Acta Veterinaria (Beograd), Vol. 44. No. 5—6, 309—318, 1994.

UDK 619:615.918

THE ADSORPTION EFFECTS OF A MINERAL ADSORBER OF THE CLINOPTILOLITE TYPE PART I: ADSORPTION OF AFLATOXINS B1 AND G2

MAGDALENA TOMAŠEVIĆ-ČANOVIĆ*, M. DUMIĆ*, OLIVERA VUKIĆEVIĆ*, P. RADOŠEVIĆ**, I. RAJIĆ*** and T. PALIĆ***,

* *Institute for Technology of Nuclear and Other Mineral Raw Materials, Belgrade ** VMA – Institute for Hygiene, Belgrade *** Faculty of Veterinary Medicine, Belgrade*

(Received, 9. March 1994.)

A new mineral adsorber, representing a concentrate of the zeolitic mineral clinoptilolite, was obtained by technological processing, from a zeolitic tuff deposit in Zlatokop (Serbia, Yugoslavia). In this paper, the adsorbing characteristics of this product for mycotoxins of the aflatoxin group (B1 and G2) are presented. The investigations were carried out at pH2 and pH7, in an electrolyte with a composition similar to animal gastric juice, in order to evaluate the influence of pH on the adsorptive characteristics of clinoptilolite.

Mycotoxin determinations were carried out in the electrolyte without and with added mineral adsorber after incubation with shaking at 37°C (in a water bath). The test solution contained 1 g of mineral adsorber and certain concentrations of the toxins (50, 100, 200 and 300 μg/g). The quantity of mycotoxin not adsorbed was detected in the supernatant, after chloroform extraction, by the highly effective liquid chromatographic (HPLC) fluorescene method. Measurement of adsorption was started at 5 minutes and continued up to 48 hours of mycotoxin-mineral adsorber contact.

The results obtained show that, immediately after contact with mineral adsorber (5 min) both mycotoxins were adsorbed more than 80%. The level of adsorption increased up to 48 hours without the occurrence of desorption. Mycotoxin B1 was better adsorbed from the neutral (pH7) than from the acidic (pH2) medium, while the mycotoxin G2 was better adsorbed in the acidic medium (pH2).

Key words: Aflatoxin, mineral adsorber, hemisorption, adsorption.

INTRODUCTION

Mycotoxins are important fungal metabolites that have been recognized within the last two decades as a potential threat to human and animal health.

Because mycotoxins are environmental chemicals which can poison both man and animals, their occurrence and also the occurrence of the fungal strains producing them have been studied by many scientists (Betina, 1984).

The aflatoxin group of mycotoxins (a structurally similar group of polysubstituted coumarins) are secondary fungal metabolites produced by the Flavus-parasiticus group of the genus Aspergillus. The toxic effect of aflatoxins has been well documented (Betina, 1984; Shull, 1985).

Many investigations have been carried out using different mineral components for prevention of aflatoxicosis. Thus Harvey et al. (1989), Philips et al. (1988) and Kubena et al. (1990,1993), used hydrated sodium-calcium aluminosilicate as a sorbent for aflatoxins. Experiments were done in vivo on swine (Harvey et al. 1989), chickens (Philips et al. 1988; Kubena et al. 1990) and turkeys (Kubena et al. 1993). The results obtained showed that hydrated Na-Ca aluminosilicate can modulate the toxicity of aflatoxins. Colvin et al. (1989) examined the effects of a high affinity aluminosilicate sorbent on the prevention of aflatoxicosis in growing pigs.

Dietary additions of zeolite, bentonite or spent bleaching clay from canola oil refining (Smith 1980, 1984) have been reported to diminish the adverse effects of T2 toxin and zearalenone in rats and immature swine. Clay and zeolitic minerals encompass a considerably complex and diverse family of aluminosilicates that possess a variety of functional properties.

The effects of zeolite in the prevention of mycotoxicosis in swine (Trajkovič, 1991) and chickens (Palić et al. 1991) have been examined in vivo in experiments on domestic animal farms. The results obtained showed positive preventive effects of a mineral adsorber obtained by technological processing of natural zeolite from the Zlatokop deposit in Serbia.

In this paper, the results of adsorption, in vitro, of aflatoxins B1 and G2 on this mineral adsorber are presented. Aflatoxins B1 and G2 were chosen as diverse toxins of this group. Aflatoxin B1 is the most toxic toxin within the group (Betina, 1984) containing a double bond in the furane ring, while aflatoxin G2 has a second lactone ring.

MATERIALS AND METHODS

The mineral adsorber examined was obtained by technological preparation of the zeolitic tuff from the Zlatokop deposit. The basic characteristics of the produc are as follows:

The particle size distribution determined on a Cyclosizer is presented in Table 1.

Mineralogical composition: the basic component is clinoptilolite with the presence of quartz and plagioclase.

Table 1. Particle size distribution of the mineral adsorber

Particle size, μm	M, %
+ 44	1.17
− 44 + 33	2.33
− 33 + 23	5.47
− 23 + 15	7.57
− 15 + 10	6.89
− 10 + 0	76.57

The chemical composition is given in Table 2.

Table 2. Chemical composition of the mineral adsorber, %

SiO_2	Al_2O_3	Fe_2O_3	TiO_2	MnO	CaO	MgO	Na_2O	K_2O	P_2O_5	L.I.
64.21	11.48	0.88	0.25	0.03	4.55	1.45	1.71	1.29	0.05	14.00

Unit cell composition (Obradović, 1987)

$Na_{0.59}K_{0.75}Ca_{2.14}Mg_{0.96}(Al_{6.05}Si_{29.10}O_{72})14.00H_2O$

The cation exchange capacity, determined by the ammonium acetate method is presented in Table 3.

Table 3. CEC and exchangeable cations of the mineral adsorber

Cation	K^+	Na^+	Ca^{++}	Mg^{++}	Total
CEC, meq/100 g	17.3	82.6	49.5	16.8	166.2

Aflatoxins B1 and G2 were produced by Sigma Chemical Co.

The electrolyte was prepared using basic inorganic components present in the gastric juice of animals, containing o,1M HCl/dm^3 and 0.05M NaCl/dm^3. The pH was adjusted with 0.1N NaOH.

The test method for determination of the mycotoxins: certain amounts (50, 100, 200 and 300 μg) of aflatoxins (B1 or G2) in methanol were added to 100 cm^3 of electrolyte. An aliquot (0.4 cm^3) was taken for the determination of the total amount of mycotoxins present in solution (C_s). To the contaminated solution of the electrolyte 1 g of mineral adsorber was added and suspension was placed in the water bath (37^{0}C) and slightly shaken. For the determination of non-adsorbed mycotoxins after a certain time (C_f) a portion was taken for analysis. The mineral product was separated by centrifuging. Mycotoxins in the supernatant solution were determined after extraction into chloroform by HPLC.

The limit of detection by this method, is 20 pg.

This method was utilized for the determination of the stability of both mycotoxins in basic electrolyte during the time range from 1 to 48 hours, as well as, for the determination of the precision and efficiency of the extraction.

RESULTS AND DISCUSSION

Mycotoxin B1 adsorption results,for all the initial (C_s) and final concentrations (C_f) per g of mineal adsorber, contact time and pH, are given in Table 4.

Table 4. Final concentrations and calculated adsorption values for aflatoxin B1 at pH2 and pH7

	Cs = 50 μg/g				Cs = 100 μg/g			
	pH 2		pH 7		pH 2		pH 7	
Conctact time, min.	Cf	A%	Cf	A%	Cf	A%	Cf	A%
5	8	84.0	7	86.0	11	88.0	13	87.0
30	8	84.0	5	90.0	17	83.0	11	89.0
60	7	86.0	4	92.0	17	83.0	9	91.0
120	6	88.0	7	86.0	17	83.0	9	91.0
180	7	86.0	4	92.0	17	83.0	9	91.0
240	9	82.0	3	94.0	24	76.0	9	91.0
300	9	82.0	3	94.0	11	89.0	6	94.0
360	9	82.0	3	94.0	15	85.0	6	94.0
24h	5	90.0	2	96.0	15	85.0	6	94.0
48h	5	90.0	2	96.0	13	87.0	6	94.0
	C_s = 200 μg/g				C_s = 300 μg/g			
5	32	84.0	32	84.0	39	87.0	40	86.7
30	37	81.5	28	86.0	66	78.0	37	87.7
60	42	79.0	23	88.5	66	78.0	30	90.0
120	47	76.5	23	88.5	92	69.3	30	90.0
180	42	79.0	18	91.0	66	78.0	30	90.0
240	42	79.0	18	91.0	66	78.0	30	90.0
300	42	79.0	18	91.0	66	78.0	30	90.0
360	37	81.5	14	93.0	59	93.0	30	90.0
24 h	32	84.0	14	93.0	46	84.0	22	92.7
48 h	28	86.0	14	93.0	39	87.0	15	95.0

C_s - total amount of added aflatoxins (μg in 100 ml electrolyte)
C_f - free amount of aflatoxins in supernatant (μg/100 ml electrolyte)
A, % - percentage of aflatoxins adsorbed on zeolite

The differences between C_s and C_f represent the amount of adsorded mycotoxin and are given as percentages (A%).

Thuis, Table 4. shows a decrease of mycotoxin B1 concentration in the supernatant, depending on the contact time, for all four investigated concentrations, at pH2 and pH7. It can be seen that, in a very short time after contact, the concentration of mycotoxin in the supernatant decreased. In the following 48 hours, no reversibility was detected. On the contrary an increase of the adsorption was evident.

Data presented in Table 5 show the decrease of G2 mycotoxin concentration in the supernatant in dependance on the contact times (at pH2 and pH7). In this case, maximum adsorption was obtained in a very short contact time, also.

In order to eliminate errors due to possible spontaneous aflatoxin B1 and G2 degradation or their sorption on the vessel walls, stability investigations were executed in the clear systems (without zeolite) for the same period of

time and under the same conditions as during the experiments. The aflatoxins B1 and G2, in both electrolytes did not show any changes in concentration during the test time. The precision of the method, in both model suspensions was better than 2.5 % for n=5 and the efficiency of the extraction was over 90%.

Table 5. Final concentrations and calculated adsorption values for aflatoxin G2 at pH2 and pH7

	C_s = 50 μg/g				C_s = 100 μg/g			
	pH2		pH7		pH2		pH7	
Contact time, min.	C_f	A%	C_f	A%	Cf	A%	Cf	A%
5	11	78.0	14	72.0	23	77.0	33	67.0
30	7	86.0	10	80.0	13	87.0	33	67.0
60	7	86.0	10	80.0	13	87.0	23	77.0
120	8	84.0	11	78.0	11	89.0	23	77.0
180	7	86.0	12	76.0	11	89.0	21	79.0
240	8	84.0	10	80.0	11	89.0	21	79.0
300	7	86.0	10	80.0	9	91.0	17	83.0
360	7	86.0	10	80.0	9	91.0	17	83.0
24 h	5	90.0	7	86.0	9	91.0	16	84.0
48 h	5	90.0	7	86.0	9	91.0	16	84.0
	C_s = 200 μg/g				C_s = 300 μg/g			
5	49	75.5	55	72.5	58	80.7	95	68.3
30	31	84.5	51	74.5	39	87.0	68	77.3
60	27	86.5	43	78.5	39	87.0	47	84.3
120	36	82.0	43	78.5	58	80.7	68	77.3
180	27	86.5	43	78.5	39	87.0	74	75.3
240	27	86.5	38	81.0	39	87.0	68	77.3
300	27	86.5	38	81.0	39	87.0	61	79.7
360	22	89.0	21	89.5	32	89.3	61	79.7
24 h	22	89.0	25	87.5	26	91.3	47	84.3
48 h	18	91.0	30	85.0	22	91.3	34	88.7

C_s - total amount of added aflatoxins (μg in 100 ml electrolyte)
C_f - free amount of aflatoxins in supernatant (μg/100 ml electrolyte)
A, % - percentage of adsorbed aflatoxins on zeolite

Thus, adsorption of mycotoxin B1 in the range of 50-300 μg/g of mineral product was from 80% to 95% depending on the intial mycotoxin concentration, contact time and pH of the electrolyte. Adsorption was higher at pH2 than at pH7.

Adsorption of mycotoxin G2, in the range of 50-300 mg/g of mineral product was from 70–90%, depending on the initial mycotoxin concentration, contact time and pH of the electrolyte. Opposite to B1, mycotoxin G2 was better adsorbed at pH2 than at pH7.

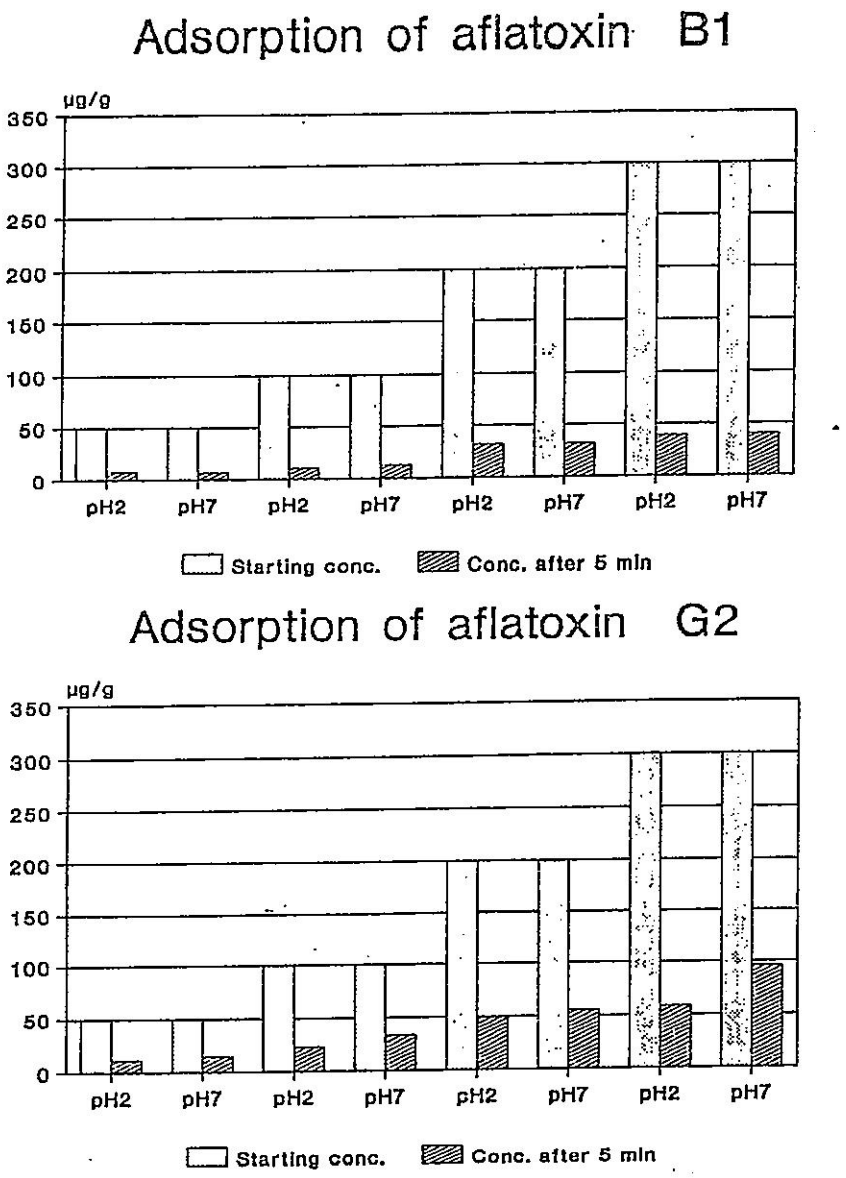

Figure 1. Adsorption of aflatoxins B1 and G2 after 5 minutes on pH2 and pH7

The difference (C_s-C_f) corresponding to the adsorbed mycotoxin, was the highest in all investigated cases five minutes after contact with the mineral adsorber. The difference of mycotoxin content in the supernatant after the first measured interval (Figure 1) clearly shows that B1 was better adsorbed at pH7 compared to mycotoxin G2, while the adsorption at pH2 was very similar for both mycotoxins. Adsorption of both mycotoxins for the test series of the contact time, for 1 h and 48 hours, showed generally similar adsorption for all concentration regions of the test solutions (Figure 2).

The complex mycotoxin/aflatoxin was stable. Thus, less than 10% of aflatoxin which was initially bound to mineral could be extracted by various organic solvents.

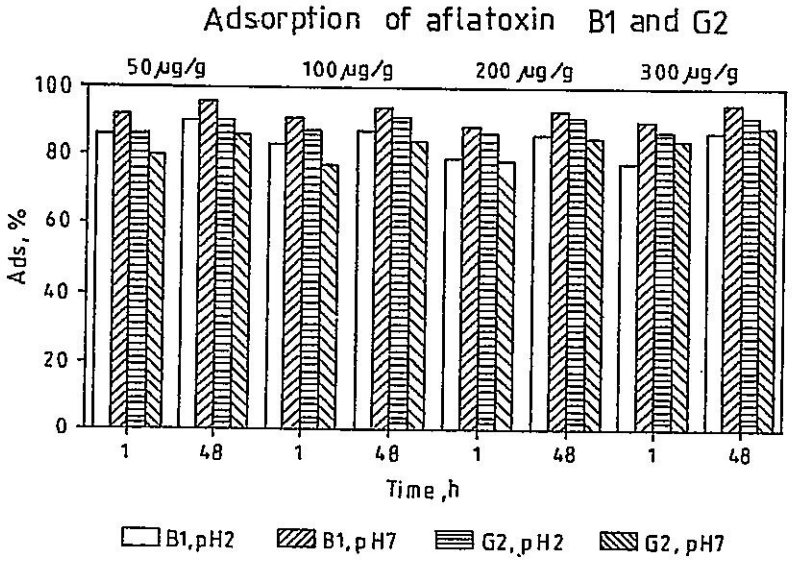

Figure 2. Percentage adsorption (A%) for aflatoxin B1 and G2 on mineral adsorber for different toxin concentrations and for 1 and 48 hours

Similar results were obtained by researchers from the Texas State College (Philips et al., (1988, 1990) using different aluminosilicate minerals both crude and modified. The best adsorption effects were obtained with chemically modified phyllosilicates (the products were patented). The products are being applied in the USA.

These in vitro results confirmed the in vivo tests (Trajković, 1991; Palić et al., 1991) with a mineral product as a mycotoxin sorbent. The tests were executed by addition of 0.2 % of mineral to contaminated animal feed.

CONCLUSION

Adsorption of the mycotoxins, aflatoxin B1 and aflatoxin G2, on mineral adsorber in electrolytes similar to animal gastric juice showed that this product could be used in the prevention of mycotoxicosis. The equilibrium of the adsorption process is reached in a very short time. Once adsorbed mycotoxins B1 of G2 have no tendency to be desorbed. This mineral adsorber could be a useful component, added to animal feed, in the prevention of mycotoxicosis of animals (swine and chickens) which has been confirmed in trials in vivo.

Acknowledgements

This work is a part of the research activities which are conducted through the projects "Interaction of the raw minerals with different organic and inorganic radicals" and "Obtaining, Valorization and Application of Natural zeolites from Yugoslavia". The authors would like to express their gratitude to the Science Fund of the Republic of Serbia and the European Economic Community DG-XII for financial support of the projects.

REFERENCES

1. *Betina V.* 1984. *Mycotoxins - Production, Isolation, Separation and Purification, Elsevier, Amsterdam.*
2. *Colvin B. M., Sangster L. T., Haydon K. D., Beaver R., Wilson D. M.* 1989. Effect of a high affinity alumosilicate sorbent on prevention of aflatoxicosis in growing pigs, *Vet. Hum. Toxicology* 31, 46–48.
3. *Harvey R. B., Kubena L. F., Philips T. D., Huff W.E. Corrier D. E.* 1989. Prevention of aflatoxicosis by addition of hydrated sodium calcium aluminosilicate to diets of growing barrows, *Am. J. Vet. Res, 50,* 416–420.
4. *Kubena L. F., Huff W. E., Harvey R. B., Yersin A. G., Elissalde M. H., Witzel D. H., Giroir L. E., Philips T. D. and Petersen H. D.* 1991 Effect of HSCAS on growing turkey poults during aflatoxicosis, *Poulty Scie, 70,* 1823–1830
5. *Kubena L. F., Huff W. E. Harvey R. B., Yersin A. G., Elissalde M. H., Philips T. D. and Rottinghaus G. E. 1993. Efficacy of a hydrated sodium calcium alumina silicate to reduce the toxicity of aflatoxin and diacetoxyscirpenol, Poul Sci. 72,* 51–59.
6. *Obradović J, Dimitrijević R.* 1987. Clinoptilolitized tuffs from Zlatokop near Vranje, Serbia, *Glas de l'Academie serbe des sciences et des arts, N°51,* 7–19.
7. *Palić T., Petrović R., Stanojlović M., Jovanović Đ.* 1991. Possibilities of Mikozel application on broilers poisioned with mycotoxin T-2 (Mogućnost primene Mikozela kod rovanja brojlera mikotoksinom T-2), *Živinarski dani,* 117–119, *Ohrid - Jugoslavija.*
8. *Philips T. D., Kubena L. F., Harvey R. B., et al.* 1988. "Hydrated sodium calcium alumosilicate:a high - affinity sorbent for aflatoxin, *Poult. Sci., 67,* 243–247.
9. *Smith T. K.* 1980. Influence of dietary fiber, protein and zeolite on zearalenone toxicosis in rats and swine.*J. Anim. Sci, 50,* 278–285.
10. *Smith T. K.* 1984. Spent canola oil bleching clay: Potential for treatment of T-2 toxicosis and shortterm inclusion in diets for immature swine, *Poult, Sci., 67,* 243–247.
11. *Shull L. R., Checke P. R.* 1985. Micotoxins - Natural toxicants in feeds and poisonous plants, West port, *Comn: AVI Publishing Co Inc.* 395–476.
12. *Trajković D.* 1991. Influence of mycotoxins on sow reproduction (Uticaj mikotoksina na reprodukciju krmača), Master Degree, Veter, Fac. Beograd.

ADSORPCIONI EFFEKTI MINERALNOG ADSORBERA NA BAZI KLINOPTILOLITA I DEO: ADSORPCIJA AFLATOKSINA B1 I G2

MAGDALENA TOMAŠEVIĆ-ČANOVIĆ, M. DUMIĆ, OLIVERA VUKIĆEVIĆ, P. RADOŠEVIĆ, I. RAJIĆ i T. PALIĆ,

SADRŽAJ

Tehnološkom preradom zeolitskog tufa Zlatokop, dobijen je novi mineralni adsorber čiju osnovu čini koncentrat minerala klinoptilolita. U ovom radu su date adsorpcione karakteristike ovog proizvoda za aflatoksine B1 i G2. Ispitivanja su vršena u elektrolitu hemijskog sastava sličnog želudačnom soku životinja. Da bi utvrdili uticaj pH na adsorpcione karakteristike ispitivanja su izvršena na pH2 i pH7.

Sadržaj mikotoksina je određivan u elektrolitu bez i u prisustvu mineralnog adsorbera. Adsorpcija je praćena na 37°C uz blago mešanje. Ispitivanom rastvoru koji sadrži 1 g mineralnog adsorbera se dodaje određena koncentracija

toksina (50, 100, 200 i 300 μg/g). Sadržaj neadsorbovanog mikotoksina se određuje u filtratu, nakon hloroformne ekstrakcije metodom tečne hromatografije. Adsorpcija je praćena u intervalu od 5 minuta do 48 časova kontakta adsorber-mikotoksin.

Dobijeni rezultati pokazuju da neposredno posle kontakta adsorber-mikotoksin (5 minuta), dolazi do adsorpcije više od 80% oba mikotoksina. Pri daljem praćenju do 48 časova adsorpcija se povećava bez pojave desorpcije. Aflatoksin B1 se bolje adsorbuje u neutralnoj sredini (pH7) nego u kiseloj (pH2), dok se aflatoksin G2 bolje adsorbuje u kiseloj sredini (pH2).