EFFECTIVENESS OF ALUMINOSILICATE-BASED PRODUCTS FOR DETOXIFICATION OF MICOTOXIN-CONTAMINATED DIETS FED TO BROILER CHICKENS

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Summary. A total of 210 one-day-old male Ross-308 broiler chickens were allocated into 7 groups, with 6 replicates per group and 5 birds per replicate. The chickens were kept in cages. Birds of all groups were fed diets of identical composition, except that diets for groups II - VII contained natural micotoxins found in ground wheat characterized by the following contamination levels: OTA - 68.9 ppb (µg/kg), DON - 60.4 ppb (µg/kg), ZEA <0.5 ppb (µg/kg). The share of contaminated wheat grain in the above diets was 50%, which resulted in the following micotoxin concentrations: OTA - 35 ppb (µg/kg), DON - 30 ppb (µg/kg) and ZEA <0.5 ppb (µg/kg). Group II birds were fed a contaminated diet without additives, while diets for groups III - VII were supplemented with four different detoxifiers with aluminosilicates as the active substance. The obtained results did not provide a basis for determining the effectiveness of the tested detoxifiers. Contaminated wheat grain used in this study showed visual symptoms of fungal spoilage, and contained micotoxins whose concentrations exceeded sevenfold the EU-recommended maximum allowable levels (as confirmed by a laboratory analysis). However, it had no significant negative effect on the production results of broiler chickens. The use of all detoxifiers resulted in a decrease in the body weights (BW) of chickens (from 3.62 to 8.24%), and it deteriorated feed conversion ratio (FCR) (from 1.59 to 6.76%), compared with the group fed a micotoxin-contaminated diet.

Keywords: broiler chickens**,** micotoxins, detoxifiers, performance.

ALIUMINIO SILIKATO PAGRINDU SUKURTŲ PRODUKTŲ VEIKSMINGUMAS NUKENKSMINANT MIKOTOKSINAIS UŽTERŠTUS VIŠČIUKŲ BROILERIŲ LESALUS

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Santrauka. Tyrimai atlikti su 210 vienadienių „Ross-308“ viščiukų broilerių, suskirstytų į 7 grupes, atliekant šešis lygiagrečius bandymus su kiekviena grupe. Viščiukai broileriai buvo laikomi narveliuose. II–VII grupės paukščių lesalų sudėtyje buvo maltuose kviečiuose randamų gamtinių mikotoksinų. Šiuose kviečiuose buvo nustatyti tokie užkrėtimo lygiai: OTA – 68,9 ppb (µg/kg), DON – 60,4 ppb (µg/kg), ZEA <0,5 ppb (µg/kg). Užkrėstų kviečių grūdų dalis lesaluose sudarė 50 proc., kurie ir nulėmė mikotoksinų koncentraciją: OTA – 35 ppb (µg/kg), DON – 30 ppb (µg/kg) ir ZEA <0,5 ppb (µg/kg). II grupės viščiukai broileriai gavo užkrėstus lesalus be priedų, o III–VII grupės lesalai buvo papildyti keturių skirtingų rūšių detoksikantais, kurių aktyvioji medžiaga buvo aliuminio silikatai. Gauti rezultatai nedavė pagrindo nustatyti išbandytų detoksikantų veiksmingumą. Tyrimui naudotuose kviečiuose buvo nustatyti grybinio sugedimo požymiai, ir mikotoksinų koncentracija juose septynis kartus viršijo ES rekomenduojamą maksimalų leistiną kiekį. Reikšmingo neigiamo poveikio viščiukų broilerių auginimo rezultatams tas neturėjo. Visų detoksikantų panaudojimas nulėmė viščiukų kūno masės sumažėjimą (nuo 3,62 proc. iki 8,24 proc.) ir pablogino pašarų energijos konversijos santykį (nuo 1,59 proc. iki 6,76 proc.) palyginti su grupe, kuri gavo mikotoksinais užkrėstų lesalų.

Raktažodžiai: viščiukai broileriai, mikotoksinai, detoksikantai, produktyvumas.

Introduction. According to Dänicke (2002), the global scale of fungal contamination of cereal grain is as high as 25 - 50%. Fungi are a diverse group of organisms and their biology has not been fully elucidated yet. Fungi are parasites that are not capable of photosynthesis and therefore they use host-derived substrates and nutrients. Fungi show a multidirectional pattern of action. They cause changes in the nutrient composition of animal feed and produce toxins, known as micotoxins. Approximately 400 compounds are currently recognized as micotoxins. Among micotoxins found in animal feed, the following are considered to be most dangerous to human and animal health: aflatoxin B_1, ochratoxin A, deoxynivalenol, zearalenone, fumonisin B_1, patulin and satratoxins (Grajewski, 2006). Micotoxins representing the group of toxic secondary metabolites produced by mold-type fungi are compounds of different chemical structure and rarely occur in isolation, which makes it difficult to control their

formation (Kołacz et al., 2004). Ochratoxin A is the most important micotoxin detected in cereal grain and animal feed. Cases of acute ochratoxicosis are rare. In poultry, ochratoxins usually cause chronic intoxication, with no visible clinical symptoms, followed by a decrease in body weight gains and laying performance, higher water intake, deficiencies of vitamins A, B_1, B_{12}, C and E, as well as a reduction in blood coagulability. The above micotoxins also contribute to the occurrence of diseases whose causes are difficult to identify. Ochratoxins are listed as human and animal carcinogens. They also may generate nephrotoxic effects, due to their high accumulation in the kidneys and liver (Huff and Hamilton, 1975). The feeding of micotoxins contaminated feed resulted in reduced broilers' growth in the grower phase, evaluation in blood uric acid levels, discoloration of breast meat, immunosupression and also in brain neurochemistry (Smith, 2009).

In the European Union, the maximum limit of fungal cells in wheat grain was set at 2 x 10^5 cfu/g, whereas the maximum permissible levels of micotoxins vary (Table 1).

Table 1. **Maximum allowable concentrations of micotoxins in wheat grain**

Micotoxin	Maximum allowable levels*, ppb (µg/kg)
Aflatoxin B_1	2
Aflatoxins $B_1 + B_2 + G_1 + G_2$	4
Ochratoxin A – OA	5
Deoxynivalenol – DON	1250
Zearalenone – ZEA	100

*source: Official Journal of the European Union L174 of 7 July 2005

A wide range of detoxifiers are offered for inactivation of fungi and their micotoxins in animal feed. The majority of these products are based on aluminosilicates, activated charcoal and special polymers (Grajewski, 2003). The mechanism of their action remains partly unknown. Their detoxifying effect involves physical adsorption, binding and trapping the toxic substances in pores (Shareef et al., 1998), followed by their elimination together with the detoxifier. The adsorption capacity of detoxifiers is determined by their structure, pore size and exposure surface area. Their binding capacity is also affected by the affinity for the adsorbed substances. In a study by Ramos et al. (1996) and Trenholm et al. (1999), activated charcoal administered in the amount of 1% showed the ability to adsorb ochratoxin A. Zaborowski (2004) demonstrated that charcoal had a strong detoxifying effect on ochratoxin when applied at 0.3%. According to Quarles (1983), Plank et al. (1990) and many other authors, the use of aluminosilicates and charcoal may be dangerous as they bind not only the particles of gases (ammonia, toxins) but also the ions of metals and vitamins, in particular vitamin A and vitamin E.

The objective of this study was to determine the effectiveness of aluminosilicate-based products, for detoxification of diets contaminated with micotoxins (in particular ochratoxin A), fed to broiler chickens.

Materials and Methods. The experimental materials comprised a total of 210 one-day-old male Ross-308 broiler chickens allocated into 7 groups, with 6 replicates per group and 5 birds per replicate (the experiment was approved by the Local Ethics Committee, 60/2007). The chickens were kept in cages until the age of 28 days. Birds of all groups were fed friable complete starter diets whose composition is presented in Table 2. Group I chickens received a non-contaminated diet. Diets for the remaining groups (II–VII) contained natural micotoxins found in ground wheat characterized by the following contamination levels: OTA - 68.9 ppb (µg/kg), DON - 60.4 ppb (µg/kg), ZEA <0.5 ppb (µg/kg). The share of contaminated wheat grain in the above diets was 50%, which resulted in the following micotoxin concentrations: OTA - 35 ppb (µg/kg), DON - 30 ppb (µg/kg). Group II birds were fed a contaminated diet without additives, while diets for groups III - VII were supplemented with detoxifiers A (Sorbix), B (Myco AD), C (Mycofix) and D (Mycosorb), available on the local market, with aluminosilicates as the active substance.

Table 2. **Composition and nutritive value of diets**

Components	%
Protein concentrate*	25
Wheat	64**
Soybean meal	8
Soybean oil	3
Energy and nutrient content	
Crude protein, %	21.36
ME, MJ	12.48
Crude fat, %	4.85
Crude fibre, %	2.88
Crude ash, %	1.81
Methionine, %	0.60
Lysine, %	1.40
Ca, %	1.00
Available P, %	0.54
Na, %	0.17

*protein concentrate contained also a vitamin and mineral premix

**in groups II-VIII, contaminated wheat grain accounted for 50%, and non-contaminated wheat grain accounted for 14%

Table 3. **Feeding program for broiler chickens, and the type and dose of aluminosilicate-based detoxifiers**

Groups	Diet/detoxifier
I	non-contaminated diet
II	contaminated diet
III	contaminated diet + detoxifier A – 0.5%
IV	contaminated diet + detoxifier A – 1.0%
V	contaminated diet + detoxifier B – 0.05%
VI	contaminated diet + detoxifier C – 0.1%
VII	contaminated diet + detoxifier D – 0.05%

The experimental design is shown in Table 3. Micotoxin concentrations were determined by high-performance liquid chromatography (HPLC) with a fluorescence detector (Shimadzu), preceded by extraction and purification on immunoaffinity columns.

Mortality rates, the final body weights of chickens and feed conversion ratio were monitored throughout the experimental period. In week 4, one excreta sample weighing approximately 100 g was collected in each treatment replicate. The samples were partially dried to determine their water content. At the completion of the experiment the birds were sacrificed, and pH was measured in selected segments of the gastrointestinal tract during a postmortem examination. The percentage share of the heart, liver, spleen in total body weight was also determined.

The experimental results were processed statistically using STATISTICA software package ver. 9.0 (StatSoft Inc., 2009). Data in tables are given as means and standard deviation.

Results. The health status of all birds was very good. No cases of diarrhea or alimentary diseases were noted. Deaths were recorded only in group I – control (3.33%) and group II - fed a contaminated diet (3.33%), and their causes (cachexia in group I and cardiac arrest in group II) were not related to the experimental factors. The water content of partially dried excreta ranged from 27.7% (group II fed a contaminated diet) to 30.9% (group VII), and showed no statistically significant differences. According to Ritz et al. (2005), the water content of 35% and higher can be considered harmful.

The production results of broiler chickens were both unexpected and interesting (Table 4). The highest performance levels were noted in group II, fed a diet containing naturally contaminated wheat grain. The tested detoxifiers, administered as recommended by manufacturers, not only did not improve the production results but even contributed to a decrease in the performance of chickens.

Table 4. **Production results of 4-week-old broiler chickens**

Parameter	Groups							*SEM*	P value
	I control	II	III	IV	V	VI	VII		
		contaminated diet	contaminated diet + detoxifiers						
			A – 0.5%	A – 1.0%	B – 0.05%	C – 0.1%	D – 0.05%		
BW, kg	1.438±0.075	1.492±0.106	1.418±0.053	1.438±0.070	1.422±0.074	1.369±0.080	1.424±0.045	*0.012*	0.222
%	*100.0*	*103.8*	*98.6*	*100.0*	*98.9*	*95.2*	*99.0*		
%		*100.0*	*95.0*	*96.4*	*95.3*	*91.8*	*95.4*		
FCR, kg/kg	1.495^{a}±0.049	1.508^{a}±0.019	1.508^{a}±0.091	1.538^{a}±0.036	1.532^{a}±0.047	1.610^{b}±0.057	1.550^{ab}±0.037	*0.009*	0.012
%	*100.0*	*100.9*	*100.9*	*102.9*	*102.5*	*107.7*	*103.7*		
%		*100.0*	*100.0*	*102.0*	*101.6*	*106.8*	*102.8*		
Mortality rate, %	3.33	3.33	–	–	–	–	–		

Values followed by different superscript letters differ significantly; abc – $P \leq 0.05$

The highest average body weight (1.492 kg) was noted in group II birds, receiving a contaminated diet. It was by approximately 3.76% higher than the average body weight in the control group (1.438 kg), and by 3.62–8.24% higher than the body weights of chickens fed diets of identical composition, supplemented with detoxifiers. The feed conversion ratio in group II was also very good, as the consumption of the contaminated diet per kg body weight gain reached 1.508 kg and it was only slightly (by 0.87%) higher than in group I fed a non-contaminated diet. The use of detoxifiers deteriorated feed conversion (from 1.59 to 6.76%), compared with contaminated feed (group II), but the observed differences were statistically non-significant.

Table 5. **Proportion of the weights of the liver, heart and spleen in total body weight, %**

Parameter	Groups							*SEM*	P value
	I control	II	III	IV	V	VI	VII		
		contaminated diet	contaminated diet + detoxifiers						
			A - 0.5%	A - 1.0%	B - 0.05%	C - 0.1%	D - 0.05%		
Liver	2.77±0.32	2.73±0.30	3.48±0.57	3.64±0.87	2.95±0.43	3.13±0.41	3.17±0.41	*0.095*	0.062
Heart	0.57±0.06	0.55±0.04	0.53±0.06	0.59±0.07	0.55±0.06	0.56±0.07	0.57±0.03	*0.009*	0.761
Spleen	0.08±0.02	0.07±0.03	0.06±0.01	0.06±0.01	0.07±0.02	0.07±0.02	0.07±0.02	*0.003*	0.716

Values followed by different superscript letters differ significantly; abc – $P \leq 0.05$

The poorest production results were obtained in group VI, where detoxifier C was used, while the best results were noted in group IV, where detoxifier A was administered at the amount of 1%. The addition of all detoxifiers caused an increase in the percentage share of liver in the carcass, in comparison with group I and group II birds, but the differences were non-significant (Table 5). The analyzed detoxifiers caused a decrease in the reaction (pH) of the gastrointestinal tract (from the gizzard to the cecum), but significant differences were observed only with respect to the gizzard in groups VI and VII (Table 6).

Table 6. **Reaction (pH) in gastrointestinal tract segments**

Specification	Groups							*SEM*	P value
	I control	II contaminated diet	III	IV	V	VI	VII		
			contaminated diet + detoxifiers						
			A - 0.5%	A - 1.0%	B - 0.05%	C - 0.1%	D - 0.05%		
Crop	4.46±0.44	4.37±0.12	4.38±0.33	4.52±0.41	4.31±0.23	4.47±0.21	4.56±0.55	*0.056*	0.924
Proventriculus	4.27±0.47	4.06±0.45	3.97±0.27	3.68±0.54	4.28±0.23	4.20±0.31	4.26±0.31	*0.068*	0.176
Gizzard	3.55^{b}±0.38	3.54^{b}±0.38	3.47^{ab}±0.34	3.05^{ab}±0.38	3.38^{ab}±0.36	2.89^{a}±0.58	2.93^{a}±0.33	*0.077*	0.034
Jejunum	6.53±1.14	6.03±0.08	5.83±0.12	5.89±0.16	5.99±0.10	5.87±0.18	5.98±0.11	*0.079*	0.250
Ileum	5.59±0.58	5.61±0.53	5.11±0.49	5.43±0.64	5.52±0.41	5.54±0.51	4.98±0.54	*0.089*	0.459
Cecum	5.97±0.46	6.13±0.35	6.00±0.14	5.96±0.18	5.86±0.25	5.76±0.37	5.75±0.32	*0.053*	0.451

Values followed by different superscript letters differ significantly; abc – $P \leq 0.05$

Discussion. The wheat grain used in this study was stored under high humidity conditions, and therefore it was characterized by poor organoleptic properties, including an unpleasant, sour aroma. The addition of contaminated wheat to experimental diets resulted in micotoxin concentrations that exceeded sevenfold the EU-recommended maximum allowable levels set for ochratoxin A. The fact that the best production results were reported in group II, fed a contaminated diet, may suggest a contribution of some unknown biological mechanisms. The fermentation process might have taken place, leading to the formation of lactic acid bacteria and volatile fatty acids. Böhm at al. (1999 and 2000) and Grajewski (2006) found that the enzymes of lactic acid bacteria may cause micotoxin biodegradation in the digestive tract, while volatile fatty acids – in particular propionic acid and lactic acid – can inactivate detoxifiers. Polak et al. (2009) demonstrated that the micotoxin ZEA was destructed as the pH in the gastrointestinal tract decreased after the application of citric acid. According to Mazurkiewicz-Zapałowicz (2006), fungi exhibit multidirectional biochemical activities. Apart from harmful micotoxins, they produce also antibiotic substances which are active against microorganisms, as well as some other biologically active substances having potential beneficial effects on human and animal health. Another possible explanation for the above data is that wheat grain could contain not only ochratoxin but also citrinin whose toxicity is relatively low, whereas the results of laboratory analyses could be treated as if they concerned ochratoxins only (Richter 2001). Our findings confirm the opinion of Kołacz et al. (2004) that micotoxins usually do not occur in isolation, and that even members of one group differ considerably with respect to chemical structure, which makes it difficult to identify them and control their formation. The fact that aluminosilicate sorbents having a total surface area of 650–800 m^2/g may interact with water to form bases which can further react with fat to produce soaps in the gastrointestinal tract, thus adversely affecting the performance levels of broiler chickens, is also an important consideration.

Conclusions. The obtained results do not provide a basis for determining the effectiveness of the tested detoxifiers. The main conclusions that can be drawn from the experiment are as follows:

1. Despite the visual symptoms of fungal spoilage and the presence of micotoxins in concentrations which exceeded sevenfold the EU-recommended maximum allowable levels (as confirmed by a laboratory analysis), contaminated wheat grain used in the study had no significant negative effect on the production results of broiler chickens.

2. The use of all detoxifiers resulted in a decrease in the body weights of chickens (from 3.62 to 8.24%), and it deteriorated feed conversion (from 1.59 to 6.76%), compared with the group fed a micotoxin-contaminated diet.

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Received 14 September 2010
Accepted 28 January 2011