INFLUENCE OF BENTONITE ON TRACE ELEMENT KINETICS IN RATS. 1. IRON

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The effect of dietary bentonite on uptake and distribution of radioiron given intragastrically was examined in the carcass and selected organs of rats. Feeding a bentonite fortified diet resulted in an increase in iron absorption in the carcass and organs (except for spleen) within a 28-day period postdosing.

Key words: rat, bentonite, radioiron, absorption, distribution.

Bentonite is a mixture of minerals of montmorillonite group. The special properties of bentonite such as hydration, swelling, water absorption, and viscosity make it a valuable material for a wide range of applications (1). Bentonite is used as a supplement in domestic animal feed. Numerous reports stress that bentonite decreases ruminal ammonia concentration and improves feed passing in the small intestine (4, 8). Moreover, a diet supplemented with bentonite affected beneficially acid-alkaline balance in the perinatal period of cows (3).

High ion exchange capacity of bentonite favours binding a wide range of cations (1). Several authors stressed that a diet supplemented with bentonite decreases the uptake of radiocontaminants from the gastrointestinal tract of animals (7, 9). It was also found that bentonite decreased markedly the absorption of cadmium administered orally (6). Furthermore, the chemical properties of bentonite also suggest an influence on intestinal absorption of minerals (9, 11, 12). For example, Schwartz and Werner (11) reported that bentonite at a high dose reduced renal and hepatic copper and zinc but increased iron and manganese incorporations into goats. The present study was carried out to determine whether bentonite as a dietary supplement affected the alimentary uptake of iron and its distribution in the carcass and organs of rats fed a moderate amounts of bentonite.
Material and Methods

Experiments involved 90 male Wistar rats weighing 212 g ± 11 g. The animals were randomly assigned into two dietary groups and were allowed *ad libitum* access to feeds and tap water. Diets for group 1 and group 2 were based on a commercial rat chow (LSM) and the same diet fortified with 2% of bentonite, respectively; the animals were fed with these diets for the whole experimental period. Body weight gain and feed intake were recorded weekly. Bentonite originated from the Polish geological sources and its composition was evaluated by Dembinski (8).

Iron chloride (labelled with iron-59, Polatom, Poland) in a 0.5 mL water solution was given daily for 28 d except weekends by intragastric tube comprising about 40 kBq per rat. All the rats were killed by immersion in gaseous carbon dioxide 6 h, 12 h, 1 d, 2 d, 4 d, 7 d, 14 d, and 28 d postdosing. The following organs were removed: liver, kidneys, small intestine (initial 15 cm), spleen, heart, testicles, brain, and muscles. The content of radioiron in the carcass (whole body without the stomach and intestines) and organs was measured in a whole-body counter ZM 701 (Polon, Poland) and in a well-type scintillation counter ZR 11 (Polon, Poland), respectively. Reference standards for quantification of carcass were prepared by intraperitoneal injection of the appropriate solution to rats which were killed 15 min thereafter.

The area under the curves (AUC) of radioiron content versus time points was calculated by the trapezoidal rule.

Data were analysed statistically using Student’s *t*-test at P<0.05.

Results

Each rat consumed about 29 g of feed per day during the whole experimental period. At the end of the experiment the two groups of rats were of comparable weight although the rats fed the bentonite enriched diet demonstrated a little higher body weight gain (Table 1).

The organ to body ratios for the liver, spleen, heart, testicles, and kidneys were similar in the two groups of rats at the end of the experiment (Table 1).

<table>
<thead>
<tr>
<th>Diet</th>
<th>Body gain (%)</th>
<th>Liver (mean ± SD)</th>
<th>Kidneys (mean ± SD)</th>
<th>Heart (mean ± SD)</th>
<th>Testicles (mean ± SD)</th>
<th>Spleen (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSM</td>
<td>83</td>
<td>3.91 ± 0.35</td>
<td>0.66 ± 0.05</td>
<td>0.26 ± 0.02</td>
<td>0.79 ± 0.17</td>
<td>0.19 ± 0.04</td>
</tr>
<tr>
<td>LSM (B)</td>
<td>92</td>
<td>3.95 ± 0.33</td>
<td>0.71 ± 0.05</td>
<td>0.26 ± 0.02</td>
<td>0.78 ± 0.09</td>
<td>0.18 ± 0.10</td>
</tr>
</tbody>
</table>

Carcass retention of iron (Table 2). The AUC values comprising the time course of radioiron in the carcass were used to compare the bioavailability of iron in the LSM and LSM(B) groups. As shown in Table 2, LSM(B) group absorbed more iron as compared to the LSM group. The carcass burden of radioiron in the LSM(B) group was higher and the differences were statistically significant at 6 h and 2 d postdosing.
Table 2
Carcass retention of Fe-59 (% dose) and AUC values

<table>
<thead>
<tr>
<th></th>
<th>3 h</th>
<th>6 h</th>
<th>1 d</th>
<th>2 d</th>
<th>4 d</th>
<th>7 d</th>
<th>14 d</th>
<th>28 d</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSM</td>
<td>10.89</td>
<td>12.35</td>
<td>11.13</td>
<td>10.03</td>
<td>11.61</td>
<td>9.34</td>
<td>10.68</td>
<td>7.21</td>
<td>6671</td>
</tr>
<tr>
<td>SD</td>
<td>4.81</td>
<td>1.35</td>
<td>2.45</td>
<td>1.81</td>
<td>3.91</td>
<td>0.56</td>
<td>2.94</td>
<td>1.64</td>
<td></td>
</tr>
<tr>
<td>LSM</td>
<td>11.18</td>
<td>17.66*</td>
<td>11.38</td>
<td>14.97*</td>
<td>10.02</td>
<td>10.25</td>
<td>11.42</td>
<td>8.40</td>
<td>7037</td>
</tr>
<tr>
<td>SD</td>
<td>3.45</td>
<td>1.34</td>
<td>2.65</td>
<td>0.82</td>
<td>2.29</td>
<td>1.17</td>
<td>2.10</td>
<td>1.60</td>
<td></td>
</tr>
</tbody>
</table>

* - indicates statistically significant differences at P<0.05

Iron content of body organs (Table 3). The content of radioiron in selected organs is expressed by AUC values. Both the LSM group and LSM(B) group accumulated the highest proportion of radioiron in the blood and liver. Negligible amounts were taken by the muscles and testicles. The AUC values of radioiron were visibly higher in the LSM(B) group than in the LSM group. The differences were related to those found in the carcass AUC values. Differences in the content of radioiron in individual organs (not shown) were in several cases statistically significant especially in the blood, liver and kidneys.

Table 3
AUC values in selected organs and tissues

<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>LSM</td>
<td>532</td>
<td>5.4</td>
<td>4.2</td>
<td>687</td>
<td>72</td>
<td>35</td>
<td>76</td>
<td>23</td>
<td>5.7</td>
</tr>
<tr>
<td>LSM(B)</td>
<td>620</td>
<td>6.9</td>
<td>5.2</td>
<td>783</td>
<td>83</td>
<td>36</td>
<td>72</td>
<td>28</td>
<td>6.4</td>
</tr>
</tbody>
</table>

Explanations: -liver, kidneys, testicles, heart, and spleen as a whole organ
-brain, small intestine, muscles and blood as 1 g samples

Discussion

Under the conditions of our experiment, a 2% addition of bentonite to the rat’s diet did not affect significantly feed and water consumption. The rats fed the diet with bentonite supplements demonstrated an improved growth rate as compared to those fed a standard laboratory chow. Similar observations were reported by others (2, 6, 12).

Few experiments have attempted to evaluate organ iron levels in animals fed a bentonite fortified diet (11); of particular importance was the observation that bentonite administered at high doses affected hepatic and renal iron retention. The results of the present studies are, at least in part, comparable with the above finding. Although similarities in these results are apparent, the use of radioiron in our studies permitted us to evaluate not only organ radioiron level but also a time-dependent distribution of radioiron in the carcass and organs. The AUC values calculated from the kinetics data indicated that the carcass and organ retention of radioiron increased within a 28-day period postdosing as a result of feeding the diet fortified with bentonite.

Because the rats fed the bentonite enriched diet absorbed more radioiron than those fed a standard diet it is concluded that bentonite may stimulate uptake of dietary iron. Alterations in iron uptake may be explained by the fact that bentonite used in our experiment comprises large amounts of iron oxide and several trace elements (2)
including copper that can interfere with dietary iron absorption (5). Evidence from experiments on the goats and sheep fed bentonite enriched diet indicated a decrease in copper level in the liver and kidneys (9, 11). Thus, as it was expected from the iron-copper interaction model (5) a lowered copper incorporation resulting from bentonite action may cause an increased iron uptake in the rats fed bentonite diet as compared to that in the rats on a standard laboratory chow.

Our results support the findings that bentonite added to diet may interfere with the metabolism of iron. Moreover, it is suggested that bentonite may affect uptake and distribution of other elements. Because bentonite has a wide-use in animal feeding this speculation points to carry out further studies considering the effect of bentonite especially on the trace elements that dietary intake may be insufficient.

References