studies in pathological material from cows with purulent-necrotic lesions of the distal limbs, *F. necrophorum*, bacteria of the genus: *Streptococcus* spp., *Staphylococcus* spp., *Clostridium* spp., as well as yeast fungi *Candida albicans* and microscopic fungi: *Aspergillus* spp. were most often found, *Penicillum* spp. and *Mucor* spp.

After individual applications of the “DB” product to cows with mild damage to the fingers and toes in the experimental group (3 heads) and “Chemi spray” in the control group (4 heads), recovery occurred on the 3rd day.

Recovery of cows with an average degree of damage occurred in the experimental group (10 heads) on days 10-15, in the control group (6 heads) on days 13-18.

As a result of research, it was found that “DB”, when applied externally, is harmless, has no side effects and is not inferior in effectiveness to “Chemi spray”. At the same time, the product we developed quickly stops the inflammatory process, accelerating the resorption of the infiltrate and stimulating regenerative processes in the tissues of the fingers and hooves. The improvement in the clinical condition of animals after the use of "DB" correlated with the results of bacteriological examination of samples from the affected areas of the hooves. Thus, if before the use of the drug a wide variety of opportunistic microorganisms, yeasts and molds were isolated from sick animals, then after the use of “DB” anaerobic microorganisms and microscopic fungi were not isolated in any case. Application of a product for dry hoof baths to the hooves, after cleaning and trimming them, has a pronounced preventive and therapeutic effect due to the synergy of adsorption, antimicrobial and anti-inflammatory activity of the developed product. In this regard, the use of the “DB” product for dry hoof baths, along with mechanical cleaning and disinfection of environmental objects, is one of the effective measures to combat diseases of the fingers and hooves of cattle.

When assessing the preventive effectiveness of “DB”, in the conditions of a dairy complex with loose housing at the beginning of work in barn No. 1, among 147 cows of the experimental group there were 50 lame animals (34.0 %). In barn No. 2, which contained 146 heads, there were, respectively, 43 lame cows (29.4 %). In the third barn, where baths were not used, among 123 cows there were 35 cows with clinical lameness (28.5 %). After 20 days of daily use of wet baths, 10 lame animals, 10 lame animals (6.8 %) remained among the animals in barn No. 2, and 5 heads (3.4 %) remained among the experimental cows (using a dry bath) in barn No. 1. In the barn where baths were not used, the number of lame cows increased to 41 heads (33.3 %).

Thus, the test results clearly showed that the use of dry baths with the developed “DB” product is more effective than the use of wet baths with copper sulfate.

During the observation of the clinical condition of cows kept in tethered dairy herds, it was found that in the first experimental group (using “DB”), the decrease in the number of lame cows occurred faster by 7 days compared to the cows of the second experimental group (wet bath with copper sulfate). On the 20th day of using dry baths with the developed product, there were 4 lame cows (2.1 %) left in the first experimental group, and 9 lame cows (4.6 %)
in the second experimental group. In the control group, 32 lame cows (17.9%) were identified.

Dry foot baths with the developed product had a pronounced antimicrobial and fungicidal effect, kept the hooves dry and clean, strengthened the tissue of the hooves, thickened the horn, prevented the risk of infection and promoted wound healing.

The use of dry baths was convenient and the animals tolerated the procedures of passing through them without fear. Dry baths created an unfavorable environment for the growth of microorganisms and reduced the possibility of infections, thereby improving the sanitary and hygienic conditions of keeping cows and significantly reducing the cost of time and money for treatment. The test results indicate the possibility of introducing the “DB” product into the system of measures to combat diseases of the fingers and toes in agricultural enterprises that are unfavorable for diseases of the distal extremities, with tethered and free-stall housing.

4 Conclusion

Thus, the use of group and individual prevention and treatment of diseases of the distal limbs in cows using the developed “DB” product for dry hoof baths helps to reduce bacterial contamination in the area of the fingers and hooves, stops the infection and creates favorable conditions for accelerating the dehydration phase of the inflammatory process and tissue regeneration. Compared to wet baths, dry baths are easy to dispense, less dirty, easier to clean, you can add product as you go, they have a wide temperature range, animals are not afraid and calmly pass through them without slipping. The results of the study indicate higher efficiency and manufacturability of using the developed product in production conditions compared to wet baths.

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