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EFFICACY OF ORGANOZEOLITE TO AMELIORATE THE TOXIC EFFECTS OF ZEARALENONE IN LAMBS

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In vitro and in vivo studies were conducted to evaluate the efficacy of organozeolite (Min-a-Zel Plus®) to alleviate the toxic effects of zearalenone (ZEN) in lambs. Min-a-Zel Plus® was able to bind >90% of ZEN in vitro at pH 3, 7 and 9. For in vivo studies, sixty four lambs were divided in to four groups which included: I - control group - basal diet containing neither Min-a-Zel Plus® nor zearalenone; II - basal diet supplemented with 8.3 mg ZEN/kg diet; III - basal diet supplemented with 8.3 mg ZEN/kg and 0.2% Min-a-Zel Plus®; IV - basal diet supplemented with 8.3 mg ZEN/kg and 0.5% Min-a-Zel Plus®. The Min-a-Zel Plus® supplement dramatically reduced the content of ZEN in liver, kidneys and muscles. The lower amount (0.2%) reduced the content of ZEN in all samples, but ZEN was still present in the organs. Addition of 0.5% Min-a-Zel Plus® eliminated ZEN from all organs, totally. These results indicate that Min-a-Zel Plus® is effective in preventing the toxic effects of ZEN, which may be present in lamb rations.

Key words: mycotoxins, zearalenone, mineral adsorbents, organo-zeolite, lambs

INTRODUCTION

Mycotoxins present a unique challenge to producers because they arise from common molds, occur in great variety and numbers, are sporadic or heterogeneous in their distribution, and because their actions in farm animals are often not diagnostic. There are no mycotoxin-specific treatments, such as vaccines.

The fungi which produce the chemical residues known as mycotoxins are common, simple organisms which have very modest requirements for their existence. Nutritional needs are met by a source of carbon and nitrogen; they require water; all need oxygen, although some require much less than others (Doerr, 2003). Thus, they need little, they reproduce easily and they have adapted highly successful survival mechanisms. Complexity begins with the nature of animal feeds (forage, grain, oilseeds, supplements, pasture, etc.), as each supplies carbon and nitrogen and contains some moisture. Molds should be considered to

have the initial advantage in any animal production system. Unfortunately when animals become sick or fail to produce, or their products are in some fashion contaminated, the comparative cost of preventive quality control measures will be seen as puny when placed against the devastating losses that mycotoxins are capable of causing.

Zearalenone (ZEN) *–14,16-dihydroxy–3 methyl–3,4,5,6,9,10–hexahydro–1H-2–benzoxacyclotetradecin–1,7(8H)–dione* is the most widely distributed Fusarium mycotoxin. It is associated primarily with corn, but occurs in modest concentrations in wheat, barley and sorghum. It is well known, that in mice and rats, ZEN is metabolized to active compounds after ingestion. The primary metabolites of ZEN are the reduced products, α- and β-zearalenol. These stereoisomers are obtained when the 6' carbonyl group is reduced to an alcohol. It is interesting that the former (mp 178–180^{o}C) is four times more active than the parent compound, while the latter isomer (mp 146-148^{o}C) is only slightly more active (IARC, 1993).

Measures used by the livestock industry to protect animals from the toxic effects of mycotoxins include grain testing, use of mold inhibitors, fermentation, microbial inactivation, physical separation, thermal inactivation, irradiation, ammoniation, ozone degradation and the use of adsorbents (Ledoux *et al*., 1999). Unfortunately most of these measures are costly, time-consuming and only partially effective. At the present time, one of the more promising and practical approaches is the use of adsorbents.

A search of the recently reviewed scientific literature reveals only limited information on mycotoxin binder studies *in vitro*. Information is mainly limited to aflatoxin binders (Phillips *et al*., 1990, Ledoux & Rottinghaus, 1999, Tomašević-Čanović *et al*., 1994), although *in vitro* binding studies for zearalenone (Daković *et al*., 2001, Lemke *et al*., 1998, Tomašević-Čanović *et al*., 2003), ochratoxin A (Daković *et al*., 2003, Rotter *et al*., 1989) and ergotamine (Tomašević-Čanović *et al*., 2003, Chestnut *et al*., 1990), have also been published.

Selected adsorbents, added to ZEN-contaminated feeds can sequester ZEN during the digestive process allowing the mycotoxin to pass harmlessly through the animal. The major advantages of these adsorbents include cheapness, safety and easy administration through addition to the animal feed (Avakumović *et al*., 2002).

Investigations of organo-zeolite added to animal feed contaminated with ZEN, on the production results (average final weight, weight gain per feeding day, feed conversion) of different categories of swine, showed that the applied adsorbent reduced the negative effects caused by ZEN in animal diets. At 0.2% of the ration organo-zeolite significantly decreased the appearance of zearalenontoxicoses, improved the state of health and economically favoured production of all categories of swine (Avakumović *et al*., 2002).

The objectives of this research were determination the efficiency of Min-a-Zel Plus® in ameliorating the toxic effects of ZEN present in lamb rations and to demonstrate that the addition of this adsorbent to the diet would not negatively affect lamb performance.

MATERIALS AND METHODS

In vitro study

ZEN was purchased from Sigma-Aldrich Co. The primary stock solution of ZEN (100 μg/ml) in methanol was diluted to 10 μg/ml for the adsorption experiments in an electrolyte containing NaCl (1g/l). The pH of the solution was adjusted to the desired pH with 0.1 M NaOH or 0.1 M HCl.

Min-a-Zel Plus® is the trade name of an adsorbent obtained by organic modification of the zeolitic mineral – clinoptilolite with a long chain quaternary ammonium salt (Tomašević-Čanović *et al*., 2000).

The *in vitro* binding ability of Min-a-Zel Plus® was tested as follows: aliquots (10 ml) of the test solutions were added to test tubes containing 100 mg of adsorbent. Controls were prepared by adding 10 ml of test solution without the addition of adsorbent. The tubes were mechanically shaken for 2 h and then centrifuged at 10 000 rpm. ZEN concentrations in electrolytes without (Ct) and with mineral adsorbents (Cf) were determined by HPLC. HPLC analyses were performed using an LKB Broma Model 215 HPLC pump equiped with a RHEODINE 7125 injector, a Bio-Rad ODS column (250 x 4.4 mm, 5 μm particle size) and a UV detector (Bio Rad 1801 UV Monitor (λ = 236 nm). The mobile phase was H_2O:CH_3OH:CH_3CN= 50:10:40 with a flow rate of 1 ml/min.

In vivo study

The experiment was conducted on 64 lambs, with equal nimbers of males and females. The lambs were cross-breds out of cigaja ewes by Ile de France rams and were divided into four groups (I, II, III and IV) with 16 lambs in each group. Group feeding was with restricted amounts of concentrate mixtures and hay. The experiment started after weaning, when the lambs were 60 days old, and lasted for 53 days.

Group I received a concentrate mixture without added ZEN or mycotoxin adsorbent Min-a-Zel Plus®. Groups II, III and IV received concentrate mixture contaminated with ZEN (8.3 mg/kg) by including corn, which contained 12.8 mg/kg of ZEN. Group II received this concentrate mixture without Min-a-Zel Plus®. Group III received this concentrate mixture together with 0.2% and group IV with 0.5 % of Min-a-Zel Plus®.

The concentrate mixture was composed of corn, extruded soybean, sunflower meal, wheat bran, alfalfa meal, chalk, salt, vitamin and microelement supplements, and contained 15.20% of total protein.

Six, randomly selected, lambs (four males and two females) from each group were killed and the liver, kidney and muscles were removed, weighed and used for determination of ZEN in tissue.

ZEN was determined in tissue by solid phase extraction (SPE). Approximately 10 g of organ was homogenized and macerated with 10 ml of 50% CH_3OH in water for 4 h. After the maceration the sample was filtered through "Whatman" 0.45 μm paper. Then the methanol was evaporated off on a Rotavapor, leaving the aqueous macerate as the basis for the extraction. SPE columns (Merck type RP

18. 10 μm granulation and 5 g of filling) were selected for extraction. The columns were conditioned first with 5 ml CH_3OH at 1 ml/min (20 drops), and then, at the same rate, with 5ml H_2O. Macerate was then applied to the column at the rate of 2 ml/min (40 drops), the column was washed 3x with 5ml H_2O and then with 5 ml CH_3OH. Finally, the column was eluted with 1 ml of a mixture (1:1) of acetate buffer (pH=5.2) and CH_3OH. After that, ZEN in the eluate was determined in the same way as the in the *in vitro* study (Schuster *et al*., 1992).

RESULTS AND DISCUSSION

In vitro study

The potential practical application of adsorbents to diminish the adverse effects of ZEN relies on the important characteristic of the adsorption index, which is defined as the ratio between the adsorbed (Ct-Cf) and total (Ct) ZEN concentration (Ledoux & Rottinghaus, 1999).

Lemke *et al*. (1998) examined the adsorption of ZEN on organobentonites with different amounts of long chain organic cations *in vitro*. They showed that adsorption of ZEN increased as the amount of organic phase increased.

In our previous paper (Dakovic *et al*., 2001) the *in vitro* adsorption of ZEN on organozeolites and organobentonites with different amounts of organic cation was also investigated. Maximum adsorption of ZEN on organozeolites was achieved using ten times less organic phase than for organobentonites.

Adsorption of ZEN on organobentonites was found to change at different pHs (Lemke et al., 1998). The effect of pH on ZEN adsorption by the organozeolite Min-a-Zel Plus® is shown in Table 1, where it can be seen that ZEN adsorption index was high (> 90%) at pH 3,7 and 9. Thus, the adsorption of ZEN on organozeolites is less pH dependent than on organobentonites.

Table 1. Influence of pH on ZEN adsorption by Min-a-Zel Plus®

	Adsorption index, %		
	pH 3	pH 7	pH 9
Min-a-Zel Plus®	93	96	92

In order to determine the optimal amount of adsorbent for the *in vivo* study, solutions of ZEN (10 ml) containing 1, 2 and 4 ppm were added to test tubes containing 40, 20, 10 and 4 mg of Min-a-Zel Plus®.

It can be seen in Figure 1 that, at all ZEN concentrations, ZEN adsorption indexes increased with increasing amount of Min-a-Zel Plus® in suspension. Thus, at the ZEN concentration of 1 ppm with 4 mg of Min-a-zel Plus® (in 10 ml of electrolyte), the ZEN adsorption index was 39% while the same concentration of ZEN and with 40 mg adsorbent (in 10 ml of electrolyte) gave an adsorption index of 91%. Similarly, 4 ppm ZEN with 4 mg of adsorbent, showed a toxin adsorption index of 28%, which increased to 89% with 40 mg of Min-a-Zel Plus®. From these results, it

can be concluded that similar adsorption indexes were achieved at ZEN concentration of 1 ppm and 4 ppm. Significant increases of ZEN adsorption were achieved only with increasing amounts of Min-a-Zel Plus® in suspension.

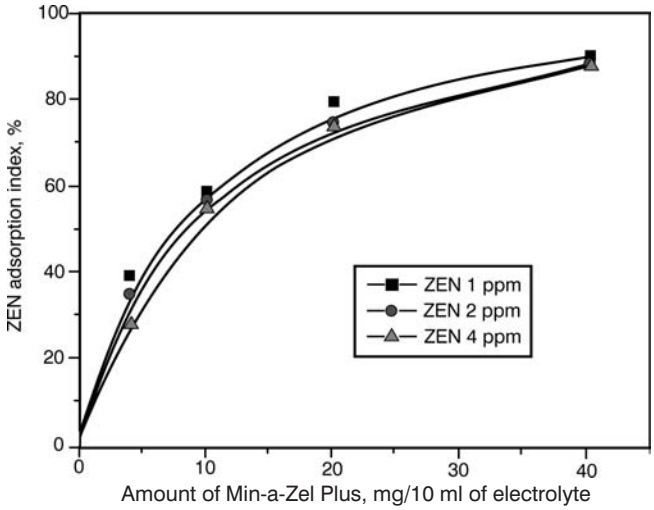

Figure 1. Adsorption of ZEN (1, 2 and 4 ppm) at different amounts of Min-a-Zel Plus®

These results suggested that, for efficient adsorption of ZEN present in animal feed, ≥0.2% of Min-a-Zel Plus® is required, whether the feed is contaminated with 1 mg/kg or with 4 mg/kg of ZEN. Thus, 0.2% and 0.5% of Min-a-Zel Plus® were used for the *in vivo* experiments.

In vivo study

In Table 2, it can be seen that the lambs in group III, which consumed the combination ZEN/Min-a-Zel Plus® (0.2%) showed the highest daily gain (209 g). The mean daily gain in group IV offered the combination ZEN/ Min-a-Zel Plus® (0.5%) showed a lower mean daily gain (179 g) than the control group (189 g), but a higher daily gain than group II (174 g) which consumed feed contaminated with ZEN.

Thus, compared with the control group, the mean daily gain, was 7.94% lower in group II and 5.29% lower in group IV, while the daily gain in group III was 10.58% higher. However the differences between treatments were not statistically significant ($p>0.05$).

Also, in Table 2, it can be seen that concentrate consumption per kg of body gain was 3.12 kg in the control group (I), while in groups II and IV it was higher by 0.27 kg (7.96%) and 0.17 kg (5.17%), respectively. However, concentrate intake per kg gain in group III was 0.30 kg lower compared to the control group (10.63%). These results indicate that addition of Min-a-Zel-a Plus® (groups III and IV), improved the utilization of concentrate mixture contaminated with ZEN (group II).

Thus, the efficiency of concentrate utilization in groups III and IV was similar to the control group (I), which received concentrate without ZEN. Similar results were obtained with swine offered feed contaminated with ZEN. Namely, Avakumović *et al*. (2002), showed that inclusion of organozeolite in a diet containing ZEN improved the state of health and economy of production of three categories of swine (sows, piglets and fattening swine).

Table 2. Effects of Min-a-Zel Plus® on the performance of lambs fed ZEN

	Group			
	I Control	II + ZEN	III + ZEN and 0.2% Min-a-Zel	IV + ZEN and 0.5% Min-a-Zel
Body weight, at the beginning the of trial, kg	17.12	17.41	17.41	17.06
Body weight, at the end of trial, kg	27.12	26.62	28.47	26.56
Daily gain, g/day	189	174	209	179
Concentrate/gain, kg	3.12	3.39	2.82	3.29
ZEN, mg / 100 kg of body weight	–	22.31	21.40	22.51

In the present study, the intake of ZEN (mg) per 100 kg of body weight of lambs in groups II, III and IV, was very similar, at 22.31, 21.40 and 22.51 mg, respectively (Table 2).

The effects of Min-a-Zel Plus® on relative liver and kidney weights of lambs fed feed contaminated with ZEN are presented in Table 3.

Table 3. Effects of Min-a-Zel Plus® on relative liver and kidney weights of lambs (n=6) fed feed contaminated with ZEN

	Group			
	I Control	II + ZEN	III + ZEN and 0.2% Min-a-Zel	IV + ZEN and 0.5% Min-aZel
Liver, g	416.67	421.67	411.67	405.83
Kidneys, g	80.03	89.25	83.23	82.00
Liver / warm carcass, %	2.60	2.71	2.70	2.64
Kidney / warm carcass, %	0.50	0.57	0.55	0.53

Compared with the control group (diet without ZEN), the mean weights of liver and kidneys where somewhat higher in lambs in group II (421.67 g and 89.25 g), offered feed contaminated with ZEN. Lower mean weights of the liver were obtained for groups III (411.67 g) and IV (405.83 g) given adsorbent. The differences were not statistically significant ($p>0.05$). The mean weights of liver and kidneys in relation to the weight of the warm carcass (%) showed the same trends in groups II, III and IV as the absolute values (Table 3).

The amounts of residues of ZEN found in organs (liver, kidney and muscles) of the lambs are shown in Figure 2.

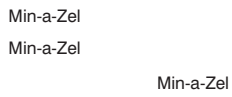

Figure 2. The amount of ZEN found in lamb organs, μg/kg

The greatest amounts of ZEN were detected in the liver (9.45 μg/kg), kidneys (8.53 μg/kg) and muscles (7.64 μg/kg) of lambs in group II, which consumed feed contaminated with ZEN without Min-a-Zel Plus®. The mean amounts of ZEN in organs (liver, kidney and muscles) of the lambs from group III (combination ZEN/Min-a-Zel Plus® – 0.2%) were significantly lower, and were 0.17, 0.09 and 0.02 μg/kg, respectively. The differences were statistically very significant ($p<0.01$). In group IV (combination ZEN/Min-a-Zel Plus® – 0.5%) residues of ZEN were not detected in all investigated organs. These results indicate that Min-a-Zel Plus® (0.2 and 0.5%) prevented the accumulation of ZEN in organs of the lambs. The higher content of Min-a-Zel Plus® (0.5%) was totally effective.

It is difficult to compare our *in vivo* results with other works because, data about the application of a mineral adsorbent – organozeolite in diets for lambs in order to prevent the toxic effect of ZEN were not found in the available literature. However, many investigations have shown that mineral adsorbents prevent the toxic effects of aflatoxin (Phillips *et al*., 1995, Resanović, 2000). Thus, addition of 0.5% of a mineral adsorbent based on an inorganic form of zeolite (Min-a-Zel) to a broiler diet markedly reduced aflatoxin B1 accumulation in the kidney, liver, muscle, stomach and spleen (Resanović, 2000).

CONCLUSION

Supplementation of mycotoxin adsorbent Min-a-Zel Plus® in concentrate mixtures for lambs (0.2 and 0.5%), contaminated with ZEN, prevented accumulation of mycotoxin residues in the liver, kidneys and muscles.

At the same time, addition of Min-a-Zel Plus® tended to act positively on the rate of gain in body weight and efficiency of concentrate utilization, which contributed to the achievement of more farourable economic and lamb meat production results.

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EFIKASNOST ORGANOZEOLITA U PREVENIRANJU TOKSIČNIH EFEKATA ZEARALENONA KOD JAGNJADI

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SADRŽAJ

Efikasnost adsorpcije zearalenona (ZEN) na mineralnom adsorbentu mikotoksina dobijenim organskom modifikacijom prirodnog zeolita (Min-Zel Plus®) je praćena u uslovima *in vitro* i ogledima *in vivo* na jagnjadima koja su dobijala hranu kontaminiranu ZEN-om.

In vitro rezultati su pokazali da Min-Zel Plus® adsorbuje ZEN sa indeksom adsorpcije 90%, na pH 3, 7 i 9. Za *in vivo* eksperimente jagnjad su podeljena u

četiri grupe, u svakoj grupi po 16 jagnjadi: I grupa – kontrolna je hranjena hranom koja ne sadrži ni ZEN ni Min-Zel Plus®, II grupa – je dobijala hanu kontaminiranu sa 8.3 mg ZEN/kg, III grupa je dobijala hranu kontaminiranu sa 8.3 mgZEN/kg i 0.2% Min-a-Zel-a Plus® i IV grupa je dobijala hranu kontaminiranu sa 8.3 mg ZEN/kg i 0.5% Min-Zel-a Plus®. Rezultati analiza sadržaja ZEN u organima jagnjadi su pokazala da je za razliku od II grupe u kojoj je značajno prisutan ZEN u svim organima, u grupama III i IV, koje su dobijale i Min-Zel-a Plus® značajno je smanjen sadržaj ZEN u jetri, bubrezima i mišićima. Ipak, pri količini od 0.2% Min-Zel-a Plus® u ispitivanim organima jagnjadi prisutne su male količine zaostalog ZEN, dok pri količini od 0.5% nije detektovan ZEN u ispitivanim organima.