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MYCOTOXIN LEVELS IN CORN AND FEED COLLECTED FROM INDONESIAN FEEDMILLS

Budi Tangendjaja[1] and Sri Rachmawati[2]

[1]Research Institute for Animal Production, PO Box 221 Bogor 16002
[2]Research Institute for Veterinary Science, Bogor, Indonesia

ABSTRACT

Mycotoxins are metabolites of fungi grown in agriculture products and have deleterious effect to animal at certain levels. Samples collection of corn and feed for mycotoxins analysis was done in three different periods, i.e: year 1982-1984, 2002 and 2004. The method of aflatoxin analysis used by Research Institute for Animal Production in the year 1982-1984 was Thin Layer Chromatography. In 2002, 12 major feedmills in Indonesia had been asked to collect 2 different corn samples (good and bad quality), that were analyzed for different mycotoxin levels using a commercial ELISA kit. In 2004, using ELISA kit that has been developed at the Research Institute for Veterinary Science, aflatoxin levels in corn and feed collected from Indonesian feedmills were measured. The survey was extended to measure aflatoxin levels in corn and feed submitted to the official laboratory of Directorate General of Animal Production. Result indicated that aflatoxin levels in corn collected in early 1980 were ranged from 88-200 ppb. Aflatoxin analysis of corn collected by feedmills in 2002 indicated that "good quality" corn has less mycotoxin than those of "bad" corn. Aflatoxin level of "good" corn ranged between 0-68 ppb while "bad" corn ranged between 2-115 ppb. Average fumonisin, ochratoxin, T2 toxin and vomitoxin of good corn were 0-6 ppb, 0-3.1 ppb, 0-10.1 ppb and 0-7.5 ppm respectively. Aflatoxin levels in corn collected in 2004 were ranged from 0-183 ppb while imported corn was 47-75 ppb. At same time, poultry feed produced from those feedmills contained aflatoxin between 0 – 175 ppm. From 134 samples of poultry feed collected from Jakarta areas contained aflatoxin between 0- 75 ppb. In conclusion, most of Indonesian corns have acceptable level of mycotoxins for poultry feed but few corn and feed samples have higher levels and may have a long term effect to animals.

Key words: Mycotoxin, aflatoxin, corn, feed, ELISA

INTRODUCTION

Mycotoxin is a metabolite product from certain fungus which is toxic to animal and human. It may cause deleterious effect such as reduction in growth rate, decrease feed intake, reduce egg production and hatchability, decrease immune response and at high level may cause mortality to animals. Mycotoxin often gets the blame for unexplainable field problems in animal production. In fact, the cause and effect is usually not very clear when mycotoxins are involved. The feed may not reveal presence of molds or toxins and problems may be related to other stressors and disease agents. Outbreaks may be seasonal during damp or hot weather when toxins are formed after raw materials

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have been received at the feed mill. If raw materials already have a low level of mycotoxins present when received, problems may by triggered by other stressors. The effect also depends on the age of the livestock, sex, species, stress status and the action or metabolism of the particular mycotoxin.

There are more than 300 known mycotoxins but only a few have been examined for toxic effects. Molds from the genera *Aspergillus, Penicillium and Fusarium* are notorious for infesting cereal grains and legumes and causing later havoc in livestock operations. Crop drought stress, frost damage, insects and nematode damage and rainy hot harvest weather all favor development of molds and mycotoxins in the field. Mycotoxin formation increase each step of the way as raw material are handled, subjected to changes in environmental conditions during transport and storage, feed manufacture and later storage in the animal production facility (Swick, 2004).

Although mycotoxin problems have been observed for many years, the problem became frequently discussed in recent year partly because of the development of technique to measure the mycotoxin content rapidly and increase the sensitivity and more awareness of human food for health.

In the past, identification and quantification of the various mycotoxins was tedious laboratory work and relied heavily on thin layer chromatography. Later, high-pressure liquid chromatography was also used to measure mycotoxin contamination. More recently, enzyme-linked-immuno-sorbent-assay kits have been developed to detect and quantify the various mycotoxins. Several kits are available in the market that can be used to measure mycotoxin level in qualitative or quantitative levels. Some of them have been approved by Grain Inspection Packers and Stockyards Administration (GIPSA) to be used in trade of agriculture products in USA.

In the past few years, several laboratories in the Southeast Asian region have analyzed and reported results of mycotoxin assays. Optimal Laboratory in the Philippines reported levels as high as 103 ppb for aflatoxin in corn and 650 ppb in corn barn. Aflatoxin in copra meal from the Philippines was found to be as high as 103 ppb as reported by the Philippine Coconut Authority. Romer Laboratories (2003) reported on 35 soybean samples from various origins. Over 50% of Argentine samples were found to be over the safety limit for zearaleone. Soybean meal samples were analyzed for T2 toxin and aflatoxin Optimal Laboratory in 2003 and found to be below the recommended allowable levels set by Khajarern.

Very little information available on Indonesian mycotoxin status, with majority on aflatoxin. Bahri *et al.*, 1994 reported that 98% of 86 poultry feed samples was content of Aflatoxin B_1 with the average of 98.3 ppb. Recent report (Bahri *et al.*, 2005) indicated that aflatoxin contamination in feed was less compare to the previous result. From limited number of samples, results indicated that 70% of 10 commercial feed samples collected from Lampung contain Aflatoxin B_1 in the range of 0-40 ppb with the average of 13.5 ppb, whereas all feed samples (18) collected from East Java content of Aflatoxin B_1 from 4.1-131.3 ppb, and average of 30.7 ppb.

This paper reported the mycotoxin level in corn and feed collected from the feedmills mainly from Java and Lampung where major feedmills are located. The result was compared with previous data collected in early 1980s.

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MATERIALS AND METHODS

Samples collection were performed 3 times, first collection was conducted at Research Institute for Animal Production in 1982-1984 for corn and rice bran that were submitted to analytical laboratory. The samples were analyzed for aflatoxin only based on Thin Layer Chromatography technique using Aflatoxin B_1 as standard. Quantification was performed based on serial standards under UV light. Second collection was conducted by asking 15 major feedmills in Indonesia to carry 0.5 kg samples of corn that classified to "good" and "poor" quality according to their specifications. The corn samples were analyzed using ELISA kit from Neogen Co. (Agriscreen). Several mycotoxins were analyzed included total aflatoxin, fumonisin, ochratoxin, T2 toxin and Vomitoxin. Third collection of samples were conducted for poultry feed and corns collected by 2 feedmills in Java, the samples submitted to Balai Pengujian Mutu Pakan Ternak- BPMPT of Directorate General of Animal Production in Bekasi and the samples collected by Balai Pengujian dan Penyidikan Veteriner (BPPV) Jogyakarta. All samples from the third collection were analyzed at their own laboratories using the ELISA technique for Aflatoxin developed at Research Institute for Veterinary Science and limit detection of the method is 0.3 ppb.

RESULTS AND DISCUSSION

Table 1. Aflatoxin level (analyzed by TLC) in rice bran and corn received at RIAP from 1982 to 1984.

Feed Ingredients	Year	No. of samples	Aflatoxin B1	
			Average Level (ppb)	std.dev.
Rice bran	1982	21	<2	
	1983	25	2	5
	1984	19	2	5
Corn	1982	78	180	190
	1983	25	88	130
	1984	25	200	177

Earlier result on the analyses of aflatoxin in corn and rice bran collected by Research Institute for Animal Production for 3 years from 1982 to 1984 is presented in Table 1. Almost all corn collected during that time were contaminated by aflatoxin at various level, with the average level between 88-200 ppb, depending upon the year. The actual level was varied significantly as indicated by high standard deviation. During that time, it resulted in difficulty to produce duck feed at acceptable limit of aflatoxin. As generally recognized that duck is less tolerance to aflatoxin compare to chicken.

Unlike corn, rice bran contamination with aflatoxin was significantly less than that for corn. The average levels were only 2 ppb and this number is close to detection limit of aflatoxin using Thin Layer Chromatography technique. The variation of aflatoxin level was also less compare to that of corn. Rice barn is widely used as major feed ingredient for feeding ducks and pig, for some farmers, rice bran was used as sole diet for pig.

Survey performed by Research Institute for Veterinary Science in 1985-1986 (Widiastuti *et al.*, 1988) indicated that the aflatoxin contamination in corn was affected by the time period in which the corn is harvested. Corn harvested during wet season

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(between December to July) has higher aflatoxin level compare to corn in dry season. The level of aflatoxin was reflected in similar pattern in feed. Of course the aflatoxin level in feed was less than that level in corn. Corn was the major contribution of aflatoxin in Indonesian feed and corn usage in poultry diet may reach >50% of final ration. Poultry feed is produced at >85% of total feed production in Indonesia and corn is the major energy source. During that period, Indonesia had imported certain amount of peanut meal as protein source from South Asia and much peanut meal was contaminated by aflatoxin. However the usage of peanut meal in poultry diet was normally limited because most of the protein source was coming from soybean meal.

Corn quality variation is common problem from the feedmill. Corn received by feedmill is normally classified into several classes based on the numerous quality measures. Most of feedmill rejected poor quality corn if it is below the standard set by the feedmill. In 2002 different quality grade of corn from feedmill was analyzed for mycotoxin level and the result of analyses is presented in Table 2.

Good quality corn had less Aflatoxin and Ochratoxin levels compare to poor quality corn but Fumonisin, T2 toxin and Vomitoxin level is similar. Most of corn collected by feedmill were originated from the local corn therefore major type of mycotoxin detected would be Aflatoxin and Ochratoxin that derived from Aspergillus sp. This fungus is common species for the tropics while for temperate areas, type of fungus grown in corn would be from Fusarium sp. that produce T2 toxin or fumonisin.

Table 2. Different mycotoxin level of corn classified "good" and "poor" quality submitted from 12 different feedmill.

	Corn quality	Average	Min	Max	Std.Dev
Aflatoxin	"good"	24.6	0.4	67.8	26.2
(ppb)	"poor"	59.4	1.9	115.2	34.4
Fumonisin	"good"	0.7	0.0	6.0	1.7
(ppb)	"poor"	0.8	0.0	3.4	1.2
Ochratoxin	"good"	1.0	0.0	3.1	1.2
(ppb)	"poor"	3.9	0.0	19.9	6.2
T2 Toxin	"good"	3.1	0.0	10.1	3.4
(ppb)	"poor"	3.6	0.0	14.2	4.5
Vomitoxin	"good"	0.8	0.0	7.5	2.5
(ppm)	"poor"	0.7	0.0	5.8	1.9

Aflatoxin level in good quality corn is less than 25 ppb with the maximum level is < 70 ppb but in poor quality corn the highest level may reach above 100 ppb as the limit of Aflatoxin set by the National Standard for Indonesia. There is no standard has been set for other mycotoxin besides Aflatoxin.

Ochratoxin level of both good and poor quality corn is also considered low, level for good corn is 1 ppb while for poor quality corn is only 4 ppb. The maximum level for poor quality corn is only 20 ppb, therefore ochratoxin may not be problem in the feedmill. Food and Drug Administration from USA suggested that the acceptable limit for Ochratoxin for poultry is 250 ppb, therefore both quality of corn is still acceptable to be used for poultry feed as far as the ochratoxin is concerned. Ochratoxin, however, has been reported to be major problem in the Philippines (Reas, personal communication) 3 years ago when the Philippines feedmill received poor quality soybean meal from South America. Very little information is available for ochratoxicosis in Indonesia.

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Other mycotoxin such as T2 toxin, fumonisin and vomitoxin level in this study was less than aflatoxin as expected. Both good and poor quality corn did not have significant level of these types of mycotoxins. Both type of corn is not different in level of this mycotoxin. The corn grading system in the feedmills is not based on the mycotoxin levels; several feedmills adopt the grading system based on other quality measures such as density, broken corn, foreign materials, damage kernel etc., therefore it can be concluded that poor quality corn has less T2 toxin, fumonisin or vomitoxin. There is however an indication that poor quality corn has more aflatoxin rather than good quality corn.

With the development of ELISA technique by Research Institute for Veterinary Science in 2003, the cost of analyses for Aflatoxin become more economical compare to the ELISA kit obtained from overseas. The aflatoxin level in corn and feed from two feedmill (A and B) had been measured and the result is reported in Table 3.

Aflatoxin level of corn in feedmill A is higher than corn in feedmill B. Average level of aflatoxin in corn in feedmill A is 68.7 ppm and the level is varied between 2-165 ppm. This result is in contrast with feedmill B that average aflatoxin level is only 0.3 ppb with maximum level is less than 33 ppb. The higher level of aflatoxin in corn is also reflected with aflatoxin level in the feed produced by those feedmills. However it is not necessary that the level of aflatoxin in feed is around 50% of the aflatoxin content in corn. The aflatoxin development may occur during manufacturing of feed or during storage after the feed has been made. However the maximum level of aflatoxin in feed from feedmill B is still below the acceptable limit according to Indonesian National Standard (50 ppb). Unlike feedmill B, the aflatoxin level in feed produced by feedmill A may reach above the acceptable limit. However it is important to note that higher level of aflatoxin in feedmill A might be due to the poor quality samples that purposely send for the analyses. From limited number of samples (3) of corn import from the feedmill, aflatoxin level of imported corn from Thailand was 47-75 ppb, while the local corns were ranged from 0-183 ppb.

Result of analyses of corn submitted to BPMPT or collected from a poultry shop by BPPV indicated that corn in poultry shop has a higher aflatoxin level. Corn submitted to BPMPT may come from feedmill that submit the sample for the analyses. The samples may be selected to obtain a better quality or probably the poor quality sample that cause the problem to animal. Therefore it is not necessary that corn from feedmill has a better aflatoxin level compare to corn from poultry shop, although feedmill should normally have a better quality assurance system. But of course it also depends very much upon the feedmill or poultry shop.

The level of aflatoxin in feed is very much depending upon the feedmills; result in Table 3 indicated that feedmill A had a higher aflatoxin level than that from feedmill B.

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Table 3. Aflatoxin level of corn and feed obtained from feedmills and samples submitted to Balai Pengujian Mutu Pakan Ternak (BPMPT), Balai Pengujian dan Penyidikan Veteriner (BPPV) (ppb).

	No. sample	Average	Minimum	Maximum	Std.Dev
Corn					
Feedmill A	19	68.7	1.8	165.0	50.7
Feedmill B	18	3.2	0.3	32.1	8.7
BPMPT (1)	10	5.5	2.2	11.2	5.0
BPPV	11	55.9	46.1	65.6	13.8
Feed					
Feedmill A	11	55.1	1.0	175.1	62.8
Feedmill B	15	4.5	0.3	26.0	6.3
BPMPT (1)	26	19.5	0.3	123.3	30.9
BPPV	11	10.2	2.0	38.0	10.6
BPMPT (2)	123	26.8	0.3	141.6	28.8

(1),(2) indicate 1st and 2nd collection

There were few feed samples contained aflatoxin > 175 ppb. The higher average level of aflatoxin in feed from feedmill A was also reflected by higher variation in aflatoxin levels. It seems that this feedmill has not conducted a strict quality assurance system and the higher level of aflatoxin in feed was related with higher level of aflatoxin in their corn. Majority of aflatoxin in feed was derived from corn as expected.

In an extensive series of assays conducted by Khajarern in 2001, 2002 and 2003, a problem with high levels of aflatoxin and T2 was found in corn in 2001 but not 2002 or 2003. Zearaleone levels above allowable were found in Argentine and Brazilian soybean meal in 2002 and 2003 and in Argentine soybean meal 2001. Levels below allowable were reported for U.S. soybean meal in all years tested. Levels of zearalenone in imported shipments of Argentine soybeans were reported to be as high 728 ppb in 2004 as measured by a non-commercial laboratory in a soybean crushing plant (Swick, 2004).

In conclusion, aflatoxin contamination in corn from Indonesia was less in recent year compared to the result in early 1980s. Poor quality corn based on feedmill specifications has higher mycotoxin levels compare to good quality corn and Aflatoxin is still the major concern relative to other mycotoxins (fumonisin, ochratoxin, T2 toxin and Vomitoxin). Depending upon the feedmill, aflatoxin levels in corn and feed were varied and it seemed that strict quality control in a feedmill would improve the aflatoxin contamination. However the average levels found in feed (~55 ppb) might not cause major detrimental effect to chicken but there were few samples had aflatoxin level > 175 ppb and may have longtime effect to chicken.

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REFERENCES

Bahri, S., Yuningsih, R. Maryam dan P. Zahari.1994. Cemaran aflatoksin pada pakan ayam yang diperiksa di laboratorium toksikologi Balitvet tahun 1988-1991. Penyakit Hewan 47:39-42

Bahri, S., R. Maryam dan R. Widiastuti. 2005. Cemaran aflatoksin pada bahan pakan dan pakan di beberapa daerah Propinsi Lampung dan Jawa Timur. JITV 10(3):236-241

Swick, R.A. 2004. Managing mycotoxin menace. Proc. US Agricultural Cooperators Conference. Bali, August 17-20, 2004. ASA Singapore

Widiastuti, R., S. Maryam, B.J. Blaney, Salfina and D.R. Stoltz. 1988. Corn as a source of mycotoxins in Indonesian poultry and the effectiveness of visual examination methods for detecting contamination. Mycopathol. 102:45-49