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MYCOTOXICOLOGICAL TESTS FROM THE ASPECT OF THE HACCP SYSTEM AND LEGISLATION

ABSTRACT: Mycotoxins, as secondary metabolic products of molds, are common contaminants of raw feed materials and compound feeds. Depending on the agro-meteorological and storage conditions, molds can contaminate grains and produce mycotoxins in the field, before and after harvest and during storage. Way of preventing animal mycotoxicoses and transfer of mycotoxins to humans through food chain is regular inspection on mycotoxicological feed safety. This paper presents the results of examination of aflatoxin, ochratoxin and zearalenone in 89 samples tested under laboratory conditions during a one year period. The analyses of types of samples, contamination, and their origin demonstrated the need for preventive control of mycotoxin content, primarily in grains. The results of testing the content of aflatoxin in milk indicate the existence of risks to human health. In order to protect humans and animals from mycotoxicological contamination, we propose the use of the system hazard analysis and critical control point (HACCP), which has been proved to be an effective strategy in food safety control. The basic principles of HACCP approach in the production of feed and foodstuffs, as well as the compliance and harmonization of legislations with those in the European Union, in the field of mycotoxicological tests, are the best prevention against mycotoxin effects on health and economy. Implementation of HACCP system currently presents one of the basic preconditions for the export of our products and their placement on the European market.

KEY WORDS: animal feed, HACCP, mycotoxins, regulations

INTRODUCTION

Raw feed materials are suitable substrates for the growth of mold and toxin production. Depending on the type of feed, growing and maturing conditions, as well as the conditions of storage, different toxins can be present in various feed materials (Table 1).

Tab. 1 – Feedstuffs and their associated mycotoxins (P e t t e r s s o n , 2004)

Feed raw material	Mycotoxins produced	
	In field during culturing	Preservation and storage
Barley	DON, NIV, Zea, HT-2, T-2	OTA, Afla, Cit
Maize	DON, Fum, Zea	Zea, Afla
Oats	DON, NIV, HT-2, T-2	OTA, Afla, Cit
Rice	Ergot	OTA
Wheat	DON, NIV, Zea, Ergot	OTA, Afla, Cit
Soya bean meal	–	Afla
Silages	Afla, DON	Zea

Afla − aflatoxins; Cit − citrinin; DON − deoxynivalenol; Ergot − ergotamin;
Fum − fumonisin; NIV − nivalenol; OTA − ochratoxin A; Zea − zearalenone.

Mycotoxins are rarely present in food at high concentrations. More common problem is chronic mycotoxicosis caused by low toxin concentrations that are consumed by animals for a longer period (P e t t e r s o n , 2004). The negative effect of mycotoxins results in a decline of productivity and their presence in food chain is of great economic importance. For the purposes of health safety and avoiding economic losses it is very important to prevent the mold growth and toxin production in cereals.

HACCP (Hazard Analysis Critical Control Point) is a food managment system, designed to prevent the safety problems, including food poisoning. It has been developed by NASA and US army with the aim of providing ”absolutely safe food“ for US astronauts, and implemented in the food industry. Today, it presents a global food safety standard. HACCP has been proven as an effective way to prevent the presence of hazardous substances that rarely occur in food, by eliminating the need for complicated analyses of final products. HACCP approach involves detailed analysis of each step in food processing using seven clearly defined principles. The presence of mycotoxins in final product is often the result of circumstances which occurred much earlier in the production chain. In addition, mycotoxins are very stable compounds that are difficult to remove once formed. The analyses are complicated, time consuming and expensive (A l d r e d et al., 2004). Therefore, prevention is more economical and convenient way of fight against mycotoxins. For this reason modern technology aims to introduce low-cost rapid tests for mycotoxicological control of the raw materials in order to avoid expensive and complicated analyses of the final product.

On the basis of the results obtained in mycotoxin examinations, the objective of this paper is to point to the necessity of prevention in the fight against mycotoxins and mycotoxicoses by introducing novelties in science and by harmonization with the legislation of the European Union (EU).

MATERIALS AND METHODS

The feed samples obtained from the feed producers, from the farms where health problems were detected or death cases occured, as well as the samples submitted by the inspection for the purpose of monitoring safety, were analyzed.

Raw milk samples were taken from five commercial dairy farms. A total of 50 samples of raw milk were examined.

Part of the animal feed (45 samples) was analyzed by thin layer chromatography (TLC, The Official Gazette of SFRY, No. 15/87), and part of the samples (44 samples) were analyzed by immunoaffinity enzymatic method (ELISA) using *Ridascreen*® kits (Art.No. R:4701; R:1311; R:1401, R-Biopharm, Germany). For the analysis of milk samples ELISA kit *Tecna*, Italy, was used.

RESULTS AND DISCUSSION

The results of feed examining obtained by TLC are presented in Table 2. In only one raw feed material sample aflatoxins were detected, and ochratoxin A was detected in two samples, but in a concentration lower than the maximum prescribed (The Official Gazette of RS, 2010). In the tested feedingstuff samples, toxin concentrations were not above the detection limit of the methods used.

Tab. 2 – The examination results of animal feed samples using TLC

Sample	No. of samples	No. of contaminated samples	Limits of detection (ppm) Range of contamination (ppm)		
			Aflatoxins	Ochratoxin A	Zearalenone
Raw feed material	9	3	<0.025 0.025	<0.02 0.02–0.1	<0.05 –
CF for broilers	15	–	<0,005	<0,02	<0.25
CF for layers	3	–	<0.01	<0.05	<0.25
CF for poultry	2	–	<0.01	<0.2	<0.25
CF for piglets	1	–	<0.005	<0.02	<0.05
CF for piglets from 1–15 kg	1	–	<0.01	<0.02	<0.05
CF for fattening pigs	5	–	<0.01	<0.04	<0.1
CF for sows	5	–	<0.01	<0.04	<0.05
Other CF	4	–	<0.005	<0.02	<0.05

CF – Complete feedingstuffs

As it can be seen from Table 3, mycotoxins were observed in a large number of the samples tested by ELISA, but no significant contamination was

Tab. 3 – Content of mycotoxins in feed samples using ELISA

Sample	No. of samples	No. of contaminated samples Range of contamination (ppb)		
		Aflatoxins	Ochratoxin A	Zearalenone
Raw feed material	23	17 (2.1–35.6)	14 (1.2–900)	21 (2.0–241)
CF for poultry	11	3 (2.6–5.6)	4 (1.1–4.6)	11 (14.2–102.2)
CF for pigs	8	5 (5.4–13.9)	7 (1–9.8)	8 (6.2–42.9)
CF for cattle	2	0 –	1 2.42	2 (10.6–20.8)

(CF – Complete feedingstuffs; Limits of detection: 1 ppb for ochratoxin A, 1.75 for total aflatoxins and zearalenone)

detected. However, in one maize sample originating from a pig farm, ochratoxin A was detected in the quantity of 900 µg/kg. This result was also confirmed by TLC method. Although the current regulation on the quality of animal feed (The Official Gazette of RS, 2010) does not prescribe the maximum amount of ochratoxin A in maize, having in mind the maximum permissible concentrations in complete feedingstuffs for piglets and pigs, this corn is not safe for the consumption. By analyzing and comparing the existing regulations in Serbia and those in the EU, it can be concluded that there are significant differences in the types of samples encompassed by the legislation, but also in the prescribed maximum permissible concentrations (Table 4). In order to comply with the EU regulations, it is necessary to update and amend the current regulations in Serbia which regulate only aflatoxins in the raw material. New directions, based on the recommendations of the European Commission are to be given for other toxins in the raw materials used for feed production. It is necessary to introduce monitoring of fumonisins in corn in Serbia because of the confirmed presence of these toxins (Jakšić, 2004; Kokić et al., 2009). The current regulation related to the trichothecenes should be defined more precisely.

When analyzing the types of tested samples, the number of complete feedingstuffs (57) significantly exceeds the number of raw feed materials (32). The number of requests made by the manufacturer makes 55% of the samples, the number of the requests by the inspections makes 37%, and the number of the requests by the veterinary services from the farms with health problems makes 8%.

Out of 50 tested samples of raw milk, aflatoxin M_1 was detected in two samples taken from different farms. The amount of aflatoxin was 7.5 ng/kg and 10 ng/kg which was significantly lower than the maximum permissible concentration (0.5 µg/kg) according to the Regulation (The Official Gazette, No. 5/92; 11/92; 32/02). The limit values, set by the EU, were not exceeded (0.05 µg/kg), but the results confirmed the possibility of excretion into milk.

Tab. 4 – Legislation on mycotoxins in animal feed and milk in the EU and Serbia (EC, 2003, 2006a, b, The Official Gazette RS, No. 4 / 2010, the Official Gazette SRY, No. 5 / 92, 11/92, 32 / 02)

Mycotoxin	Sample	Maximum content (ppm) EU	Maximum content (ppm) Serbia
Aflatoxin B_1	Feed materials; com. and compl. feedingstuffs for cattle, sheep and goats	0.02	0.05
	Com. feedingstuffs for dairy animals	0.005	0.01 (and compl.)
	Com. feedingstuffs for calves and lambs	0.01	0.01
	Com. and compl. feedingstuffs for pigs and poultry	0.02	0.03
	Other com. feedingstuffs	0.01	0.01
	Other compl. feedingstuffs	0.005	0.01
Aflatoxin M_1	Milk and milk products	0.05×10^{-3}	0.5×10^{-3}
		Guidance values (ppm)	**Maximum content (ppm)**
DON	Feed materials, cereals with exception of maize by-products	8	–
	Maize by-products	12	–
	Compl. and com. feedingstuffs with the exception of:	5	–
	for pigs	0.9	0.5
	for calves, lambs and kids	2	–
Zearalenone	Feed materials, cereals with exception of maize by-products	2	–
	Maize by-products	3	–
	Compl. and com. feedingstuffs for piglets and gilts	0.1	0.5
	Compl. and com. feedingstuffs for sows and fattening pigs	0.25	1.0
	Compl. and com. feedingstuffs for calves, dairy cattle, sheep and goats	0.5	3.0
Ochratoxin A	Feed materials, cereals	0.25	–
	Compl. and com. feedingstuffs for pigs	0.05	0.1 pigs (0.2 fattening and sows)
	Compl. and com. feedingstuffs for poultry	0.1	1 (0.25 for layers)
Fumonisin	Feed materials, maize and maize by-products	60	–
	Compl. and com. feedingstuffs:		
	for pigs, horses, rabbits and pet animals	5	–
	for fish	10	–
	For poultry, calves, lambs and kids	20	–
	For ruminants and mink	50	–
Trichothecenes	Feedingstuffs for broilers, pigs, calves	–	0.30
	Feedingstuffs for sows, cattle, poultry	–	0.60
T_2, DAS and derivates	Com. feedingstuffs for pigs and poultry	–	1.0
	Com. and compl. feedingstuffs for broilers, piglets and calves	–	0.5

Com. – complete; Compl. – complementary; DON – deoxynivalenol, DAS – diacetoxyscirpenol, – not legislated

Our results point to the following data: the analyzed number of samples of feed raw material is smaller in comparison to complete feedingstuffs, feed raw material is contaminated by the mycotoxins not enompassed in the regulations, there is a suspicion of mycotoxicosis on the farms, and the excretion of toxins in milk has been proven. Therefore, it can be concluded that well organized preventive mycotoxic control is needed.

Combining Good Agricultural Practice, Good Manufacturing Practice, Good Storage Practice, and applying the seven HACCP principles whithin the framework of the quality systems such as ISO 9000, it is possible to introduce *the most cost-effective prevention* of mycotoxicosis (F A O , 2001). Although a large number of mycotoxins has been discovered so far, only around ten have been proven to have impact on human health, and are marked as hazardous. The critical control points are extreme conditions during crop growth and harvesting, the conditions in distribution, processing and storage of food (A l d r e d et al., 2004). Critical limits may be maximum values of mycotoxins prescribed by the regulations, or simply measurable parameters that influence the production of toxins, such as temperature, humidity and toxin producing molds. The procedures for monitoring critical control points in this case are the methods for determining the parameters. The corrective actions include implementation of all available measures for combating mold and preventing toxin production, which includes implementation of appropriate agricultural practice in the field, and use of feed additives like adsorbents, antioxidants, vitamins or enzymes. The verification procedures are also necessary within the HACCP system applied in the control of mycotoxins, as a way of checking that the system works in all control points. Finally, all the stages that describe monitoring procedures and corrective actions, as well as routine records of parameters, must be documented.

We believe that the information obtained by monitoring of mycotoxins in Serbia is very important to obtain a complete picture of the frequency and amounts of certain toxins in some samples, in the agrometeorological climatic condition of this area. All the results obtained by monitoring are to be taken into account when assessing risk and compliance with European regulations.

The methods for mycotoxin determination have significant role in monitoring and therefore must meet certain standards. Due to different chemical structures, a large number of mycotoxins, and matrix heterogeneity it is difficult to choose fast, simultaneous and precise method for routine determination. To assure the quality of analysis, it is necessary to validate and verify test methods according to the European requirements (E C , 2006c, 2010), to accredit the methods and carry out interlaboratory exchange programs, and to use certified reference materials.

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МИКОТОКСИКОЛОШКА ИСПИТИВАЊА СА АСПЕКТА НАССР СИСТЕМА И ПРАВНЕ РЕГУЛАТИВЕ

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Резиме

Микотоксини, као секундарни метаболички продукти плесни, чести су контаминенти биљних хранива и смеша за исхрану животиња. У зависности од агрометеоролошких услова и услова чувања, плесни могу да контаминирају житарице и продукују микотоксине на пољу пре жетве, као и након жетве у току складиштења. Основни начин превенције микотоксикозе животиња и преношења микотоксина ланцем исхране до људи је редовна контрола микотоксиколошке исправности хране за животиње. У раду су приказани резултати испитивања афлатоксина, охратоксина и зеараленона у 89 узорака хране за животиње пристиглих у лабораторију у току једне године. Анализом врсте узорака, контаминираности, као и анализом порекла узорака, доказана је потреба за превентивном контролом садржаја микотоксина, пре свега у биљним хранивима. Резултати испитивања садржаја афлатоксина у млеку показују постојање ризика по здравље људи. У циљу заштите људи и животиња од микотоксиколошке контаминације, предложена је примена система анализе опасности и контроле критичних тачака (НАССР), која се показала као ефикасна стратегија за контролу исправности хране. Основни принципи НАССР приступа у производњи хране за животиње и намирница, поштовање и усклађивање законских прописа у области микотоксиколошких испитивања са Европском унијом, најбоља су превенција здравствених и економских последица микотоксина. Имплементација НАССР система је тренутно један од основних предуслова за извоз наших производа и освајање европског тржишта.