EFFECT OF ORAL ADMINISTRATION OF ZINC SULPHATE WITH SIMULTANEOUS USE OF NONSPECIFIC IMMUNOSTIMULATION ON THE COURSE OF TRICHOPHYTOSIS IN BEEF CATTLE

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Abstract

The paper assesses the treatment of cutaneous fungal infection in young beef cattle caused by Trichophyton verrucosum using orally administered zinc sulphate for 28 d at 2 g per animal per day and subcutaneous application of 2.5 mg/kg levamisole administered three times every 7 d. The study was conducted on animals at the age of 110 to 122 d, which were divided into two experimental groups: group I (animals suffering from ringworm with low levels of zinc in serum) and group II (animals suffering from ringworm with normal levels of zinc in serum). The remaining animals (control group) were divided into three subgroups: K (clinically healthy animals, which had normal serum levels of zinc), KI (animals infected with ringworm and showing a reduced level of zinc), and KII (animals suffering from ringworm with normal serum zinc levels). During the study the following parameters were investigated: clinical signs of fungal infection, zinc content in serum, percentage of phagocytic cells, and phagocytic index. The symptoms of the disease were observed only in group II on day 56. In group I, an increase in the percentage of phagocytic cells was observed, as compared to the original values, on days 7, 21, and 56 and a fall in this parameter was noted on days 14 and 28. In group II, there was a decrease on day 14 and an increase in comparison to the original values, followed by a gradual fall till day 56 of the experiment. In group I, phagocytic index remained on a stable level till day 56 and in group II until day 28, and subsequently began to increase gradually. It was found that oral supplementation of zinc sulphate combined with nonspecific immunostimulation may be applied in fighting skin fungal infection in cattle, and that zinc and levamisole may exhibit synergism affecting the evolution of nonspecific immunity.

Key words: cattle, trichophytosis, zinc, levamisole.

Trichophytosis (skin ringworm) in cattle, caused by Trichophyton verrucosum, is a significant problem in the rearing of young cattle. In herd, in which it is endemic, the disease causes economic losses resulting from a reduction in weight gain of the fattened animals and reduction in the use value of skins, as well as from costly and not always effective treatment. Among many risk factors that predispose to the development of trichophytosis, a vital role is played by the efficiency of immune system, especially the natural mechanisms of antifungal resistance (4, 15). Treatment and control of trichophytosis in commercial cattle rearing system, which applies chemotherapeutic agents, is difficult, time-consuming and not always effective. The lack of effect is associated with high fungal spore resistance to environmental factors and disinfectants, and difficulty in penetration of antifungal drugs to the site of the infection. Moreover, some of these drugs are highly toxic at the same time and have an immunosuppressive effect. Therefore, the most effective method of combating skin ringworm in cattle farms is specific prophylaxis and therapy using vaccines with concomitant administration of topical treatment, and devastation of the fungus in the external environment, as well as improvement of environmental conditions and balance of the ration, particularly in terms of zinc content (14). Zinc deficiency in cattle trichophytosis significantly impairs the efficiency of antifungal resistance mechanisms of the infected animals, contributing to a chronic disease, thus creating difficulties in its control (12). Often the economic reasons and limited access to biopreparations, hinder immunoprophylaxis and specific immunotherapy of cutaneous mycosis in cattle. Hence, in its treatment nonspecific active immunostimulation is increasingly applied to strengthen the natural (innate) resistance using natural or synthetic immunomodulators. As a result of the strengthening of immunopotentiation of the
immune response and an increase in its duration are observed, or both of these effects are obtained simultaneously. One of the commonly used immunopotentiation catalysts is levamisole, which is left-handed isomer of tetramisole from imidazo thiazole group, known for many years as an effective antiparasitic drug (10). In vivo and in vitro studies demonstrated that levamisole used in low doses (2.5 mg/kg) stimulates the functions of T lymphocytes, macrophages, and monocytes phagocytic activity, chemotaxis, and mobility, especially in states of impaired immune response (2, 11).

The aim of this study was to evaluate the effectiveness of combination therapy consisting of oral zinc sulfate administration and subcutaneous levamisole injections in the treatment of trichophytosis in young beef cattle with normal and low levels of zinc in serum.

**Material and Methods**

The study was conducted in the autumn on 30 cows and bulls of Simmental and Piemontese breeds, aged of 3–4 months. The animals came from a large-lot production farm, where trichophytosis caused by T. verrucosum was stationary, as confirmed by mycological examination. On the basis of the clinical picture and serum zinc concentrations used in the study, the animals were classified into two experimental groups. Group I (n = 6) consisted of those suffering from ringworm with low levels of zinc in serum, whereas in group II (n = 6) were animals suffering from ringworm with normal levels of this element in serum. The animals from both groups had orally administered zinc sulfate in the form of 4% aqueous solution at a dose of 2 g/d per animal for 28 d, combined with a subcutaneous injection of 7.5% levamisole at a dose of 2.5 mg/kg, three times on 7-d intervals, i.e. at the beginning of the experiment (0), and then on days 7 and 14 after the onset of the experiment. The remaining animals (control group) were divided into three subgroups, namely: a subgroup K (n=6) with clinically healthy animals, which had normal serum levels of zinc, subgroup KI (n=6) - animals infected with ringworm and showing a reduced level of zinc, and subgroup KII (n=6) animals suffering from ringworm with normal serum zinc levels. The animals of KI and KII groups accounted for a negative control, for experimental groups I and II, respectively.

The animals in both experimental and control groups were subjected to clinical trials, which were performed on 0, 7, 14, 21, 28, and 56 d of observation. In order to objectify the clinical course of the disease, a certain number of points have been attributed to particular types of skin lesions according to the schema described hereinafter, and their symbols were applied to the diagrams included in the surveys created specifically for the experiment.

Types of efflorescence and surface area and volume, which they occupied, were taken into account. Erythema, which described the possible exacerbation of inflammation, was assigned 4 points, scab - 2 points, and alopecia - 1 point. When both alopecia and scab occurred, only alopecia was taken into account. The number of points obtained in each of the occupied areas (right side of the head, left side of the head, neck, right side, left side of the neck, back, right side of the chest, left chest, right abdomen, left abdomen, right buttok, left page rump, limbs) was summarised and completed with the number of points depending on the size of the occupied areas of the skin: 1%-10% - 1 point, 11%-20% - 2 points, etc. - to a maximum of 10 points. The number of points obtained this way increased with the severity of the disease. When the clinical condition of the animals improved the number of points decreased and was 0 in clinically healthy individuals.

In addition, serum zinc level and phagocytic activity of leukocytes were determined in all animals on 0, 7, 14, 21, 28, and 56 d of the experiment. The levels of zinc were determined by flame atomic absorption spectrometry apparatus (Unicam 939), while the phagocytic activity (percentage of phagocytic cells) and phagocytic index were determined by Ładosz and Dąbska method (7). For all results, the mean (x) and standard error of mean (SE) were calculated. Statistical analysis of the results was performed using Statistica 6.0 programme (StatSoft Inc. 2003). The significance of differences was calculated by Mann-Whitney method at P≤0.05. Correlation of results was calculated using the Kolmogorov method at P≤0.05.

### Table 1

Clinical score in experimental and control groups during 56 d of study

<table>
<thead>
<tr>
<th>Day of study</th>
<th>0</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
<th>56</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>7.4</td>
<td>3.44</td>
<td>7.4</td>
<td>3.44</td>
<td>6.8</td>
<td>3.62</td>
</tr>
<tr>
<td>II</td>
<td>9.8</td>
<td>3.02</td>
<td>9.6</td>
<td>3.8</td>
<td>8.6</td>
<td>3.91</td>
</tr>
<tr>
<td>K</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>KI</td>
<td>8.83</td>
<td>1.66</td>
<td>8.17</td>
<td>1.25</td>
<td>7.17</td>
<td>0.98</td>
</tr>
<tr>
<td>KII</td>
<td>10.6</td>
<td>1.63</td>
<td>8.6</td>
<td>1.96</td>
<td>7.6</td>
<td>1.88</td>
</tr>
</tbody>
</table>

x - mean, SE - standard deviation, * - statistically significant compared to baseline, I – experimental group with ringworm and low level of zinc in serum, II – experimental group with ringworm and normal level of zinc in serum, K - clinically healthy control group with normal level of zinc in serum, KI - control group with ringworm and normal level of zinc in serum, KII - control group with ringworm and normal level of zinc in serum.
Fig. 1. Percentage of phagocytic cells (phagocytic activity) during experiment.
• - statistical significance in comparison to baseline; symbols of groups are explained in the footnote to Table 1.

Fig. 2. Phagocytic index during experiment.
• - statistical significance in comparison to baseline; symbols of groups are explained in the footnote to Table 1.
Results

Results of the clinical trial are illustrated in Table 1. The presented data show that, in animals from group I, clinical symptoms of trichophytosis persisted throughout the experiment, although the number of points that describe the severity of the disease began to decline since the 14th day of the experiment. However, in group II, from the 7th day, a gradual decrease in the number of points describing the severity of the disease was observed and on the 56th day of the study no clinical signs of trichophytosis were found in all animals. In group II, negative correlation \( r = -0.40 \) between zinc levels and the severity of the clinical symptoms was observed.

Test results of phagocytic activity expressed in percentage of phagocytic cells and phagocytic index are illustrated by Fig. 1 and 2. The highest percentage of phagocytic cells was observed in the control group K on the first day of the experiment, while in the other groups its mean values were similar and significantly lower than in group K. In subsequent periods of research, in animals from group I, fluctuations in the percentage of phagocytic cells showing an upward trend in its average values on days 7, 21, and 56 and a downward trend on days 14 and 28 of the study, compared to baseline were observed. However, in group II, the percentage of phagocytic cells at similar levels to baseline values persisted for 7 days, after which a substantial decline (statistically significant compared to baseline) occurred, and on day 21, it reached the output values. After this time, the percentage of phagocytic cells underwent a gradual decline, and on day 56 of the study, its lowest average value over the whole experience was found statistically significantly lower in relation to the baseline. In turn, there were no statistically significant differences in mean percentage of phagocytic cells between group I and control groups K and KI with the exception of the start of the experiment.

The results of the average values of phagocytic index are shown in Fig. 2. In group I, average values of phagocytic index throughout the study were at a level similar to the baseline. Only on day 21 of the study, a statistically significant decrease in this parameter, compared to baseline, was observed. Similarly, in group II, the average values of phagocytic index until day 28 of the observation were at the output level, and only after that time they gradually underwent substantial increase. However, in control group KI, which included sick animals with low levels of zinc, an upward trend in the values of phagocytic index in the subsequent stages of research compared to baseline, and statistically significant increase on day 56 were noted. In turn, in the control group KII of animals with trichophytosis and normal zinc levels, the increase in the phagocytic index was first found on day 7 and until day 28 remained at a slightly lower level than baseline; after that time it underwent a gradual increase to the baseline values.

Discussion

Therapeutic efficacy of oral administration of zinc sulphate in combination with levamisole immunostimulation proved satisfactory only in the case of animals with clinical form of trichophytosis and normal serum zinc levels (group II). Based on the results of the clinical trial obtained in group II, and a negative correlation between serum zinc levels and the severity of clinical symptoms, it can be assumed that the use of inter alia levamisole, as an immune stimulator, enables to achieve satisfactory results in the treatment of trichophytosis in cattle. It is evidenced by the fact that in this group of animals, the clinical symptoms of ringworm disappeared completely in all subjects on the last day of testing. This is probably the result of increased phagocytic activity of peripheral blood neutrophils, which constitute the first line of defence, under the influence of levamisole immunostimulation. This medicine is used inter alia in order to increase the effects of immunisation with bacterial and viral vaccines in cattle, sheep, and poultry, to prevent diseases in calves and pregnant mares, and to stimulate non-specific defence mechanisms of newborn foals (3, 5, 6). Wawrzkiwicz et al. (13) obtained a positive effect of the use of levamisole in cattle vaccinated with bivalent inactivated vaccine containing T. verrucosum and T. mentagrophytes strains. It resulted in more rapid restoration of normal percentage of T lymphocytes and values of leukocyte suicidal index in animals compared to the group receiving only the vaccine. It also cannot be excluded that additional supplementation of zinc sulphate, in accordance with the hypothesis of Namazi (8), causes the synergistic effect with levamisole, elevating the size of cellular immune response. Effect of zinc on the formation of the percentage of phagocytic cells in cattle has already been the subject of previous studies (1, 12), while the positive effects of levamisole immunostimulation on phagocytic activity in calves was stressed by Paulik et al. (9). Oral administration of preparations containing zinc causes, among other things, an increase in animal weight gain and in the percentage of phagocytic cells and phagocytic index value (1, 14).

In conclusion, it should be noted that oral administration of zinc sulphate with simultaneous non-specific immunostimulation in cattle with clinical form of trichophytosis enables to achieve satisfactory results in treating this disease. The obtained results indicate that this method can be successfully used as a supplement to immunotherapy, particularly in the cases where satisfactory therapeutic effects are not achieved. Moreover, zinc and levamisole may exhibit synergism in the stimulation of non-specific immune response.

References


