*M i r o s l a v I. U r o š e v i ć[1], I g o r M. J a j i ć[2],
Ž e l j k a G. M i l i č i ć[1]*

[1] Agriculture School Futog, Carice Milice 2, 21410 Futog, Serbia
[2] Department of Animal Science, Faculty of Agriculture,
  University of Novi Sad, Trg D. Obradovića 8, 21000 Novi Sad, Serbia

MYCOTOXINS IN HORSE FEED – INCIDENCE OF DEOXYNIVALENOL IN OAT SAMPLES FROM STUD FARMS

ABSTRACT: Reports concerning mycotoxins in horse feed are very rare and are typically restricted to fumonisins. As a non-ruminant monogastric species, horses may be more sensitive to adverse effects of mycotoxins, but the most severe effect of fumonisin B1 (FB1) in equines is that it causes fatal leucoencephalomalacia. In recent years, the European Food Safety Authority (EFSA) has evaluated several mycotoxins as "undesirable substances in animal feed" with the aim of establishing guidance values for the feed industry. In its evaluation of deoxynivalenol (DON), EFSA concluded that this toxin exhibited toxic effects in all species, but that horses were more tolerant towards this toxin than pigs. According to the available data, a systematic survey on mycotoxins in horse feed in Serbia has not been published. Therefore, the aim of this study was to investigate the incidence of mycotoxins in horse feed in Vojvodina. Samples of oats for horse consumption, collected in 2010, were analyzed by enzyme immunoassays (ELISA) for deoxynivalenol contamination. Twelve samples of oats were taken from twelve horse studs, with sport, school and hobby horses.
KEY WORDS: deoxynivalenol, feed, horse, mycotoxins

INTRODUCTION

There are two main groups of fungi that should be considered in the equine world. They are the field fungus *Fusarium*, which produces toxins such as fumonisin, deoxynivalenol (DON), zearalenone and T2, and the storage fungus *Aspergillus*, which produces toxins such as aflatoxin and ochratoxin. Important toxigenic fungus related to the horse disease is *Fusarium* because it is a potent producer of mycotoxins (Q u i n n et al., 2002).

Horses are the species which are most sensitive to fumonisin B1 (FB1) toxicity. The target organs in horses are central nervous system, liver and heart (V o s s et al., 2007). Two syndromes caused by FB1 have been described in horses, namely, the neurotoxic form of leukoencephalomalacia (ELEM) and the hepatotoxic form. These may appear independently or simultaneously. ELEM is characterized by the sudden onset of one or more of the following

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signs: frenzy, incoordination, aimless circling, head pressing, paresis, ataxia, blindness, depression and hyperexcitability (R o s s et al., 1991). Mortality is usually high and death may occur without clinical signs. The primary pathological feature is liquefactive necrosis of white matter of the cerebral hemispheres. To determine the levels of FB1 in feed associated with ELEM, R o s s et al., (1991) studied 45 confirmed equine leukoencephalomalacia cases. FB1 concentrations ranged from <1 to 126 ppm, with the majority of the samples being above 10 ppm. The hepatotoxic syndrome occurs much less frequently than the neurotoxic form and usually takes 5–10 days from the time of onset of clinical signs to death. Clinical symptoms include loss of appetite and depression followed by oedema of the head and a prominent icterus (V o s s et al., 2007). Elevated serum bilirubin concentration and liver enzyme activities are typically present. Neurotoxicity and hepatotoxicity have been reproduced experimentally in horses, ponies and donkeys by feeding them with naturally contaminated feed (R o s s et al., 1993), fumonisins containing culture material (W i l s o n and M a r o n p o t , 1971; B r o w n i e and C u l l e n , 1987) and purified FB1 (M a r a s a s et al., 1988; K e l l e r m a n et al., 1990). Deoxynivalenol (DON) is a member of the trichothecene family of mycotoxins. The occurrence of deoxynivalenol is associated primarily with *Fusarium graminearum* (*Gibberella zeae*) and *Fusarium culmorum*, both of which are important plant pathogens commonly found in cereals and other crops (J E C F A , 2001). Although DON is among the least toxic of the trichothecenes, it is the most frequently detected one throughout the world and its occurrence is considered to be an indicator of the possible presence of other, more toxic trichothecenes (L o m b a e r t , 2002). Although animals are differentially sensitive to DON (P e s t k a , 2007), reduced weight gain is universally observed in monogastric species including mice, rats, pigs, dogs and cats chronically exposed to the toxin at low doses (F o r s y t h et al., 1977; H u g h e s et al., 1999; M o r r i s s e y et al., 1985; P e s t k a and S m o l i n s k i 2005). The Commission of the European Communities (Commission Regulation No. 576/2006) established the tolerance values for DON in unprocessed durum wheat and oats (1750 ppb), and other unprocessed cereals (1250 ppb).

J o h n s o n et al. (1997) investigated the effects of horse feeding by DON-contaminated grain. The five healthy adult horses were provided with barley naturally contaminated with DON for 40 days. The barley had been severely contaminated and analysis showed that the concentration of DON was between 36 and 44 ppm. No adverse effects, such as feed refusal, or altered serum chemistry or haematology, were identified during the feeding trial. The results suggested that, like ruminants but unlike other species, horses are relatively resistant to the adverse effects of DON.

In a further study (R a y m o n d et al., 2003) horses were fed with grain naturally contaminated by Fusarium mycotoxins (DON, fumonisin, zearalenone) to investigate the effects of these mycotoxins on feed intake, serum immunoglobulin (IgA, IgG, IgM) concentrations, serum chemistry, and haematology. Such contaminated diet for horses resulted in reduced feed intake and increased serum gamma-glutamyl transferase (GGT) activities. Serum

levels of GGT were significantly higher in horses consuming contaminated grain which was sampled on the 7th and 14th day of supplementation, but not on the 21st day. The lack of difference in serum activities of GGT on 21st day implied that the horses may have adapted to the hepatotoxicity caused by the combination of Fusarium mycotoxins. According to J o h n s o n et al. (1997), serum levels of IgA, IgG and IgM were not affected by the diet.

Another study investigated the effects of feeding mature, exercised horses with grain naturally contaminated with Fusarium mycotoxins (R a y m o n d et al., 2005). In contrast to the findings of J o h n s o n et al. (1997), the results suggested a 35% decrease in feed intake, higher than the 65% reported in the previous experiment (R a y m o n d et al., 2003), possibly reflecting the increase in energy requirements due to exercise. Moreover, weight loss and unchanged serum activities of the hepatic membrane-associated enzyme, GGT, observed in horses fed with the contaminated diet as opposed to the control group (R a y m o n d et al., 2005), were in contrast with the authors' previous work (R a y m o n d et al., 2003). The results obtained by R a y m o n d et al., (2003 and 2005) suggested a relatively high degree of reduced feed intake when horses are simultaneously exposed to different fusariotoxins. However, the presence of fumonisin in the test diet (R a y m o n d et al., 2003) should have been addressed in the context of possible synergistic interactions between fumonisin and DON.

B a r n e t t et al. (1995) studied the correlation of mycotoxins with the incidence of colic in horses. Feed samples from farms experiencing possible feed related incidences of colic and control farms were analyzed. DON was found in the concentrates in 100% of the colic cases at levels ranging from 0.20 to 8.3 ppm and in 70% of the control group concentrates (0–2.5 ppm). However, the cause-effect relationship of mycotoxins and equine colic remains unclear although it should certainly be considered as a possibility, especially when a series of colic cases are observed on a single farm (N e w m a n and R a y m o n d, 2005; C a l o n i et al., 2009).

B u c k l e y et al. (2007) agreed with previous reports which suggested that zearalenone and deoxynivalenol (DON) can cause problems in horses. However, authors suggest that most horses will not eat feed contaminated with these mycotoxins unless they are starved, or they are in another feed ingredient that masks the taste and smell of mycotoxins.

There is little published information about the incidence of mycotoxins in horse feed in Serbia, but there are assumptions about their presence. Therefore, the aim of this study was to investigate the incidence of deoxynivalenol in oats for horse feeding in Vojvodina. Twelve samples of oats were taken from five private horse owners and seven equestrian ranches (horse clubs). Five samples were produced in the harvesting period in the summer of 2009 and rest (seven samples) in 2010.

MATERIALS AND METHODS

Oats for feeding horses in horse farms were kept in bulk or in bags in a dry place, and all samples were taken in a plastic bag which was sealed. Immediately after sampling, 500 g of each sample was prepared by grinding in a laboratory mill, and the sample was homogenized by mixing. Samples prepared in such a way were packed in plastic bags and stored in a freezer at -20^{0}C until analysis. Prior to each analysis, the samples were allowed to reach the room temperature. Extraction and clean-up of 20.0 g of the sample were extracted with 100 ml of distilled water and shaken on Ultra Turrax (IKA, Germany) for 3 minutes. After filtration through Whatman #1 filter paper, deoxynivalenol was analyzed by competitive direct enzyme-linked immunosorbent assays (ELISA). We used Veratox DON HS, Quantitative High Sensitivity Test (Neogen, USA) according to the manufacturer's instructions. The limit of quantification was 25 µg/kg (ppb).

RESULTS

From twelve samples used in our study, four samples (30%) were positive for DON, and we found the following concentrations: 0.005; 0.017; 0.094 and 0.150 ppm. It was remarkable, however, that none of the above mentioned symptoms of Mycotoxicosis were observed during the feeding with oats, used in our research. None of the 96 animals showed any reduced feed intake, feed refusal, reduced weight gain or clinical sign of hepatotoxicity.

DISCUSSION

Based on information received from the resident veterinarians and/or horse owners, we created a database about horses, their nutrition and care, and health. These data are discussed together with the findings of oats from the laboratory.

Our findings of DON in samples are in accordance with most studies on the occurrence of these mycotoxins in cereals (E F S A 2004 and 2005; L i e s e n e r, 2010; B u c k l e y, 2007). Our investigation included a total of 96 horses from the equestrian clubs, and according to the category, they belonged to different breeds (Thoroughbred, Standard bred, Lipizzaner, pony and cross-breeds) and were aged between three and 20 years. The horses included 64 mares, 15 stallions and 7 castrates. Data about different equine category (mares, stallions, castrate) are important because there is a gender predisposition in relation to the occurrence of clinical symptoms in mycotoxicoses. Based on some investigations (G o n z a l e z - Č a b r e r o et al., 1990) non castrated male animals (stallions) are more susceptible to this toxin than their female counterparts.

It is also important to note that the average level of DON was quite low (0.067 ppm), and that all samples met the requirements concerning guidance values for DON in cereals for animal feed industry (Commission Regulation No. 576/2006). This value for product intended for animal feed is 8 ppm. Nevertheless, the highest concentration of DON found (0.150 ppm) in oats for horse feeding in our study was below this level, and the majority of other samples was below 0.100 ppm.

Unfortunately, there are no data on no-effect or maximum level of DON in the "FSA – Opinion of the scientific panel" related only to horse feeding. There are only recommendations that other animal species, including rabbits, horses, cats and dogs, seem to have higher tolerance towards DON than pigs. On the other hand, at present, the available data do not allow the establishment of a no-effect level for pigs. The lowest reported levels with negative effect on feed intake vary between 0.35 and 0.9 ppm.

The initial adverse effect observed after the exposure to DON is reduced feed intake. These effects lead to a reduced body weight gain, particularly in growing animals. DON affects the immune response, and the release of pro-inflammatory cytokines is one of the earliest manifestations of the exposure (E F S A , 2007).

Our data showed that animals from five out of twelve horse farms (42%) were, besides forage (hay, clover, straw), fed with oats as feed concentrates. On the other seven horse studs, horses were fed with pellets, corn, barley, triticale and sugar beet pulp. Obviously, the individual toxin concentration of oat raw material would be much more important for the first group above mentioned (42%) than the added oats in the concentrated feed (pellets) and/or mixed with other cereals in the meal.

In conclusion, the results of first systematic survey demonstrate that mycotoxins are present in horse feeds in Serbia. In fact, the vast majority of these samples is considered as "food quality", since those toxins are present at the levels that are below the respective EU maximum levels for foodstuffs in the feeding industry. Although these findings are reassuring, it has to be acknowledged that very little is known about the adverse effects of other mycotoxins in horses in Serbia. Even though our study has not reported it, colic can be most common disease of the digestive system of horses in this area. In this sense, C a l o n i (2010) emphasizes that the possible cause-effect relationship between *Fusarium* mycotoxins and equine colic requires further investigation. Additionally, further extensive studies concerning mycotoxin intake from commercial and non-commercial sources of horse feed seem to be advisable.

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МИКОТОКСИНИ У ХРАНИ ЗА КОЊЕ – ДЕОКСИНИВАЛЕНОЛ У УЗОРЦИМА ОВСА СА ФАРМИ ЗА КОЊЕ

Мирослав И. Урошевић[1], Игор М. Јајић[2], Жељка Г. Миличић[1]

[1] Пољопривредна школа са домом ученика Футог,
Царице Милице 2, 21410 Футог, Србија
[2] Департман за сточарство, Пољопривредни факултет Нови Сад,
Универзитет у Новом Саду, Трг Д. Обрадовића 8, 21000 Нови Сад

Резиме

Извештаји о појави микотоксина код коња су веома ретки и обично се односе на фумонизин. Као моногастрична врста, а непреживари, коњи би требало да су осетљивији на деловање микотоксина, а најопаснији је фумонизин B1 (FB1) који код коња изазива леукоенцефаломалацију са фаталним последицама.

Протеклих година Европска Агенција за безбедност хране („EFSA") је испитала велик број микотоксина као „нежељених супстанци у сточној храни", делом и са циљем да успостави граничне вредности на индустрију сточне хране. У истраживањима о деоксиниваленолу (DON) у „EFSA" је закључено да он код свих врста изазива токсичне поремећаје, али да су коњи мање осетљиви на овај токсин него свиње.

На основу доступних података, до сада није било публикација о систематском истраживању микотоксина у храни за коње у Србији. Стога је циљ ове студије био да испита појаву DON-a у храни за коње у Војводини. Узети су узорци овса за исхрану коња, сакупљени у 2010. години, и анализирани помоћу ензимског имуно-везујућег теста (ELISA) на контаминацију деоксиниваленолом. Сакупљено је дванаест узорака овса из дванаест запата коња, који се користе за спорт, школу јахања и рекреацију.