EFFEC TS OF DIET SUPPLEMENTED
WITH SOME TRACE ELEMENTS ON THE
CONCENTRATION OF THE ELEMENTS AND IMMUNE
INDICES IN PIGS

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This work aimed to study the effect of organic-bound (metallic proteinate) trace elements – Zn, Mn, Cu, Fe, and Se - on live weight, concentrations of the elements in the blood serum, as well as phagocytosis, mitogen stimulation of lymphocytes, and the immunogenicity of inactivated oil vaccine against Aujeszky’s disease. During the experiment, 15-week-old pigs were fed a diet with organic mineral admixture at a dose of 1 g per kg of the diet. The experimental and control animals were given inactivated oil vaccine against pseudorabies in the 5th and 7th week of the experiment. Feeding the organic-bound trace elements resulted in a favourable effect on weight gain, content of trace elements in the blood, indices of phagocytic activity, and the response of lymphocytes to Aujeszky’s disease virus.

Key words: pigs, feed supplements, trace elements, immunity, mineral profile.

Trace elements are necessary for maintaining body functions, optimal growth, reproduction, and the immune system (5). Trace mineral nutrition can therefore determine the health of the animals and their body homeostasis. On the other hand, lack of these minerals leads to a significant decrease in production (5). Trace elements are components of many active substances involved in metabolic pathways, they play an important role in the production of hormones and enzymes with direct or indirect effects on immunocompetence and response to stress (14). The addition of trace elements in inorganic forms to the diet (chlorides, oxides, sulphates, carbonates) is a commonly used method of pig diet supplementation. However, absorption of nutrients from the
intestine and their incorporation into cellular biochemical pathways are influenced by many physical and chemical factors, which have adverse effects on both absorption and metabolic utilization (biological availability) of these elements by the cells (6). Some of these effects are directly related to certain chemical forms of the elements, or to the presence of other inorganic ions, which have the same absorption mechanism (10).

The binding of metallic ions to organic substances (ligands) through donor atoms (oxygen, nitrogen, sulphur) leads to increased biological availability of these elements. This is probably caused by a different absorption mechanism via peptide and aminoacid absorption mechanisms (2), or by alternate absorption mechanisms (9).

Comparative studies of the biological availability of organic and inorganic forms of trace elements (1, 16, 3, 12) showed organic-bound trace elements to be more resistant to the effects of other chemical substances. These forms are more soluble and, therefore, better absorbed and incorporated into biological systems and body structures. Presently, there is an increasing attention to organic, or complex forms of trace elements. In such forms, the trace element is bound to a certain ligand, which is usually a part of an amino acid or peptide.

This study attempted to define the effect of feed supplementation with organic-bound (metallic proteinate) trace elements (Zn, Mn, Cu, Fe, and Se) on live weight, concentrations of the elements in the blood, as well as phagocytosis, mitogen stimulation of lymphocytes, and the immunogenicity of inactivated oil vaccine against Aujeszky's disease in pigs.

**Material and Methods**

**Animals.** In the experiment lasting ten weeks, eight 15-week-old pigs were used. The animals, from the same litter, were divided into two groups, each group consisting of three males and one female. The animals were housed in two pens in the clinic and reared under identical standard conditions. The pigs were fed a restricted diet corresponding to their age category, three times daily (dry feed mixture OŠ-03; TAJBA a. s., Čaňa, Tables 1 and 2).

**Experimental design.** The experimental animals were fed diet supplemented with organic-bound trace elements (Zn, Mn, Cu, Fe, Se) in the form of commercial feed admixture (Piglet Booster, Alltech Inc., Table 3), at a dose of 1 g per kg of the diet. The control animals were fed only the dry feed mixture OŠ-03. In the 5th and 7th week of the experiment, both groups were treated with inactivated oil vaccine Inavak (Mevak, Nitra) against Aujeszky's disease (2.5 ml).

Once a week, the animals were weighed and sampled for the blood from the orbital sinus (7). In the experimental group, the first blood collection was done before supplementation with organic trace elements. Concentrations of trace elements in the blood serum and
immunological indices were analysed up to the 8th and 10th week of the experiment, respectively.

**Determination of trace element concentrations in the blood serum.** The concentrations of minerals were analysed by atomic absorption spectrophotometry. Concentrations of Fe, Cu, and Zn were analysed by flame atomic absorption spectrophotometry (Perkin Elmer A Analyst 100) and concentrations of Se and Mn by flameless atomic absorption spectrophotometry in an inert argon atmosphere (Perkin Elmer 4100 ZL).

**Immunological tests.** The functional activity of phagocytic cells was determined by the iodo-nitro-tetrazolium reductase test and lymphocytes by the leukocyte migration inhibition test.

a) Iodo-nitro-tetrazolium reductase test (INT). A quantitative evaluation of the tetrazolium-reductase activity of leukocytes for the evaluation of the metabolic activity (MA) of phagocytes during phagocytosis was carried out according to the method described earlier (11). The results are described in the form of an index of metabolic activity (IMA) based on the ratio of the mean optical density of leukocyte suspensions with starch (n = 3) and the leukocyte suspensions without starch (n=3).

b) Leukocyte migration inhibition assay (LMIA). LMIA was used to analyse the reaction capacity of lymphocytes to Aujeszky’s disease virus stimulation and was carried out according to method described earlier (11). The leukocyte suspensions were tested in concentration of $2.10^8$ ml$^{-1}$ under agarose with $10^6$ inactivated Aujeszky’s disease virus strain, identical with that used in the vaccine, and without antigen. The migration index (MI) was determined as a ratio of the mean of the migration areas of leukocytes with and without antigen.

**Determination of antibodies.** Concentrations of virus-specific antibodies were determined by a routine virus-neutralization test (VNT). The test was done on the cell line PK 15 with Aujeszky’s disease virus at a dilution of 1:1000 TCID$_{50}$. The sera examined were diluted by a double dilution from 1:2 to 1:1024.

**Statistical analysis.** Concentrations of trace elements in the blood serum and immunological indices were evaluated by the Student’s $t$-test.

### Table 1

| Mineral composition of OŠ-03 (mg/kg) |
|-----------------|---|---|---|---|
| Fe              | Cu | Zn | Mn | Se  |
| 100             | 10 | 70 | 30 | 0.20|

### Table 2

<table>
<thead>
<tr>
<th>Amount of feed and mineral admixture corresponding to the weight of pigs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

live weight a  8 - 17  17 - 30  30 - 40  40 - 50  50 - 60
OŚ-03 b  0.84  1.50  1.75  1.90  2.10
Piglet Booster  0.84  1.50  1.75  1.90  2.10

a - kilogram; b - gram

<table>
<thead>
<tr>
<th></th>
<th>Fe</th>
<th>Cu</th>
<th>Zn</th>
<th>Mn</th>
<th>Se</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp.</td>
<td>19605</td>
<td>4253</td>
<td>18439</td>
<td>9600</td>
<td>0.147</td>
</tr>
</tbody>
</table>

Table 3
Concentrations of trace elements in the Piglet Booster (mg/kg)

Results

At the beginning of the experiments, blood serum Fe levels ranged within the physiological limits. A decrease in Fe level observed in the 2nd week was followed by an increase in Fe content, with a peak value (18.38 mol/l) in the 7th week (Table 4). In the control group, moderate sideropenia was observed for the first three weeks, and again in the 6th and 8th week. Statistically significant differences between the groups were recorded in the 6th and 7th week.

<table>
<thead>
<tr>
<th>Samplin g (weeks)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp. x</td>
<td>16.11</td>
<td>15.89</td>
<td>16.34</td>
<td>16.57</td>
<td>16.56</td>
<td>17.01</td>
<td>*</td>
<td>18.38 *</td>
</tr>
<tr>
<td>sd</td>
<td>1.90</td>
<td>1.72</td>
<td>0.98</td>
<td>1.35</td>
<td>0.78</td>
<td>0.64</td>
<td>1.01</td>
<td>0.64</td>
</tr>
<tr>
<td>Control x</td>
<td>15.89</td>
<td>15.44</td>
<td>15.89</td>
<td>16.11</td>
<td>16.79</td>
<td>15.89</td>
<td>16.34</td>
<td>15.89</td>
</tr>
<tr>
<td>sd</td>
<td>1.33</td>
<td>1.47</td>
<td>1.33</td>
<td>0.63</td>
<td>0.98</td>
<td>0.39</td>
<td>0.98</td>
<td>0.98</td>
</tr>
</tbody>
</table>

*P < 0.05

The feed admixture affected Cu concentrations during the whole experiment (Table 5). Initially, both groups suffered from hypocupraemia. The levels of serum Cu reached the lower limit of the reference range only in the experimental group (24 mol/l) in the 7th week of the experiment. Significant differences between the groups were recorded from the 5th week onward.

Table 5
The supplementation with organic-bound trace elements had no significant effect on Mn serum levels (Table 6). The first blood sampling in the experimental group showed decreased levels of Mn, which were restored during the following weeks of the supplementation. In the control group, deficient Mn levels were observed in the 3rd, 4th, and 6th week of the experiment. In both groups, the highest Mn levels were recorded in the 2nd week of the experiment.

**Table 6**
Concentration of manganese in blood serum (µmol/l)

<table>
<thead>
<tr>
<th>Sample</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp</td>
<td>0.37</td>
<td>0.46</td>
<td>0.41</td>
<td>0.42</td>
<td>0.42</td>
<td>0.43</td>
<td>0.42</td>
<td>0.41</td>
</tr>
<tr>
<td>s</td>
<td>0.04</td>
<td>0.10</td>
<td>0.07</td>
<td>0.06</td>
<td>0.05</td>
<td>0.05</td>
<td>0.04</td>
<td>0.05</td>
</tr>
<tr>
<td>Control</td>
<td>0.40</td>
<td>0.41</td>
<td>0.37</td>
<td>0.37</td>
<td>0.38</td>
<td>0.38</td>
<td>0.41</td>
<td>0.41</td>
</tr>
<tr>
<td>s</td>
<td>0.05</td>
<td>0.06</td>
<td>0.02</td>
<td>0.01</td>
<td>0.02</td>
<td>0.02</td>
<td>0.04</td>
<td>0.02</td>
</tr>
</tbody>
</table>

From the beginning throughout the whole experiment, both groups showed hypozincemia. Significantly higher serum Zn levels were observed from the 4th week onward (Table 7). During the first three weeks of the experiment, there were no significant differences between the groups.

**Table 7**
Concentration of zinc in blood serum (µmol/l)
Both in the experimental and control groups, Se levels ranged below the normal limits (Table 8). Significant changes were observed from the 3rd to the last week of the experiment.

### Table 8
Concentration of selenium in blood serum (µmol/l)

<table>
<thead>
<tr>
<th>Sampling (weeks)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Exp.</strong> x</td>
<td>0.30</td>
<td>0.35</td>
<td>0.38∗∗</td>
<td>0.38∗∗</td>
<td>0.37∗</td>
<td>0.38∗∗</td>
<td>0.38∗</td>
<td>0.38∗</td>
</tr>
<tr>
<td>sd</td>
<td>0.06</td>
<td>0.05</td>
<td>0.03</td>
<td>0.04</td>
<td>0.04</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>Control</strong> x</td>
<td>0.25</td>
<td>0.27</td>
<td>0.26</td>
<td>0.26</td>
<td>0.27</td>
<td>0.28</td>
<td>0.27</td>
<td>0.27</td>
</tr>
<tr>
<td>sd</td>
<td>0.04</td>
<td>0.04</td>
<td>0.01</td>
<td>0.03</td>
<td>0.03</td>
<td>0.04</td>
<td>0.03</td>
<td>0.02</td>
</tr>
</tbody>
</table>

*P < 0.05; ** P < 0.01

During the whole experiment we did not observe any significant difference in the live weight between the groups (Table 9). During the 8 weeks, the average live weight increased by 11.95 kg in the control and 14.02 kg in the experimental group that is by 2.07 kg more. The largest difference between the groups was observed in the 7th week of the experiment (4.62 kg).

### Table 9
Average live weight of the pigs (kg)

<table>
<thead>
<tr>
<th>Weighing (weeks)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Exp.</strong> x</td>
<td>22.48</td>
<td>24.13</td>
<td>25.45</td>
<td>26.58</td>
<td>28.40</td>
<td>31.38</td>
<td>33.75</td>
<td>36.50</td>
</tr>
<tr>
<td>s</td>
<td>4.36</td>
<td>4.71</td>
<td>5.05</td>
<td>5.60</td>
<td>5.67</td>
<td>6.72</td>
<td>7.04</td>
<td>8.30</td>
</tr>
</tbody>
</table>
The administration of organic-bound trace elements had a favourable effect on the index of phagocytic activity of phagocytes, because significantly higher values were recorded in the experimental group from the 6th week onward (Table 10). By the migration-inhibition test, the responsiveness of lymphocytes to Aujeszky's disease virus in the experimental and control groups was detected on the 7th and 14th day after re-vaccination, respectively. The differences between groups were significant (Table 11). Virus-specific antibodies were detected in both groups one week after the vaccination. On average, titres of specific antibodies were higher in the experimental group, however, the difference was not significant because of the large individual variation in the titres (Table 12).

**Table 10**
Mean indices of phagocyte activity in the peripheral blood (INT)

<table>
<thead>
<tr>
<th>Sampling (weeks)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp. x</td>
<td>2.34</td>
<td>2.23</td>
<td>2.20</td>
<td>2.34</td>
<td>2.49</td>
<td>2.53*</td>
<td>2.57*</td>
<td>2.53</td>
<td>2.54*</td>
<td>2.44*</td>
</tr>
<tr>
<td>s</td>
<td>0.06</td>
<td>0.05</td>
<td>0.09</td>
<td>0.09</td>
<td>0.12</td>
<td>0.07</td>
<td>0.13</td>
<td>0.10</td>
<td>0.12</td>
<td>0.10</td>
</tr>
<tr>
<td>d</td>
<td>2.33</td>
<td>2.34</td>
<td>2.24</td>
<td>2.22</td>
<td>2.28</td>
<td>2.23</td>
<td>2.28</td>
<td>2.31</td>
<td>2.26</td>
<td>2.21</td>
</tr>
<tr>
<td>s</td>
<td>0.07</td>
<td>0.12</td>
<td>0.09</td>
<td>0.09</td>
<td>0.06</td>
<td>0.05</td>
<td>0.09</td>
<td>0.12</td>
<td>0.07</td>
<td>0.10</td>
</tr>
</tbody>
</table>

*P < 0.05; ** P < 0.01

**Table 11**
Average values of migration-inhibition indices after stimulation of peripheral blood lymphocytes by Aujeszky's disease virus (MIT)

<table>
<thead>
<tr>
<th>Sampling (weeks)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp. x</td>
<td>0.94</td>
<td>0.93</td>
<td>0.91</td>
<td>0.93</td>
<td>0.93</td>
<td>0.84</td>
<td>0.80</td>
<td>0.59*</td>
<td>0.51*</td>
<td>0.48</td>
</tr>
<tr>
<td>s</td>
<td>0.04</td>
<td>0.03</td>
<td>0.03</td>
<td>0.08</td>
<td>0.02</td>
<td>0.05</td>
<td>0.03</td>
<td>0.07</td>
<td>0.04</td>
<td>0.07</td>
</tr>
<tr>
<td>d</td>
<td>0.93</td>
<td>0.94</td>
<td>0.95</td>
<td>0.91</td>
<td>0.93</td>
<td>0.86</td>
<td>0.88</td>
<td>0.73</td>
<td>0.65</td>
<td>0.53</td>
</tr>
</tbody>
</table>
Table 12
Average titres of specific antibodies in blood serum of pigs
immunized with Inavak G1 vaccine

<table>
<thead>
<tr>
<th>Sampling (weeks)</th>
<th>Exp. s</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>d</td>
<td>2.45</td>
<td>21.2</td>
<td>34.8</td>
<td>13.8</td>
<td>17.4</td>
<td>4</td>
<td>7</td>
<td>6</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Control s</td>
<td></td>
<td>2</td>
<td>20</td>
<td>48</td>
<td>48</td>
<td>30</td>
<td></td>
<td>6</td>
<td>5</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>d</td>
<td>0</td>
<td>12</td>
<td>16</td>
<td>16</td>
<td>21.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5</td>
</tr>
</tbody>
</table>

*P < 0.05

Discussion

The risk of potential dietary deficit in one or more trace elements is increasing with the closed system of pig rearing during the whole fattening period. This change in production practice without grass yards, has eliminated the possibilities of obtaining some important mineral substances from soil and grass. Moreover, the use of a slatted floor reduces the possibility of obtaining some vitamins and minerals from the faeces (coprophagia).

In our experiment, supplementation with organic-bound trace elements resulted in a significant increase in blood serum concentrations of Fe, Cu, Zn, Se at the beginning of pig fattening period. In both groups, Mn levels were approximately the same and within the normal range (15). In the experimental animals, there was a tendency to a higher body weight gain, however, the difference of 2.07 kg was not significant. The higher indices of phagocytic activity in the experimental animals indicate an improvement in the natural resistance to infections. Following the response of peripheral blood lymphocytes to pseudorabies virus after vaccination, it could be concluded that there was an increased responsiveness of immunocompetent cells after the vaccination and, in this way, an increase in the immunogenicity of the vaccine itself.

Some other experiments on various pig categories have shown that including the organic mineral substances (bioplexes) into the diet has resulted in improved production indices. Supplementation of pregnant and lactating sows with Fe-bioplex has led to higher embryonic survival, larger litters, better sucking of piglets, weight gain, and higher body
weight of weanlings (4, 5). In fattening pigs (60 – 90 kg), feeding of Cu-Zn-bioplex has led to reduced excretion of the elements through the faeces (13), which is enormously important in environmental protection. The use of Se-bioplex has resulted in better pork quality (4) in fattening pigs (60 – 90 kg) and a lower number of still-born piglets (8).

The requirements for trace elements in intensively reared pigs depend on their quantitative and qualitative proportions in the diet. In our experiment, the supplementation of organic forms of trace elements had favourable effects on the mineral profile of pigs with consequent increase in phagocytic activity and lymphocyte responsiveness to antigen stimulus. This indicates increased body resistance to infection. The optimization of homeostasis was manifested by the higher weight gain during the experiment.

References


