

**EFFICACY OF MINERAL AND ORGANIC ADSORBENT IN ALLEVIATING HARMFUL EFFECTS OF ZEARALENONE ON PIGS PERFORMANCE AND HEALTH**

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(Received 4. April 2007)

*Two trials, 31 and 29 days long, were conducted with the aim to pursue performance and health of pigs treated with different amounts of zearalenone and to investigate the feasibility of utilizing a modified clinoptilolite and esterified glucomannane in alleviating and/or preventing its harmful effects. Total of 64 pigs (32 each trial), 60 days old, divided into four groups, each containing 8 pigs were used. Both control groups (K) received feed without zearalenone, while experimental groups received feed supplemented with 3.84 mg/kg in the first trial and 5.12 mg/kg of zearalenone in the second trial. Pigs in the first experimental groups (O-I) were given feed with the toxin only. At the same time, a modified clinoptilolite (MC) in the amount of 0.2% was introduced in the contaminated feed of the second experimental groups (O-II) and an esterified glucomannane (EGM) in the amount of 0.1% in contaminated feed of the third experimental groups (O-III) of both experiments.*

*Zearalenone exerted its negative effects on performance of pigs in concentrations used in the experiments, proportionally to the amount of toxin in the feed (weight gain was 19.86% lower in experiment I and 22.27% in experiment II, feed intake rate 5.89% lower in experiment I and 1.52% in experiment II, feed consumption ratio was higher for 14.82% in experiment I and 21.08% in experiment II). Addition of adsorbents only partially alleviated the harmful effects of zearalenone in the feed.*

*Signs of intoxication in female pigs, known as the estrogenic syndrome, were manifested by inflammatory changes of the external genital organs (vulvovaginitis) and with histological findings of epithelial hyperplasia and submucous oedema. Also nonspecific symptoms could be seen – gastrointestinal disorders followed by diarrhea. Use of adsorbents as toxin binders mostly alleviated these negative effects.*

*Key words: adsorbents, health, performance, pigs, zearalenone*

## INTRODUCTION

According to toxicity and frequency of appearance in nature, zearalenone (F-2 toxin, ZON) is one of the most important mycotoxins of *Fusarium* fungi that are widely spread in Europe (Bottalico and Perrone, 2002; Logrieco *et al.*, 2002). Corn is usually affected, especially hybrids with a long vegetation and high humidity in the moment of harvest, but the fungi growth and toxin synthesis extends further during storage (EFSA, 2004).

Biological activity of this toxin can be explained as competition with 17- $\beta$ -oestradiol for the specific binding sites at estrogenic receptors and as interference with enzymes that are involved in the metabolism of steroids (Mitterbauer *et al.*, 2003). F-2 toxin exerts its effects in all metabolic processes that are under the influence of estrogenic hormones, usually on the genital tract and in reproduction (D'Mello *et al.*, 1999). Doses that are much greater than concentrations which have hormonal effects may have genotoxic and carcinogenic effects (National Toxicology Program, 2002). Pigs are more sensitive (Gaumy *et al.*, 2001) than other animal species, primarily manifesting hyperestrogenism in females, as well as gastrointestinal lesions, effects on the liver and alterations of serum clinical chemical parameters (D'Mello *et al.*, 1999; Döll *et al.*, 2003, Döll *et al.*, 2005).

Hyperemia and edema of vulva with a lightly clouded mucilaginous vaginal discharge are symptoms of intoxication. In severe cases vaginal and rectal prolapses can be seen. Clinical signs are more expressed in immature than in mature animals (Doll *et al.*, 2003). Diarrhoea, vomiting, feed refusal, body weight loss and haemorrhages also can be seen in the clinical picture, but not only as the consequence of zearalenone influence, yet by it is synergistic effect with other toxins present in feed contaminated with *Fusarium* fungi (D'Mello *et al.*, 1999).

The use of adsorbents is the most frequent way of alleviating and/or preventing harmful effects of mycotoxins. Adsorbents are substances nonresorbable from the gut that can physically bind some chemicals and thus block their resorption. Several commercially available adsorbents which claimed to bind mycotoxins were tested for both *in vitro* and *in vivo* adsorption. Most studies have used aluminosilicates such as modified clinoptilolite (Tomašević-Čanović *et al.*, 2003; Döll *et al.*, 2004, Döll *et al.*, 2005). The feasibility of utilizing organic adsorbents was recently examined. Esterified glucomannan, which belongs to this group, (Devegowda *et al.*, 1998; Swamy, 2002) is isolated from the inner layer of yeast cell wall and which possesses a significant capability for mycotoxin adsorption.

The primary objective of this study was not only to investigate the performance and health of pigs treated with different amounts of F-2 toxin, but also to assess the feasibility of utilizing a modified clinoptilolite and esterified glucomannane aimed at alleviating and/or preventing its harmful effects.

## MATERIAL AND METHODS

*Animals.* Two trials were performed on a total of 64 pigs of Swedish and Dutch Landrace cross-breed. Pigs were of both sexes with 14.40 kg and 16.30 kg average body weight, 60 days old and kept in breeding pens. Microclimate and hygienic conditions were appropriate for this category of pigs.

*Diets.* All groups of pigs were fed with a commercial feed mixture (grower 15-25 kg) which consisted of standard feedstuffs and contained enough nutrients to satisfy the requirements of this category. Corn naturally contaminated with zearalenone (12.8 mg/kg) was used in the amount of 30% in experimental groups of the first trial and 40% in the second trial. Zearalenone free corn was given to pigs in the control groups. Mineral adsorbent Minazel Plus<sup>®</sup> was introduced in the contaminated feed of the second experimental groups and organic adsorbent Mycosorb<sup>®</sup> in the contaminated feed of the third experimental groups of both trials.

*Experimental design.* The trials, 31 and 29 days long, were conducted on a total of 64 pigs (32 each), divided into four groups, each containing 8 pigs. Both control groups (K) received feed without zearalenone while experimental groups received feed supplemented with 3.84 mg/kg in the first experiment and 5.12 mg/kg of zearalenone in the second experiment. Pigs in the first experimental groups (O-I) were given feed with toxin only. At the same time, a modified clinoptilolite in the amount of 0.2% was introduced in the contaminated feed of the second experimental groups (O-II) and an esterified glucomannane in the amount of 0.1% in the contaminated feed of the third experimental groups (O-III) in both experiments.

*Data and sample collection.* The following data were monitored during the trials: body weight (BW), weight gain (AWG), feed intake (FI) and feed conversion ratio (FCR). Control measurements of the experimental pigs were done on a technical scale with 10<sup>-2</sup> kg precision. According to these results average body weight was calculated. Total weight gain was obtained from body weight difference and daily weight gain was calculated according to duration of the trials. The amount of mixes given to each group was measured precisely during both experiments. Feed intake was identified after adding of daily amounts of feed at the end of the trials. Feed conversion ratio was calculated according to feed intake and weight gain data for both experiments. Every day observations were done with special interest to occurrence of diarrhea and vulvovaginitis. Vulvovaginitis severity was determined according to vulvar index (Evans, 2000) which was the result of the multiplication of vulvar width and length. Six pigs per group were sacrificed at the end of the trials. Immediately after sacrifice necropsy and detailed macroscopic examinations were carried out. In the shortest possible period samples of vagina were taken for histological investigations. Vagina samples were fixed in 10% neutral formalin and absolute alcohol, embedded in paraffin, sections of 5-8 µm thickness cut and stained using the standard hematoxylin eosin method (Scheuer and Chalk, 1986).

*Statistical analysis.* All data were statistically processed (Snedecor and Cochran, 1971) and an appraisal was made of the significance of differences in mean values between the groups of pigs.

## RESULTS AND DISCUSSION

Pigs were of adequate body weight for their breed and age at the beginning of the trials and differences were not statistically significant ( $p > 0.05$ ). Performance differed at the end of the trials between control and experimental groups, and also between trials depending on toxin concentrations. Pigs in the control groups achieved the largest body weight, top weight gain and the lowest feed conversion ratio. According to literature data it was concluded that these results were common for breed, sex, age and rearing conditions.

By comparing experimental groups of pigs fed with contaminated feed with control groups it can be noted that zearalenone had a harmful influence on body weight which was decreased proportionally to its amount in the feed ( $p > 0.05$ ) i.e. for 10.84% in the first trial and for 11.13% in the second trial.

Although body weight is a respectable parameter, daily weight gain is reputed as a better indicator of feed quality. It is particularly the indicator of zootechnical conditions and animal health (Table 1). By comparing experimental groups (O-I) to control groups (K) considerably lower daily weight gain, for 19.86% ( $p < 0.05$ ), was seen in the first experiment and 22.27% in the second one.

Table 1. Weight gain\* of pigs during the trials, [kg]

Group n		Measurements of variation					
		X	±	Sx	Sd	Cv	Iv
I trial							
K	6	0.569	a	0.03	0.08	14.12	0.452-0.661
O-I	6	0.456	b	0.04	0.13	27.39	0.200-0.581
O-II	6	0.510		0.01	0.03	6.74	0.445-0.548
O-III	6	0.516		0.03	0.07	14.07	0.435-0.645
II trial							
K	6	0.503	a	0.02	0.07	13.30	0.431-0.655
O-I	6	0.391	b	0.04	0.12	30.00	0.138-0.531
O-II	6	0.469		0.03	0.08	17.32	0.369-0.586
O-III	6	0.464		0.02	0.06	13.65	0.352-0.552

\* Value expressed as  $\bar{x} \pm Sd$

a, b, c, d  $p < 0.05$

Application of adsorbents ensured a significant recompense of lost body weight, i.e. it provided satisfactory daily growth. Average body weight and daily weight gain of pigs fed with contaminated feed with addition of adsorbents were still lower than control values, but at the same time higher than average body

weight and daily weight gain of pigs fed with contaminated feed without protectors. There was not any difference in the results due to type of adsorbent. It could be concluded that utilizing modified clinoptilolite and esterified glucomannane was not enough for performance of usual body weight, i.e. it could not entirely prevent harmful effects of zearalenone, even at its lowest dosage. But, usage of adsorbents alleviated harmful effects of F-2 toxin also in the case of its higher content in the feed.

Presence of F-2 toxin reduced feed intake for 5.89% in the first trial and 1.52% in the second trial, but the application of adsorbents entirely eliminated harmful effects. Pigs fed with feed contaminated with a lower quantity of zearalenone with addition of adsorbents achieved larger consumption than pigs fed with the same feed without protectors, but it was still lower than in the control groups. Pigs fed with feed contaminated with higher amount of zearalenone with addition of adsorbents reached larger feed intake, compared to pigs in the control groups. There was no significant difference in the results due to type of adsorbent.

Feed conversion ratio, as interaction of weight gain and feed intake, differed between control and experimental groups (Table 2). Experimental groups fed with contaminated feed reached a higher feed conversion ratio, proportional to zearalenone feed content, i.e. 14.82% in the first trial and 21.08% in the second one.

Table 2. Feed conversion ratio during the trials, [kg]

Trial	M i x			
	K	O-I	O-II	O-III
I	1.793	2.105	1.918	1.915
II	1.827	2.315	2.004	2.009

Utilization of adsorbents significantly alleviated harmful effects of zearalenone, but not entirely. Feed conversion ratio of pigs fed contaminated feed with the addition of modified clinoptilolite was still higher than control values for 6.52% in the first trial and 8.83% in the second trial, but it was lower than feed conversion ratio of pigs who received contaminated feed only. Feed conversion ratio of pigs fed contaminated feed with the addition of esterified glucomannane was also higher than control values (6.37% in the first experiment and 9.06% in the second) and it was lower than conversion ratio in groups of pigs which did not get adsorbents.

Döll *et al.* (2003) also reported that feed intake and, consequently, body weight gain of female weaned piglets receiving *Fusarium* toxin contaminated diet (ZON 0.42 mg/kg and DON 3.9 mg/kg) were significantly decreased, while the feed conversion ratio was not affected by this treatment.

Regarding clinical signs this intoxication was manifested by vulvovaginitis in female pigs, but also by nonspecific symptoms of gastrointestinal disorders followed by diarrhea.

Vulvovaginitis occurrence was noticed in all experimental groups of pigs since the third day of ingesting contaminated feed. Vulvar index (Evans, 2000) showed the severity of symptoms with success (Table 3). Vulvar oedema could be seen in groups of pigs fed with contaminated mixture without of adsorbents for 10-15 days after dispossession of this feed. This symptom was less obvious by addition of adsorbents and it was present for 5-10 days after disuse of contaminated feed.

Table 3. Vulvar index

Group	n	Measurments of variation [mm <sup>2</sup> ]					
		X	±	Sx	Sd	Cv	Iv
K	6	257.33	x	7.50	18.36	7.13	240.00-280.00
O-I	6	620.00	a, y	55.14	135.06	21.78	480.00-780.00
O-II	6	441.67	b	22.97	56.27	12.74	375.00-500.00
O-III	6	446.33	b	19.73	48.33	10.83	384.00-480.00

\*Value expressed as  $\bar{x} \pm Sd$

a, b, c, d  $p < 0.05$

x, y, z, w  $p < 0.01$

Morphological examination of the genital organs of pigs fed with zearalenone contaminated feed showed macroscopic changes in the form of vulvovaginitis primarily manifested with oedema. Histological findings of epithelial hyperplasia and submucous oedema were present mostly in pigs that were not given any feed protectors. Use of adsorbents as toxin binders, especially the organic one, fairly alleviated negative effects of zearalenone.

The outbreak of gastrointestinal disorders was also observed. It was noticed since the third day of usage of contaminated feed without added adsorbents, and since the seventh day in groups of pigs that were given protectors. The majority of affected pigs was seen in groups with no added adsorbents in the feed. It is believed that this might be result of the presence of other *Fusarium* mycotoxins which co-occur in the feed. In general, combinations of *Fusarium* mycotoxins result in additive effects, but synergistic and/or potentiating interactions have been observed and are of great concern in livestock health and productivity. (D'Mello *et al.*, 1999; Jadamus and Schneider, 2002).

In practice F-2 toxicosis is usually expressed in a nonspecific form as chronic primary mycotoxicosis followed by impaired growth, decreased body weight, lower feed intake and consequently increase of feed conversion ratio (Humphreys, 1988). Observing performances cumulatively for both experiments it can be found that zearalenone provoked harmful effects proportionally to its content in the feed (Doll *et al.*, 2003), although this was not obviously clinically noted in the trials considering their similar duration and only a 1.16 mg/kg dosage difference.

Application of the inorganic adsorbent alleviated the harmful influence of zearalenone to a certain extend, what is in agreement with remarks of Coenen and Boyens (2001), Tomašević-Čanović *et al.* (2003), Döll *et al.* (2004) in *in vitro*

conditions, but much less information is available about *in vivo* trials (Kyriakis *et al.*, 2002; Döll *et al.*, 2005). Similar results are observed by utilizing an esterified glucomannane, but still lower than those reported by Devegowda and Aravind (2002) and Aravind *et al.* (2003)

Based upon the obtained results, it could be concluded that zearalenone induces detrimental alterations of health and performance in pigs. Addition of modified clinoptilolite and esterified glucomannane in the feed significantly alleviates harmful zearalenone effects. Thus, the use of adsorbents as toxin binders could be an efficient approach to reduce mycotoxin effects in animals to a certain extent. However, only absolute disuse of this kind of feed is a safe and efficient method of avoiding mycotoxins.

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#### **EFIKASNOST MINERALNOG I ORGANSKOG ADSORBENTA U UBLAŽAVANJU NEGATIVNIH EFEKATA ZEARELENONA NA PROIZVODNE REZULTATE I ZDRAVSTVENO STANJE PRASADI**

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#### **SADRŽAJ**

Dva ogleđa, u trajanju od 29 i 31 dan, izvedena su sa ciljem da se ispituju proizvodni rezultati i zdravstveno stanje prasadi tretirane različitim dozama zearalenona i da se utvrdi mogućnost upotrebe modifikovanog klinoptilolita i esterifikovanog glukomanana u ublažavanju i/ili preveniranju njegovih štetnih efekata. Korišćeno je ukupno 64 prasadi (32 po ogleđu), starosti 60 dana, podeljenih u četiri grupe, po 8 u svakoj. Prasad kontrolne grupe (K) u oba ogleđa su hranjena smešom bez zearalenona, dok su prasad eksperimentalne grupe hranjena hranom koja je sadržavala 3.84 mg/kg u prvom, odnosno, 5.12 mg/kg hrane zearalenona u drugom ogleđu. Prasad prve eksperimentalne grupe u oba ogleđa su dobijala hranu samo sa toksinom. Istovremeno, modifikovani klinoptilolit (MC) u količini 0.2% dodat je u hranu za prasad obe druge eksperimentalne grupe (O-II), dok je u smeši obe treće eksperimentalne grupe (O-III) bio prisutan esterifikovani glukomanan (EGM) u količini od 0.1%

Zearalenon ispoljava negativne efekte na proizvodne rezultate u koncentraciji upotrebljenoj u ispitivanjima, i to proporcionalno njegovoj količini u hrani (pri-

rast je bio 19.86% slabiji u prvom i 22.27% u drugom ogledu, konzumacija niža za 5.89% u prvom i 1.52% u drugom eksperimentu, a konverzija hrane je bila viša za 14.82% u prvom i 21.08% u drugom ogledu), dok su dodavanjem adsorbenata delimično uklonjeni ovi štetni efekti.

Simptomi intoksikacije, poznati kao estrogini sindrom, klinički su se manifestovali vulvovaginitisom kod ženske prasadi, a histološkim ispitivanjem utvrđena je epitelijalna hiperplazija i submukozni edem. Takođe, javili su se i nespecifični simptomi u vidu gastrointestinalnih poremećaja, praćenih dijarejom. Upotreba adsorbenata za vezivanje toksina uspešno je ublažila negativne posledice prisustva zearalenona u hrani.