EFFECT OF BENTONITE ON SUPPLEMENTAL ZINC UPTAKE AND DISTRIBUTION IN RATS

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Abstract

Rats were offered ad libitum zinc in twice the level of the standard diet (23 mg/kg diet), and bentonite (2% additive) for 28 d, together with traces of zinc chloride (labelled with zinc-65) given intragastrically. Results provided evidence that bentonite increased the body retention of zinc in comparison to the diet without this agent. Furthermore, the addition of bentonite did not influence feed intake, organ to body ratios, and haematological values, although a visible decrease in body weight gains following bentonite feeding was noted. These findings may be useful when bentonite fertilised diet is provided to animals pastured in areas with higher zinc levels.

Key words: rat, organs, carcass, bentonite, supplemental zinc.

Bentonite is a clay comprising minerals resulted from in situ devitrification of volcanic ash. Its name is derived from the Fort Benton cretaceous rocks in Wyoming (USA) where it was first found in about 1890. The composition of bentonite may vary according to its geographical location but as a mixture of minerals it does not have any chemical formula. It comprises mainly of hydrated aluminium silicates, smectite minerals such as montmorillonite (named after the town Montmorillon in Central France), and nontronite (14). Bentonite has strong colloidal and absorptive properties; it swells to about 12 to 15 times of its volume when coming into contact with water, and one gram of bentonite has a surface area of about 800 sq. meters. These properties make it a valuable material for a wide range of applications (10). The beneficial action of bentonite consists of the adsorption of toxins and trace elements including iron, calcium, selenium, calcium, selenium, and manganese and inclusion of bacteria and certain viruses, including intestinal influenza virus present in the gastrointestinal tract (1–6, 10, 12, 14).

Little is known about the influence of bentonite on zinc absorption and metabolism. Ma and Uren (8) in their in vitro experiments reported an entrapment of zinc in ditrigonal cavities of bentonite, making it less available for other processes. Schwarz and Werner (11) supported this finding showing that prolonged oral application of high doses of bentonite reduced zinc incorporation into the liver and kidneys of goats. On the other hand, our earlier report showed that dietary bentonite may moderately enhanced zinc bioavailability from the intestinal lumen (6). The present studies were, therefore, undertaken to investigate the influence of bentonite on zinc uptake and distribution in rats exposed to a higher dose of this metal.

Material and Methods

Ninety male Wistar rats weighing 205 g ±10 g were used. After an acclimatisation period of one week, the animals were randomly assigned into two dietary groups, each of 45 rats. All the rats were offered a tap water (less than 1 µg Zn/L) supplemented with zinc up to 23 mg Zn/L, and a standard rodent chow: LSM ad libitum (Fodder Manufacture Motycz, Poland), containing 23.3 g Zn/kg according to the manufacturer, except that the diet for rats in group II was modified to contain 2% of bentonite; the total daily zinc intake in these groups was two times higher in comparison to the rats fed standard diet, comprising of non-supplemented tap water and the standard LSM chow. The used bentonite originated from Polish geological sources. The animals were kept on the experimental diets for 28 d. Body weight gains and feed and water consumption were recorded weekly. In addition, blood samples were collected weekly by cardiac puncture at a volume of 1 ml into a tube containing calcium disodium versenate as anticoagulant. Erythrocyte and leukocyte counts, haematocrit value, and haemoglobin level were determined in the samples.

Rats of both groups were also given intragastrically traces of zinc chloride labelled with zinc-65, (Polatom, Poland), which was administered in a 0.5 ml of water solution comprising about 20 kBq per rat

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daily for 28 d, except weekends. The animals were killed by immersion in gaseous carbon dioxide 3 h, 6 h, 1 d, 2 d, 4 d, 7 d, 14 d, and 28 d after dosing. Radiozinc in the carcasses (whole body without the stomach and intestines) was measured using a whole-body counter ZM 701 (Polon, Poland) and that in the blood, liver, kidneys, duodenum, spleen, heart, testicles, brain, and muscles in a well-type scintillation counter ZR 11 (Polon, Poland). Reference standards for quantification of carcass radiozinc were prepared by intraperitoneal injection of the appropriate solution to rats, which were killed 45 min thereafter.

The area under the curves (AUC) of radiozinc content versus time points was calculated by the trapezoidal rule. Data were analysed statistically using Student’s t-test. The significance of differences was set up at P<0.05.

**Results**

Rats in all groups demonstrated similar water and feed intake but the final body weight gain was a little lower (by about 8%) in rats fed the diet supplemented with bentonite (data not shown).

The organ to body ratios for the liver, spleen, heart, testicles, and kidneys were similar in the two groups of rats and did not differ significantly from those found in our earlier reports (4-7).

The blood values including erythrocytes, haematocrit, haemoglobin, and leukocyte for the rats were also similar in the two examined groups of animals. Moreover, these values were also similar to those reported for normal rats (data not shown).

Retention of zinc-65 in the carcasses within a 28-d period after the exposure is shown in Fig. 1. The radio-zinc content varied between the examined groups. Comparison of these AUC values indicates that the bentonite supplemented diet increased zinc-65 retention in the carcasses by more than 50%. The differences were significant within the whole experimental period.

The highest content of supplemental zinc was found in the liver; markedly lower in the testicles and kidneys. The lowest content of zinc was found in the blood, duodenum, muscles, spleen, and brain.

The content of zinc-65 in selected organs, expressed as the AUC values, is presented in Table 1. These values, showing integrated exposure to zinc-65, the in rats fed bentonite diet, indicate that the content of zinc-65 in the examined organs was markedly higher in comparison to group I; the differences were significant nearly in all tissues and time-points and reflected those reported for carcass.

![Fig. 1. Mean zinc content in the carcasses (percentage of total dose); ★ – P<0.05](image-url)
between bentonite and zinc is limited.

...of bentonite, up to 10%, 20%, and 50% (10).

...maintained on diets composed of higher concentrations supplemented diet were also reported in animals lower growth responses following bentonite-location and dominant elements (10, 14). Furthermore, bentonite, which may vary according to its geographical inconsistency may be related to the composition of studies involving rats (3–6). It suggests that this experiment was found to increase body growth in earlier differences were not significant but it is interesting to in comparison to animals fed a non-fortified diet. The bentonite demonstrated a visibly lower growth response in comparison to animals fed the diet fortified with bentonite. However, rats fed the diet fortified with bentonite demonstrated a visibly lower growth response in comparison to animals fed a non-fortified diet. The differences were not significant but it is interesting to note that the 2% supplement of bentonite used in our experiment was found to increase body growth in earlier studies involving rats (3–6). It suggests that this inconsistency may be related to the composition of bentonite, which may vary according to its geographical location and dominant elements (10, 14). Furthermore, lower growth responses following bentonite-supplemented diet were also reported in animals maintained on diets composed of higher concentrations of bentonite, up to 10%, 20%, and 50% (10).

The scientific literature on the interaction between bentonite and zinc is limited. In vitro studies revealing a decrease in extractability of zinc trapped by bentonite (8) may suggest that bentonite used as a dietary additive makes zinc less available for absorption from the gastrointestinal tract. These findings seem to be consistent with those obtained by Schwartz and Werner (11), who found a decrease in zinc incorporation into the liver and kidneys of goats exposed to high doses (2,000 mg/kg b.w.) of bentonite for 160 d. On the other hand, no effects of bentonite upon zinc metabolism were reported in pregnant rats (16), lambs (13), and diary cows (17).

The presented study gave contradicting results, showing that rats may enhance zinc absorption from the gastrointestinal tract in response to bentonite present in their diet. Moreover, comparison of the findings with an earlier report (6) showed that bentonite elevates more markedly zinc uptake when the metal intake is increased. The evidence may suggest that the implicated relationship between bentonite and zinc absorption from the gastrointestinal tract is not clear and may involve several factors including the structure of bentonite and its composition.

The presented results provided evidence that bentonite increased zinc uptake especially when zinc intake is increased. A prolonged use of zinc fertilisers, its input from sewage and other pollution sources including zinc smelters (7), enhances zinc content in soils, food, and foodstuffs. Thus, these experimental findings may be useful when bentonite fertilised diet is provided to animals pastured in areas with high zinc levels.

**Discussion**

Parameters of physiological functions including growth, organ to body ratios, and blood values in rats exposed to higher doses of zinc were similar to those reported for normal rats in our earlier reports (3–6). This may indicate that higher zinc intakes used in the presented study did not increase the risk of zinc excess (9). Rats in our experiment obtained about 3 mg of zinc daily, which is far less than 160 mg Zn/kg of body weight, causing no adverse symptoms in these animals (15)

However, rats fed the diet fortified with bentonite demonstrated a visibly lower growth response in comparison to animals fed a non-fortified diet. The differences were not significant but it is interesting to note that the 2% supplement of bentonite used in our experiment was found to increase body growth in earlier studies involving rats (3–6). It suggests that this inconsistency may be related to the composition of bentonite, which may vary according to its geographical location and dominant elements (10, 14). Furthermore, lower growth responses following bentonite-supplemented diet were also reported in animals maintained on diets composed of higher concentrations of bentonite, up to 10%, 20%, and 50% (10).

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**References**

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**Table 1**

AUC values as a measure of zinc-65 retention in selected organs and tissues

<table>
<thead>
<tr>
<th></th>
<th>Blood</th>
<th>Duodenum</th>
<th>Muscles</th>
<th>Liver</th>
<th>Kidneys</th>
<th>Heart</th>
<th>Spleen</th>
<th>Brain</th>
<th>Testicles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>4.3</td>
<td>6.5</td>
<td>6.7</td>
<td>194</td>
<td>20.6</td>
<td>20.6</td>
<td>5.8</td>
<td>7.9</td>
<td>49.2</td>
</tr>
<tr>
<td>Group II</td>
<td>5.9</td>
<td>11.4</td>
<td>9.0</td>
<td>331</td>
<td>43.3</td>
<td>43.3</td>
<td>10.7</td>
<td>12.5</td>
<td>75.9</td>
</tr>
</tbody>
</table>

Explanations: Liver, kidneys, testicles, heart, and spleen as a whole organ

Blood as a 1 ml sample

Brain, duodenum, muscles as 1g samples

Muscles as 1g samples

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**Discussion**

Parameters of physiological functions including growth, organ to body ratios, and blood values in rats exposed to higher doses of zinc were similar to those reported for normal rats in our earlier reports (3–6). This may indicate that higher zinc intakes used in the presented study did not increase the risk of zinc excess (9). Rats in our experiment obtained about 3 mg of zinc daily, which is far less than 160 mg Zn/kg of body weight, causing no adverse symptoms in these animals (15)


