Зборник Матице српске за природне науке / Jour. Nat. Sci, Matica Srpska Novi Sad, № 124, 325—332, 2013

UDC 582.281/.282:615.9]:636.085
DOI: 10.2298/ZMSPN1324325S

Igor M. Stojanov, Miloš C. Kapetanov, Dubravka V. Potkonjak, Milica M. Živkov Baloš, Sandra M. Jakšić, Jasna Z. Prodanov Radulović

Scientific Veterinary Institute "Novi Sad", Rumenački put 20, 21000 Novi Sad, Serbia*

CORRELATION BETWEEN THE LIMIT VALUES OF LABORATORY AND CLINICAL MYCOTOXICOSIS

ABSTRACT: Analysis of feed for the presence of fungi and mycotoxins is a request necessary to meet in order to ensure a healthy and economical production in livestock. These tests are related to legal regulation which prescribes the maximum legislated content (MLC), both for the presence of mycotoxins and the total number of fungi in certain feeds. Health problems that can occur during the production of animals are sometimes caused by the presence of mycotoxins in the feed. Laboratory testing is a good practice to confirm a suspicion, and allows timely treatment of contaminated feed.

Potential problems arise under circumstances when there is a clinical outcome of mycotoxicosis and animal and laboratory findings suggest that the obtained values are below the level that is within the MLC. For these reasons, the subject of our research was to investigate the occurrence of mycotoxins and mold in feed, as well as the clinical presentation for animals that were fed with the feed with allowed values of these agents according to the recommended levels. The aim of this paper was to highlight the problems associated with clinical correlation of sick animals and laboratory findings, and suggest their overcoming.

In the period of one year, a total of 176 samples of feed (complete mixture for broilers, corn and soy products) were examined for the presence of fungi, 106 samples were examined for the presence of mycotoxins and 26 flocks of broilers and turkeys were clinically observed. Standard methods were used for isolation of molds and the ELISA test was used for the detection of mycotoxins. Clinical and pathomorphological observation of the flocks was done to determine the natural indicators of production.

Studies indicated a problem because clinical and pathomorphological findings in some cases were not correlated with laboratory findings of molds and mycotoxins in the feed, and in some cases it did not necessarily mean that the animals were healthy. Synergism and cumulative effects of mycotoxins, on the one hand, and the characteristics of each species and product category on the other hand, can create specific circumstances that can lead to disease and can increase even though the values prescribed by legislation have been met.

KEY WORDS: clinical findings, feed, molds, mycotoxins

INTRODUCTION

Modern animal production requires the implementation of optimal zootechnical measures and the use of safe feed. Feeds are especially worth atten-

* Corresponding author: Igor Stojanov-E-mail: igor@niv.ns.ac.rs

tion because of their nutritional importance. As a source of essential biological and minerals, it can be a vector of transmission of various microorganisms and their metabolites. Feed can be contaminated at various stages of production and processing, and the diversity of present microorganisms depends on some of its properties, water activity, presence of oxygen, pH and nutritional potential (M a c i o r o w s k i et al., 2007). The most important factors that contribute to the presence or production of mycotoxins in feed are often related to storage and environmental conditions which, under the control of people, can be reduced (D u a r t e et al., 2011). Presence of molds and mycotoxins in foods indicates that the contamination occurred at some point in the production of feed, and that such feed is a threat to the health of animals. Mycotoxins as secondary metabolites of molds (A v a k u m o v i ć et al., 2007) require special attention when the quality of the feed is considered. Most commonly, mycotoxins enter the body through the digestive tract during the process of eating contaminated feed, but the possibility of inhalation of toxic spores and direct dermal contact should also not be ignored (Z i n e d i n e, M a ñ e s, 2009).

The legislation specified the method for determination of the presence of fungi and mycotoxins in feed and set the threshold limit value of certain toxins in animals and different product categories (Službeni Glasnik, 2010). Potential problems arise under circumstances when there is a clinical picture of mycotoxicosis in animals but laboratory findings suggest that the obtained values are below MLC. 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 of time (P e t t e r s o n , 2004). For these reasons, the subject of our research was to investigate the occurrence of mycotoxins and mold in feed, as well as the clinical results of animals which consumed the feed with allowed values of these agents. The aim of this paper was to highlight the problems associated with clinical correlation of sick animals and laboratory findings, and to suggest their overcoming.

MATERIAL AND METHODS

During a one year long period (autumn, 2011 – autumn, 2012) we examined a total of 176 samples of complete feeding mixtures for broilers and young turkeys used in the diet for fattening poultry and turkeys. This included a total of 103 mixtures for fattening broilers and young turkeys, 29 samples of corn and 54 soybean products (meals and cakes). Isolation of fungi was performed according to the methodology defined by international standards (EN ISO 21527-2:2011). Tests for the presence of mycotoxins in the feed used for young birds were carried out on 106 samples. It included 45 samples of mixtures for chickens and turkeys, 47 corn samples and 14 samples of corn and soybean products. Content of total aflatoxins, ochratoxin A, deoxynivalenol and T2 toxin were determined by the enzymatic immunoaffinity (ELISA) method,

using Ridascreen® test kits (R-Biopharm, Germany) (J a k š i ć et al., 2012). Methods are verified according to the European requirements (E C, 2006b).

Clinical examinations were done in 11 turkeys from fattening flocks (But Big-6) and 15 flocks of broilers (Ross, Cobb), for which standard method of clinical diagnosis, observation, pathoanatomical overview and insight into the production indicators (quantity and length of feed intake, feed conversion and mortality) was done (A v a k u m o v i ć et al., 2007; P a l i ć et al., 1994).

RESULTS AND DISCUSSION

In Table 1 the results of feed tested for the presence of mold are summarized. During the experiment, the complete feeding mixtures for broilers and turkeys, and corn and soybean products were analyzed.

Tab. 1 – Distribution of feed samples according to mold counts and the number of faulty samples

Feeds	No. feed samples in the given values				number of faulty feed
Complete feeding mixtures	$<10^2$	10^2	10^3	total	
Turkeys	6	4	5	15	0
Broilers	33	7	48	88	10
Total	39	11	53	103	
Corn	0	5	24	29	0
Soybean products (meals, cakes)	31	11	12	54	0

Table 1 shows that a small number of feed was contaminated with mold in amounts that exceeded the MLC. *Fusarium*, *Aspergillus* and *Penicillium* were the most common species of fungi isolated in the examined feed, which is in accordance with the findings of other authors (B i n d e r, 2007), and the *Fusarium* species were the most common (over 30%). This finding corresponds to *Fusarium* results obtained from the studies conducted in different countries (Z i n e d i n e et al., 2007). Out of all tested feeds, complete mixture for fattening turkeys did not contain molds above the MLC while in the mixture for broilers 10 (11.36%) out of 88 nutrients examined were inadequate. In case of corn and soybean products, it can be seen that the tested samples of corn and soya products were within the acceptable limits for molds, according to the current Rulebook. Our findings are in agreement with the results of other researchers who have concluded that a small number of contaminated mixtures with mold is a sign of good quality feed. Satisfactory finding in soy products indicated that the heat treatment caused a significant decrease in mold (A s t o r e c a et al., 2011).

It is important to note that a significant number of samples, over 50% samples of feed for broilers and 60% samples of feed for turkeys, contained mold counts within the acceptable limits, but for the rest of the tested samples we could not claim that they were entirely free from mold because the detec-

tion threshold of the methods applied was $<10^2$. Similar observations are valid for the examined samples of corn and soybean products.

Table 2 shows the data related to the presence of mycotoxins in feedstuffs which were studied for the presence of molds.

Table 2 also contains data for the levels of certain mycotoxins that are allowed by our legislation and regulatory legal acts of the European Union (Službeni Glasnik, 2010, E C , 2003, E C , 2006a). Legal regulations from our country set the allowed levels for the mixtures that are used for feeding of young animals, and the levels allowed according to the EU regulation are given only for aflatoxin while for other mycotoxins only maximum limits are defined.

Tab. 2 – Mycotoxin content in feed samples in comparison to legislated values
Results of the tests of complete mixtures

Complete feeding stuffs for broilers and turkeys		Maximum legislated mycotoxin content (μg/kg)			
		Aflatoxins	Ochratoxin A	Deoxynivalenol	T2 toxin
Serbian regulations*		10	1000	300	
EU directive**		10	100	5000	
Detection limit		<2	<1	<74 or <222	
No. of samples					
Total number of samples	45	44	37	19	34
Number of faulty samples		1 (2 close to the limit)	0	2	0
				2	
				0	
Maize		Maximum legislated mycotoxin content (μg/kg)			
		Aflatoxins	Ochratoxin A	Deoxynivalenol	T2 toxin
		50			
EU directive**		20	250	12000	
No. of samples					
Total number of samples	47	39	35	43	31
Number of faulty samples		2	0	0	0
Soybean products (meals and cakes)		Maximum legislated mycotoxin content (μg/kg)			
		Aflatoxins	Ochratoxin A	Deoxynivalenol	T2 toxin
Serbian regulations*		50			
EU directive**		20	250	8000	
No. of samples					
Total number of samples	14	12	8	10	6
Number of faulty samples		0	0	0	0

* Sl. Glasnik RS, br. 4/2010 Pravilnik o kvalitetu hrane za životinje, čl. 99. (Sr)
** EC (European Commision) (2003, 2006a)

Table 2 gives data on the presence of mycotoxins in the samples of complete mixtures for broilers, corn and soybean products. It can be seen that only 3 full feed mixtures for broilers meal and 2 samples of corn were inadequate regarding the allowed levels of mycotoxins, while the other samples were within the set limits.

The results of laboratory tests indicated that the number of feeds that were not appropriate according to the allowed levels was not large; however, direct production, and fattening can lead to the problem when the symptoms of mycotoxicosis exist in spite of using the feed that meets legal requirements. The significance of the obtained results can be observed from the aspect of impact of small concentrations of mycotoxins and their synergistic effects on individuals (T a m m e r a et al., 2007).

From the 15 broiler flocks monitored, and 11 flocks of fattening turkeys, clinical and pathomorphological findings revealed changes that correspond to mycotoxicosis in 4, i.e. 2 flocks, respectively. Data on clinical, pathomorphological findings and production indicators pointed to the problems caused by mycotoxins (A v a k u m o v i ć et al., 2007; P a l i ć et al., 1994). Clinical symptoms observed in the specimens included drowsiness, contamination of feathers around the cloaca, ataxia and necrotic lesions on the hard palate, tongue and mucous membrane of the turn of the horny beak. Touching of these points was reported to cause pain in the animals. Pathomorphological changes were characterized with prominent blood vessels, carotid, dark colored anticoagulated blood, atrium overfilled with blood, bleeding, subcapsular liver with rounded edges. Natural indicators have pointed to changes in mortality, prolonged period of fattening, the altered feed conversion efficiency and lower weight in the final stage of fattening. Our findings are consistent with the results reported in the works of other authors (K a p e t a n o v et al., 2012).

Necrotic lesions in the oral cavity led to a prolonged period of food and water consumption, and feed conversion was increased because more food was needed to gain 1 kg. Mortality in flocks was increased and the feeding was extended. Laboratory tests and identified health problems on the ground indicated that mycotoxins usually did not cause acute illnesses, and if it did occur it was usually due to the multiple interactions of different factors which could cause the toxicity of these substances (M o r g a v i and R i l e y, 2007). The influence of toxins can be synergic (K u b e n a et al., 1997) or depend on the amount of mycotoxins present (D ö l l and D ä n i c k e, 2011), and their cumulative effect can cause health problems.

CONCLUSION

The results obtained in the course of this research suggest that the problem of the presence of fungi and mycotoxins in the feed cannot be approached only from the aspect of their allowed limits, but the harmful effect on the health of animals and subsequent consequences should be considered as well. It is obvious that mycotoxins pose numerous threats to different types of animals

and products. Also, in addition to laboratory tests which should be practiced in order to avoid mistakes by relying only on the given threshold, clinical findings and data on pathoanatomical and production indicators are necessary steps. Results of the laboratory tests of feed point to the fact that even if the nutrient values seem to be appropriate, the health and production problems in animals can still be reported.

REFERENCES

A s t o r e c a, L. A. A. M. D a l c e r o, V. F e r n á n d e z P i n t o, G. V a a m o n d e. (2011): A survey on distribution and toxigenicity of Aspergillus section Flavi in poultry feeds, International Journal of Food Microbiology 146: 38–43.

A v a k u m o v i ć, Đ., N e š i ć S., P e r o v i ć, J. (2007): Mikotoksikoze živine, Patentkomerc Beograd. (Sr)

B i n d e r, E. (2007): Managing the risk of mycotoxins in modern feed production, Animal Feed Science and Technology 133: 149–166.

D ö l l, S., S v e n, D. (2011): The Fusarium toxins deoxynivalenol (DON) and zearalenone (ZON) in animal feeding, Preventive Veterinary Medicine 102:132–145.

D u a r t e, S., L i n o, C., P e n a, A. (2011): Ochratoxin A in feed of food-producing animals: An undesirable mycotoxin with health and performance effects, Veterinary Microbiology 154: 1–13.

EC (European Commision) (2003): Directive 2003/100/EC of 31 October 2003 amending Annex I to Directive 2002/32/EC of the European Parliament and of the Council on undesirable substances in animal feed. Official Journal, L 285: 33-37.

EC (European Commision) (2006a) Commision Recommendation of 17 August 2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding. 2006/576/EC, Official Journal, L 229: 7-9.

EC (European Commision) (2006b): Commision Regulation of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. 2006/401/EC, Official Journal, L 70: 12-34.

J a k š i ć, S., A b r a m o v i ć, B., J a j i ć I., Ž i v k o v - B a l o š, M., M i h a l j e v, Ž., D e s p o t o v i ć, V., Š o l i ć, D. (2012): Co-occurrence of Fumonisins and Deoxynivalenol in Wheat and Maize Harvested in Serbia. Bull. Environ. Contam. Toxical. 89: 615-619.

K a p e t a n o v, M., P o t k o n j a k, D., S t o j a n o v, I., Ž i v k o v – B a l o š, M., J a k š i ć, S. (2012): Importance of clinical and Pathological diagnostics of Mycotoxicosis in fattening turkeys caused by T-2 trichothecenes, 15th Interntaional Feed Technology Symposium. Proceedings: 304-309.

K u b e n a, L. F., E d r i n g t o n, T. S., H a r v e y, R. B., P h i l l i p s, T. D., S a r r, A. B., R o t t i n g h a u s, G. E. (1997): Individual and combined effects of fumonisin B_1 present in *Fusarium moniliforme* culture material and diacetoxyscirpenol and ochratoxin A in turkey poults. Poul. Sci. 76: 256-264.

M a c i o r o w s k i, G. K., H e r r e r a, P., J o n e s, F.T., P i l l a i, S.D., R i c k e, S.C. (2007): Effects on poultry and livestock of feed contamination with bacteria and fungi, Animal Feed Science and Technology 133: 109–136.

M o r g a v i, D. P., R i l e y, R.T. (2007): An historical overview of field disease outbreaks known or suspected to be caused by consumption of feeds contaminated with *Fusarium* toxins, Animal Feed Science and Technology, 137: 201–212.

P e t t e r s s o n, H. (2004): Controlling mycotoxins in animal feed. In: Magan, N., Olsen, M., Eds., *Mycotoxins in food*, Woodhead publishing limited, Cambridge, England, 262-304.

P a l i ć, T., R a j i ć, I., N i k o l i ć, Z o r a. (1994): Bolesti živne – poremećaji ishrane, Savez živinara Srbije. (Sr)

R - b i o p h a r m: Ridascreen enzyme immunoassay the quantitative analysis of mycotoxins. Date of acces: 15 December 2012 Available from: www.r-biopharm.de

Službeni Glasnik RS br. 4/2010: Pravilnik o kvalitetu hrane za životinje, čl. 99. (Sr)

SRPSKI standard (2011): Mikrobiologija hrane i hrane za životinje, Horizontalna metoda za određivanje broja kvasaca i plesni – Deo 2: Tehnika brojanja kolonija u proizvodima sa aktivnošću vode manjom od 0,95 ili jednakom 0,95 : SRPS EN ISO 21527-2:2011 / Institut za standardizaciju Srbije. (Sr)

B e a t e, *T.,* L e h m a n n a, I., N i e b e r b, K., A l t e n b u r g e r, R. (2007): Combined effects of mycotoxin mixtures on human T cell function, Toxicology Letters 170: 124–133.

A b d e l l a h, Z., M a ñ e s, J. (2009): Occurrence and legislation of mycotoxins in food and feed from Morocco, Food Control 20: 334–344.

A b d e l l a h, Z., M i g u e l S o r i a n o, J., C a r l o s M o l t o, H., M a n e s, J. (2007): Review on the toxicity, occurrence, metabolism, detoxification, regulations and intake of zearalenone: An oestrogenic mycotoxin, Food and Chemical Toxicology 45: 1–18.

КОРЕЛАЦИЈА ГРАНИЧНИХ ВРЕДНОСТИ ЛАБОРАТОРИЈСКИХ НАЛАЗА И КЛИНИЧКЕ СЛИКЕ МИКОТОКСИКОЗА

Игор М. Стојанов, Милош Ц. Капетанов., Дубравка В. Поткоњак, Милица М. Живков-Балош, Сандра М. Јакшић, Јасна З. Проданов Радуловић

Научни институт за ветеринарство „Нови Сад“, Руменачки пут 20, 21000 Нови Сад, Србија

Резиме

Анализа хране за животиње на присуство микотоксина и гљивица је захтев који је непходно испунити како би се обезбедила здрава и економична производња у сточарству. Могући проблеми настају у околностима када се јавља клиничка слика микотоксикоза код животиња а лабораторијски налаз указује да су добијене вредности испод граничних нивоа, односно у оквиру дозвољених вредности. Из наведених разлога предмет нашег истраживања је праћење присуства микотоксина и плесни у храни за животиње, као и приказ клиничке слике које се јавља код животиња које су конзумирале храну са дозвољеним вредностима ових агенаса према правилнику. Циљ рада је да истакне проблеме везане за корелацију клиничке слике болесних животиња и лабораторијских налаза и како их превазићи.

У периоду од једне године укупно је прегледано 176 узорака хране за животиње (потпуне смеше за тов подмлатка живине, кукуруза и производа од соје) на присуство плесни, 106 узорака на присуство микотоксина и опсервирано је 26 јата бројлера и ћурића. За испитивања су коришћене ИСО методе за изолацију плесни и ELISA тест за доказивање микотоксина. Јата су прегледана клиничким и патоморфолошким методама и одређивањем натуралних показатеља производње.

Истраживања су указала на присутан проблем, да клинички и патоморфолошки налаз у неким случајевима не мора бити у корелацији са лабораторијским налазом плесни и микотоксина у хранивима као и да дозвољене вредности присуства ових материја у неким случајевима не представљају сигурност за здравље животиња. Синергизам и кумулативно дејство микотоксина са једне стране и карактеристике појединих врста животиње и производних категорија са друге стране, могу створити специфичне околности које ће довести до појаве болести и онда када су задовољене вредности прописане правним регулативама.

КЉУЧНЕ РЕЧИ: клиничка слика, микотоксини, плесни, храна за животиње

ACKNOWLEDGMENT

This investigation was financially supported by the Ministry of Education, Science and Technological Development, Republic of Serbia, Project No. TR 031071.