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ADSORPTON EFFECTS OF MINERAL ADSORBENTS; PART III: ADSORPTION BEHAVIOUR IN THE PRESENCE OF VITAMIN B6 AND MICROELEMENTS

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Mineral adsorbents based on natural zeolite and bentonite may be used in animal diets in order to prevent poisoning caused by mycotoxins. In this paper vitamin B6 and microelement adsorption by six different samples of mineral adsorbents was considered. The results indicated that mineral adsorbents based on natural zeolite had a low efficiency to bind vitamin B6 in vitro (from Cα=5% for sample 2 to Cα=22% for sample 3). This process is dependent on crystallinity and the mineralogical composition of the zeolitic samples. On the contrary, vitamin B6 was tightly bound to the mineral adsorbent based on bentonite (HSCAS)-Cα=98%. The investigation of microelement adsorption in vitro, at pH 3.5 and 5, by zeolite and bentonite showed that Cu, Zn, Co and Mn were bound less tightly to zeolite than to bentonite. The obtained data suggest that bentonite material would reduce micronutrient availability more than zeolite.

Key words: vitamin B6, microelements, adsorption, zeolite, bentonite

INTRODUCTION

Mycotoxins are fungal elaborated poisons that are toxic when consumed by animals, including human beings. These toxins can accumulate in corn, cereals, soybeans, and other food and feed crops. The toxins may occur in storage under conditions favorable for the growth of the toxin-producing fungus or fungi (Hiroshi 1978, Betina 1984, IARC 1993, Smith & Moss 1985).

There are many methods for detoxification of mycotoxin contaminated crops. They include extraction, physical separation, thermal inactivation, microbiological degradation and treatment with different chemicals. However, many of these methods are impractical, ineffective and potentially unsafe.

Results *in vitro* showed that a variety of aluminas, zeolites, silicas, phyllosilicates and chemically modified phyllosilicates were capable of adsorbing mycotoxins from aqueous solutions. However, the mycotoxin binding capacities and the stabilities of the sorption complexes were significantly different for many of these compounds (Harvey et al. 1989, 1994, Phillips et al. 1988, 1990, 1995). Aflatoxin B1 and G2 adsorption on clinoptilolite was described in Part I (Tomašević-Čanović et al. 1994).

Also, many experiments *in vivo* showed that using mineral adsorbent in animal feed blocked and immobilized mycotoxins in the gastrointestinal tracts of animals. Thus, the concentration of 5 g/l of clinoptilolite based mineral adsorbent in colostrum led to a significantly higher absorption of colostral immunoglobulin G in newborn calves (Stojić et al. 1995).

Although these mineral adsorbents are added to animal diets to prevent negative effects of mycotoxins, their influence on other feed components (vitamins, aminoacids and microelements) is not well known. Chung and Boker (1990) reported that montmorillonite did not impair phylate and inorganic phosphorus utilization. Chung et al. (1990) reported that addition of 0.5% and 1% HSCAS to the basal diet did not impair the utilization of riboflavin, vitamin A and Mn. They noted only a slight reduction in Zn utilization. In our previous study (Part II) we showed that a mineral adsorbent based on natural zeolite had no affinity to adsorb tryptophan, phenylalanine, vitamins A, D and E (Tomašević-Čanović et al. 1996).

The objective of the present study was to determine *in vitro* adsorption of microelements: Cu, Zn, Co and Mn and vitamin B6, in aqueous electrolyte, by mineral adsorbents based on natural zeolite. Considering that natural bentonite may be used for same purpose i.e. for mycotoxin prevention, results obtained for both types of adsorbents are presented here.

MATERIALS AND METHODS

The following samples from different origin and with different accessory minerals were used for the investigation of vitamin B6 adsorption:

Sample 1. Natural zeolite from Zlatokop deposit (Yugoslavia). The content of clinoptilolite was over 90% and cation exchange capacity (CEC) was 146 meq/100g. Accessory minerals were quartz and feldspar.

Sample 2. Natural zeolite from Novakovići (Serbian Republic Bosnia). The content of clinoptilolite was 90%. CEC of this zeolite was 170 meq/100g and accessory minerals were feldspar and quartz.

Sample 3. Natural zeolite from Novakovići (Serbian Republic, Bosnia). Cation exchange capacity was 110 meq/100g. In this sample the content of clinoptilolite was 60%, bentonite 30% and calcite in small amounts.

Sample 4. H^+ form of zeolite obtained by treatment 10 g natural zeolite (Zlatokop) with 100 cm^3 1M HCl for 2 h at 60^oC. After that, the sample was centrifuged, washed with deionized water until Cl^- ions were not detected and dried.

Sample 5. The commercial product for mycotoxin adsorption, HSCAS (NovaSil) is based on bentonite-layered aluminosilicate.

For determination of the effects of mineral adsorbents on reduction of microelements availability (Cu, Zn, Co and Mn) in animal feed two samples were used:

Sample 1. Natural zeolite (Zlatokop, Yugoslavia)

Sample 6. Natural bentonite from Šipovo deposit (Serbian Republic, Bosnia). In bentonite, the content of montmorillonite was 90% and CEC was 96 meq/100g. Accessory minerals were quartz and calcite. All these mineralogical data were obtained by XRPD analysis.

The adsorption of vitamin B6 and microelements was examined *in vitro* in an electrolyte of composition similar to the gastric juice of animals. The electrolyte contained 0.1 M HCl and 0.05 M NaCl. The pH was adjusted with 0.1 M NaOH.

Pharmaceutical grade vitamin B6 was added to the electrolyte at 5 mg per g of adsorbent. That corresponded to 10 mg/kg diet (10 ppm) when the adsorbent was incorporated in the diet at the level of 0.2%.

The capacity of adsorbents for microelements was determined by adding various quantities of each element to the electrolyte. Cu, Zn, Co and Mn were added as chloride solutions. Two ratios microelement/adsorbent were examined according to nutrient normatives needed in animal diets as given in Table 1.

Table 1. Amounts of microelements in contact with 1 % suspensions of adsorbent

Micro element	Requirement for animal diet (mg/kg)	Microelement added (mg)	
		5g adsorbent/kg diet	2 g adsorbent/kg diet
Cu	10	2	5
Zn	100	20	50
Co	0.8	0.16	0.4
Mn	80	16	40

Determination of vitamin B6 and microelements: A certain amount of each examined component was added to 100 cm^3 of the electrolyte and an aliquot was taken for determination of the total concentration present in solution (*Ct*). Then, 1g of mineral adsorbent was added to the electrolyte and the suspension was slightly shaken for two hours. The concentration of non-adsorbed component was then determined, after centrifugation, in the supernatant (*Cf*).

Total and non-adsorbed concentrations of vitamin B6 were determined by HPLC method (Hewlett Packard Chromatograph, Series 1100 with DAD detector).

Total and non-adsorbed concentrations of microelements were determined by atomic absorption spectrophotometry (Perkin Elmer, Model 703).

RESULTS AND DISCUSSION

The spectra of vitamin B6 determined in an aqueous electrolyte before (st B6) () and after addition of different mineral adsorbents based on zeolites and bentonites (····) are shown in Figure 1.

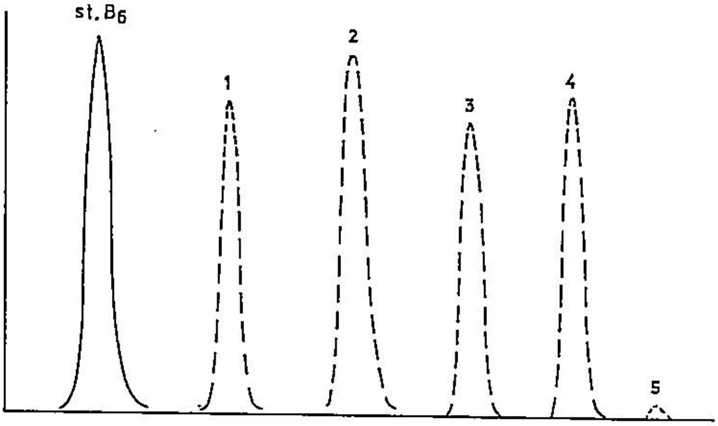

Figure 1. Vitamin B6 chromatograms:
without mineral adsorbents (—),
in the presence of mineral adsorbents (....);
sample 1-natural zeolite from Zlatokop deposit;
sample 2-natural zeolite from Novakovići;
sample 3-natural zeolite from Novakovići (zeolite with 30% of bentonite);
sample 4-H+ from of zeolite;
sample 5-HSCAS

It can be seen that the mineral adsorbents showed different ability to bind vitamin B6 from aqueous electrolyte at pH 3.5.

An adsorption index (Cα) was derived from the initial ligand concentration (Ct) and the concentration of non-adsorbed ligand (Cf):

$$C\alpha = \frac{Ct\ Cf}{Ct} \cdot 100$$

Cα=100% indicates total adsorption.

The calculated vitamin B6 adsorption indexes are given in Table 2.

Table 2. The vitamin B6 adsorption indexes for different mineral adsorbents

	Sample				
	1	2	3	4	5
Cα (%)	18	5	22	15	98

It can be seen, from these results that for the investigated mineral adsorbents, the adsorption indexes ranged from 5% for sample 2 to 98% for sample 5. Samples 1-3 are natural zeolites with different amounts of clinoptilolite. The crystallinity of zeolitic samples may have had an influence on adsorption of vitamin B6. According to the results of XRPD analysis the crystallinity of the zeolitic samples decreased in the following order:

$$2 > 1 > 3$$

Thus, from the obtained data it can be observed that with decreases in crystallinity the adsorption of vitamin B6 increases. Furthermore, the increase of vitamin B6 adsorption at the active centers of the mineral adsorbent appears to be related to increases in the bentonite content in the samples. Thus sample 3 (zeolite with 30% of bentonite) had an adsorption index of 22% while sample 5 based on bentonite, layered aluminosilicate, showed the highest adsorption of vitamin B6 ($C\alpha$=98%). Also, the acid treated zeolite (sample 4) showed an adsorption index 15% compared with 18 % for the starting zeolite (sample 1) indicating that exchangeable cation, in zeolite, had a small influence on vitamin B6 adsorption.

Chung et al. (1990) investigated the utilization of essential nutrients in poultry, using Zn and Mn as a model trace elements. Addition of 0.5 and 1% of HSCAS did not impair the utilization of Mn, but there was a slight reduction of Zn utilization in the presence of HSCAS. Data on adsorption of microelements on mineral adsorbents *in vitro* were not found in the literature.

The data obtained for microelement adsorption *in vitro* by natural zeolite and bentonite in aqueous electrolyte, at pH 3.5 and 5 are presented in Table 3 (0.2% adsorbent) and Table 4 (0.5% adsorbent).

Table 3. Microelement adsorption indexes; 0.2% adsorbent in diet

Micro element	Ct (mg/g)	$C\alpha$ (%)			
		zeolite	bentonite	zeolite	bentonite
		pH 3.5	pH 3.5	pH 5	pH 5
Cu	5	25	51	31	56
Zn	50	3	17	0	12
Co	0.4	11	73	23	73
Mn	40	5	12	3	12

Table 4. Microelement adsorption indexes; 0.5% adsorbent in diet

Micro element	Ct (mg/g)	$C\alpha$ (%)			
		zeolite	bentonite	zeolite	bentonite
		pH 3.5	pH 3.5	pH 5	pH 5
Cu	2	23	80	30	88
Zn	20	0	18	5	22
Co	0.16	10	87	2	85
Mn	16	2.5	15	1	18

Cation adsorption to the active centers of the mineral adsorbent occurred via an ion exchange mechanism. It can be seen in Table 3 and 4 that the adsorbent based on natural bentonite showed higher microelement adsorption than zeolite material. Copper and cobalt demonstrated high adsorption by bentonite at both examined pH values. Cu was adsorbed from 56 to 88% and Co from 73 to 87%. Zinc and manganese were not bound to bentonite as strongly as Cu and Co (Zn about 22% and Mn about 18%). However, all investigated microelements were bound less strongly to the zeolite, at both examined pH values. Copper adsorption by zeolite was about 25% at pH 3.5 and 30% at pH 5. Zn and Mn showed negligible binding to zeolite (max. 5%). Co adsorption was higher at pH 5 ($C\alpha$=23%). The adsorption indexes ($C\alpha$) for microlement adsorption by zeolite decreased in the following order:

$$Cu > Co > Mn > Zn$$

The obtained results are very important for practical application of mineral adsorbents. Mineral adsorbents based on natural zeolite may be used for removal of aflatoxins from aqueous electrolytes at pH 2 and at pH 7. Also, this mineral adsorbent did not impair utilization of tryptophan, phenylalanine or vitamin A, D and E. The *in vitro* results for vitamin B6 adsorption showed that vitamin B6 exhibited slight binding to zeolite materials. However, vitamin B6 is very strongly adsorbed by bentonite material. The *in vitro* results for adsorption of microelements suggest that bentonite material would greatly reduce micronutrient availability compared to zeolite material.

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ADSORPCIONI EFEKTI MINERALNIH ADSORBENATA; III DEO: ISPITIVANJE U PRISUSTVU VITAMINA B6 I MIKROELEMENATA

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SADRŽAJ

U ovom radu je ispitana *in vitro* adsorpcija vitamina B6 i mikroelemenata (Cu, Zn, Mn i Co) na mineralnim adsorbentima na bazi zeolita i bentonita. Mineralni adsorbenti na bazi zeolita su pokazali mali indeks vezivanja vitamina B6 (od 5% za uzorak 2 do 22% za uzorak 3). Zapaeno je da indeks adsorpcije vitamina B6 raste sa opadanjem stepena kristaliniteta zeolitskih uzoraka kao i sa porastom sadraja bentonita u uzorcima. Tako, indeks adsorpcije vitamina B6 za uzorak 3 (zeolit sa 30% bentonita) iznosi 22% dok za HSCAS (adsorbent na bazi bentonita) ovaj indeks iznosi 98%. Ispitivanja *in vitro* adsorpcije mikroelemenata su pokazala da se Cu, Zn, Mn i Co slabije adsorbuju na adsorbentu na bazi zeolita. Pri istim uslovima mineralni adsorbenti na bazi bentonita značajno adsorbuju ispitivane mikroelemente.