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INFLUENCE OF BENTONITE ON TRACE ELEMENT KINETICS IN RATS. II. CALCIUM

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Abstract

The suggestion that bentonite known as an ion-exchanging agent affects calcium absorption from the gastrointestinal tract was tested. Dietary bentonite (2% additive) given for 28 d together with traces of calcium chloride (calcium-45) produced moderate but persistent decreases in the calcium-45 organ content. In contrast, brentonite additives did not influence feed intake, organs to body ratios, and haematological values although an improved body weight gain following bentonite treatment was found.

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

Bentonite is a natural clay that comes from volcanic ash (1). Because of properties and accessibility, bentonite is widely used as a feed additive. It helps eliminate aflatoxins, cadmium and radiocaesium, and ameliorate food allergies, mucus colitis, spastic colitis, viral infections, such as stomach flu, and parasites that are unable to reproduce in the presence of the clay (1, 3, 5, 7, 9-11, 12). Moreover, this mineral taken internally supports feed passing and proper ruminal ammonia concentration in the intestinal system (8). The purpose of the present study was to investigate the effect of dietary bentonite on the calcium distribution, water and feed consumption, blood values and some body parameters in rats. The basis of the studies refers to earlier information that bentonite has strong adsorptive powers and its consumption may render unavailable some of the necessary nutrients by adsorbing them in the alimentary tract. It was also found that at physiological pH bentonite may substitute its sodium for several elements (4). In the previous work (6) we reported alterations in iron absorption and retention in rats fed a diet fortified with bentonite. These study is complementary to the above findings.

Material and Methods

Experiments involved 90 male Wistar rats weighing 212 g ± 11 g. The animals randomly assigned into two dietary groups (LSM – controls; LSM(B) – bentonite exposed) were allowed *ad libitum* access to feed and tap water. Diets for LSM group and LSM(B) group were based on a commercial pelleted rat LSM chow (Fodder Manufacture at Motycz, Poland) adequate in all nutrients and the same diet fortified with 2% of bentonite. The total calcium content of the LSM diet was 1.14% according to the manufacturer. However, the detailed composition of the LSM chow is not accessible without the manufacturer permission. The bentonite used originated from the Polish geological sources; its CaO content was about 4.14% (2), The animals were on these diets for the whole experimental period. Body weight gains and feed and water intake were recorded weekly during the feeding period.

Calcium chloride (labelled with calcium-45, Polatom, Poland) in a 0.5 mL water solution comprising about 100 kBq per rat was given daily for 28 d except weekends by intragastric tube to all the rats. The blood was collected at a volume of 1 mL by cardiac puncture directly into a tube containing calcium disodium versenate as anticoagulant from day 0 through 28. Weekly blood samples were analysed for erythrocyte and leukocyte counts, haematocrit value, and haemoglobin level. Rats were killed by immersion in gaseous carbon dioxide 6 h, 1 d, 2 d, 4 d, 7 d, 14 d, and 28 d postdosing. Radiocalcium content was measured in the liver, kidneys, small intestine (initial 15 cm), spleen, heart, testicles, brain, and muscles using the liquid scintillation counter Packard 2500TR. Blood values were examined using Auto Counter AC 920 (Swelab).

The area under the curves (AUC) of organ radiocalcium 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. At the end of the experiment the final body weight in the two groups of rats differed; the rats fed the bentonite enriched diet demonstrated a marked increase in their body weight gain as compared to that noted in the controls (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).

The blood value changes during the period of bentonite exposure are shown in Table 2. The values obtained for the rats fed bentonite enriched diet are all little lower than those found in the controls. However, differences were not statistically significant.

The content of radiocalcium in the organs examined is expressed by AUC values in Table 3. The rats fed both the standard and bentonite fortified diet accumulated the highest proportion of radiocalcium in the liver. Markedly lower amounts were taken by the kidneys, small intestine, and testicles. Smallest amounts were found in the spleen.

The kinetics data (content versus time) that are presented in Table 4 only for liver shows a single statistically significant alteration in the calcium content. The distribution of calcium-45 in the remaining organs was not shown although small decreases in the kinetics data were noted in the rats fed the bentonite diet as compared to those found in the controls.

Table 1
Body weight gain and organ to body ratio of selected organs (%) (mean ± SD)

Diet	Body gain	Liver	Kidneys	Heart	Testicles	Spleen
			(%)			
LSM	88	3.71 ± 0.49	0.71 ± 0.07	0.28 ± 0.02	1.01 ± 0.15	0.19 ± 0.03
LSM (B)	114	4.06 ± 0.46	0.69 ± 0.06	0.27 ± 0.02	0.81 ± 0.10	0.18 ± 0.03

Explanations: LSM – rats fed the standard laboratory LSM diet
LSM(B) - rats fed LSM fortified with bentonite

Table 2
Blood values in the controls and bentonite treated rats

	0 d	7 d	14 d	21 d	28 d
	Erythrocytes (x 10^{12}/L)				
LSM	6.51 ± 0.28	6.86 ± 0,42	6.71 ± 0,53	7.36 ± 0.50	7..94 ± 0.33
LSM (B)	6.29 ± 0.37	6.41 ± 0.38	6.54 ± 0.44	6.91 ± 0.49	7.14 ± 0.51
	Haematocrit (L/L)				
LSM	0.41 ± 0.02	0.40 ± 0.01	0.42 ± 0.04	0.43 ± 0.03	0.44 ± 0.03
LSM (B)	0.36 ± 0.03	0.35 ± 0.03	0.35 ± 0.05	0.38 ± 0.41	0.38 ± 0.04
	Haemoglobin mmol/L				
LSM	8.23 ± 0.17	8.23 ± 0.29	8.50 ± 0.74	9.10 ± 0.53	9.48 ± 0.54
LSM (B)	8.11 ± 0.12	8.04 ± 0.15	8.32 ± 0.26	8.56 ± 0.35	8.52 ± 0.37
	Leukocytes 10^9/L				
LSM (B)	8.11 ± 1.99	8.26 ± 1.87	9.74 ± 2.01	9.26 ± 1.92	7.64 ± 2.44
LSM	8.06 ± 2.25	8.75 ± 2.98	10.05 ± 2.85	10.82 ± 1.27	9.77 ± 1.07

Explanations: as in Table 1.

Table 3
The content of calcium-45 in selected organs and tissues (AUC values)

	Intestine	Muscles	Liver	Kidneys	Heart	Spleen	Brain	Testicles
LSM	12.3	7.5	233	28	8.0	3.2	7.8	34.8
LSM (B)	14.1	6.6	190	23	7.1	2.2	7.0	29.0

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

Table 4
Hepatic distribution of calcium-45 (% dose in the whole organ)

	3 h		6 h		1 d		2 d	
	mean	SD	mean	SD	mean	SD	mean	SD
LSM	0.51	0.06	0.39	0.06	0.29	0.08	0.46	0.03
LSM (B)	0.45	0.04	0.41	0.04	0.24	0.05	0.39	0.03

	4 d		7 d		14 d		28 d	
LSM	1.09	0.22	0.261	0.225	0.261	0.046	0.297	0.023
LSM (B)	0.67*	0.11	0.338	0.089	0.338	0.059	0.223	0.031
mean	SD	mean	SD	mean	SD	mean	SD	

Explanations: - * indicates a statistically significant difference at $P<0.05$

Discussion

This study has demonstrated that a 2% addition of bentonite to the rat's diet did not affect significantly feed and water consumption, relative weight of the liver, kidneys, heart, spleen and testicles, and selected blood values. On the other hand, feeding the bentonite supplemented diet caused a markedly better growth rate as compared to that in the rats fed a standard laboratory chow. A higher growth response caused by bentonite supplemented diets has been reported in several animal species (7, 8, 9). A beneficial effect of bentonite on body gain seems to be dose dependent; for example, when rats were fed a 5% dietary additive of bentonite (higher than that reported in the above studies) body weight gains were markedly lower than those in the controls (5). In the present experiment bentonite did not affect visibly feed consumption; an increase in body weight gains reported here may be related to an improved feed conversation efficiency, which was noted by others (2, 14).

Few experiments have attempted to evaluate calcium metabolism in animals fed a bentonite fortified diet. Southern *et al*. (14) reported no changes in tibia calcium content in chicks fed nutrient-deficient diets with sodium bentonite. Waltz *et al.* (15) noted only small differences in hepatic, renal, and phalanx bone calcium concentrations in lambs fed bentonite fortified diet. Similarly, results provided by Schell *et al.* (12) indicated only moderate effects of bentonite upon calcium absorption and distribution in growing pigs. The results of the present studies are, at least in part, comparable with the above findings. Although similarities in these results are apparent, the use of radiocalcium in our studies permitted us to evaluate the time dependent distribution of radiocalcium in the organs. The AUC values calculated over a 28-day period following calcium-45 exposure indicated that the organ retention of radiocalcium was smaller as a result of feeding the diet fortified with bentonite. A lower retention of calcium suggests that bentonite used as a dietary additive may decrease calcium uptake from the gastrointestinal tract and its organ retention in rats.

Mineral metabolism was found to be affected by bentonite used in diet. Moreover, the response of mineral metabolism to bentonite treatment seems to be variable with respect to the element involved (13). The results from the recent and earlier studies with the use of rats have demonstrated a significant but temporal alterations in iron absorption and organ distribution, slight disturbances in calcium kinetics and significant decreases in cadmium retention (5, 6). The mechanism through which bentonite affects the absorption of toxic and indispensable elements from the gastrointestinal tract is not well understood. Basically, differences in the element uptake may be explained by the fact that bentonite used in our experiments comprises large amounts of various trace and macro elements (2). Thus, considerable amounts of elements delivered into the intestine with bentonite may be in permanent competition for mucosal binding sites and transport into the blood stream with those provided with feed. Finally, when the luminal profile of these elements is markedly changed by dietary bentonite, a significant disturbation in the absorption of elements offered with feed may be expected.

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 the dietary intake of which may be marginal or insufficient.

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