EFFECT OF DIFFERENT LABORATORY STORAGE CONDITIONS OF ANIMAL FEED SAMPLES ON MYCOTOXIN DETECTION: A CASE STUDY

SUHAIMI D.[1]*, WAN SYAHIDAH H.[1], LILY SUHAIDA M.S.[2] AND TERJUDDIN G.[1]

1 Veterinary Public Health Laboratory, Department of Veterinary Services, Bandar Baru SalakTinggi, 43900 Sepang, Selangor

2 Veterinary Research Institute, 59, Jalan Azlan Shah, 31400 Ipoh, Perak

* Corresponding author: suhaimidollah@dvs.gov.my

ABSTRACT. The mycotoxin test data base (2005–2009) of the Veterinary Public Health Laboratory (VPHL), Department of Veterinary Services, Malaysia (DVS) showed that there was a significant increase (51%) of overall aflatoxin occurrences in various types of animal feed samples, especially those formulated from agricultural by-product, for the year 2008. A study was thus conducted to investigate if there could be some sources of mycotoxin contamination during the period of sample handling. Three different laboratory storage conditions were chosen for the study within a period of fourteen days i.e 4 °C, room temperature (in light) with mean relative humidity of 62.5%, and room temperature (in dark) with mean relative humidity of 55.7%. The observations showed that there were no significant differences in total aflatoxin, zearalenone, and fumonisin detections in all storage conditions as screened by the ELISA technique. However 11–50% inconsistencies of the mycotoxin concentrations detected were observed within the samples.

Keywords: mycotoxin, animal feed, storage condition

INTRODUCTION

Mycotoxins are toxic and/or carcinogenic compounds produced by many fungal species that grow on various agricultural commodities (Cullen and Newberne, 1994). These commodities can be contaminated either in the field or in storage. Many countries regulate the maximum level of mycotoxins that can occur in foods and feeds. Most regulations are concerned with controlling aflatoxin because it is considered the most toxic and carcinogenic of the naturally occurring mycotoxins. The FAO (1995) has suggested that it is important to detect and quantify the mycotoxin concentration in foods and feeds destined for human and animal consumption. Studies on a wide variety of agricultural products indicate that the sampling step is usually the largest source of variability associated with the mycotoxins test procedure, especially for small samples sizes. In research, quality

assurance, and regulatory activities, correct decisions concerning the fate of commercial lots can only be made if the mycotoxin concentration in the lot can be determined with a high degree of accuracy and precision. The objective of this study was to observe the effect of different storage conditions of animal feeds for mycotoxin analysis within a certain period of time of sample handling in the laboratory.

MATERIALS AND METHODS

Sample collection and testing

Twenty samples of various types of animal feeds (Table 1) were randomly selected from the samples received by the laboratory for routine mycotoxin analysis in the month of November 2010. The weights of the samples were between 0.25-1.0 kg. All samples were ground and homogenized using Ultra centrifugal Mill, Retsch with sieve size of 5 mm. and tested for their moisture contents and total nitrogen contents respectively as per method by FAO Animal Production and Health Manual, 2011. The samples were then screened for aflatoxin, fumonisin, and zearalenone by using enzyme-linked immunosorbent assay (ELISA) methods: (1) Ridascreen Aflatoxin Total, Damstadt, Germany, (2) MaxSignal Fumonisin ELISA Test Kit, and (3) MaxSignal Zearalenone ELISA Test Kit, both from BIOO Scientific Corp. The samples were divided into three groups, each weighing 20 g and placed in three different storage conditions i.e. 4 °C (chiller), room temperature (in dark), and room temperature (in light). Each sample was kept in a 50 ml clean glass tube. After a 14-day trial period, all the samples were screened again for aflatoxin, fumonisin, and zearalenone by the same ELISA methods. For the room temperature storage condition (in light), samples were placed on the working laboratory bench, and for the room storage condition (in dark), samples were wrapped with aluminium foil and kept in the laboratory drawer. Relative humidity and room temperature were recorded each day using laboratory hygrometer (1–90% scale) and laboratory thermometer (-10 °C–120 °C scale).

Data analysis

The concentrations of mycotoxins were calculated using the software provided accordingly by the ELISA kits suppliers. These concentrations were classified into 6 categories namely ND (not detected), <20 ppb, 20–100 ppb, 100–200 ppb, 200–300 ppb and >300 ppb. The cut-off points of quantitation were 5 ppb for aflatoxin and fumonisin; and 0.5 ppb for zearalenone. All data collected were qualitatively analysed using Microsoft Excel. Inconsistencies of the concentration detected between each storage condition of the samples for each type of mycotoxin, as compared to the base line concentration (Day 0), were presented in percentages. Student *t* test was used where appropriate.

RESULTS

TABLE 1. Moisture and Crude Protein contents (%) of animal feed samples used to determine the effect of different laboratory storage conditions on mycotoxin detection

Sample No.	Feeding Stuffs	Moisture content (%)	Crude Protein (%)
1	PKC (Palm kernel cake)	(not available)	(not available)
2	Puppy dog food	4.3	37
3	Adult dog food	6.8	27.7
4	Soybean hull pellet	11.4	3
5	Broiler starter	7.2	12.3
6	Broiler starter	21.7	10.5
7	Cattle pellet	8.7	37
8	Cattle pellet	8	16.6
9	Bean	1	6.2
10	DCP (Dairy cattle pellet)	1.6	15.4
11	PKP(Palm kernel pellet)	1.2	15.4
12	DCP (Dairy cattle pellet)	6	14.8
13	PKP(Palm kernel pellet)	6.6	14.8
14	Soybean hull pellet	7.8	12.3
15	Grower pellet	10.1	12.3
16	PKC (Palm kernel cake)	10.5	12.3
17	Broiler pellet	7.5	16.6
18	Rice	8.1	12.3
19	Maize	7.9	12.3
20	Maize	8.8	13

TABLE 2. Detection of aflatoxin total (ppb) after 14 days in three different storage conditions

		AFLATOXIN TOTAL (ppb)		
Sample Type	**0 Day**	**4C**	**Room T(Dark)**	**Room T(Light)**
PKC	***ND***	*20-100*	20-100	ND
Puppy dog food	***ND***	ND	ND	ND
Adult dog food	***ND***	ND	ND	ND
Soybean hull pellet	***ND***	<20	ND	ND
Broiler starter	***ND***	ND	ND	ND
Broiler starter	***<20***	<20	*ND*	*ND*
Cattle pellet	***<20***	<20	<20	*ND*
Cattle pellet	***<20***	<20	ND	*ND*
Bean	***ND***	ND	ND	ND
DCP	***20-100***	20-100	20-100	20-100
PKP	***ND***	ND	<20	ND
DCP	***<20***	<20	<20	<20
PKP	***ND***	ND	ND	ND
Soybean hull pellet	***ND***	ND	ND	ND
Grow er pellet	***<20***	20-100	<20	20-100
PKC	***20-100***	20-100	*<20*	*<20*
Broiler pellet	***ND***	<20	ND	ND
Rice	***ND***	ND	ND	ND
Maize	***ND***	ND	ND	ND
Maize	***ND***	ND	ND	ND

TABLE 3. Detection of fumonisin (ppb) after 14 days in three different storage conditions

		FUMONISIN (ppb)		
Sample Type	**0 Day**	**4C**	**Room T (Dark)**	**Room T (Light)**
PKC	***ND***	>300	>300	>300
Puppy dog food	***>300***	*100-200*	>300	*100-200*
Adult dog food	***200-300***	>300	*100-200*	>300
Soybean hull pellet	***ND***	100-200	100-200	100-200
Broiler starter	***>300***	*20-100*	>300	*20-100*
Broiler starter	***20-100***	100-200	*<20*	*<20*
Cattle pellet	***200-300***	200-300	>300	>300
Cattle pellet	***200-300***	>300	>300	*100-200*
Bean	***ND***	100-200	20-100	200-300
DCP	***200-300***	200-300	200-300	>300
PKP	***ND***	20-100	ND	100-200
DCP	***100-200***	100-200	200-300	>300
PKP	***ND***	20-100	200-300	?
Soybean hull pellet	***ND***	<20	200-300	?
Grow er pellet	***20-100***	20-100	100-200	100-200
PKC	***ND***	>300	>300	200-300
Broiler pellet	***>300***	>300	*ND*	*ND*
Rice	***ND***	ND	<20	ND
Maize	***<20***	<20	<20	ND
Maize	***20-100***	*<20*	20-100	*ND*

TABLE 4. Detection of zearalenone (ppb) after 14 days in three different storage conditions

		ZEARALENONE (ppb)		
Sample Type	**0 Day**	**4C**	**Room T (Dark)**	**Room T (Light)**
PKC	***<20***	*ND*	*ND*	*ND*
Puppy dog food	***20-100***	20-100	20-100	20-100
Adult dog food	***20-100***	20-100	20-100	20-100
Soybean hull pellet	***20-100***	20-100	*<20*	*<20*
Broiler starter	***20-100***	20-100	20-100	*ND*
Broiler starter	***<20***	*ND*	<20	*ND*
Cattle pellet	***<20***	<20	<20	<20
Cattle pellet	***<20***	<20	*ND*	*ND*
Bean	***ND***	ND	ND	ND
DCP	***<20***	*ND*	*ND*	*ND*
PKP	***<20***	*ND*	*ND*	*ND*
DCP	***<20***	*ND*	*ND*	*ND*
PKP	***<20***	*ND*	*ND*	*ND*
Soybean hull pellet	***<20***	*20-100*	*20-100*	*ND*
Grow er pellet	***20-100***	20-100	*ND*	*ND*
PKC	***ND***	ND	ND	ND
Broiler pellet	***<20***	*ND*	*ND*	*ND*
Rice	***ND***	ND	ND	ND
Maize	***ND***	ND	ND	ND
Maize	***ND***	ND	ND	ND

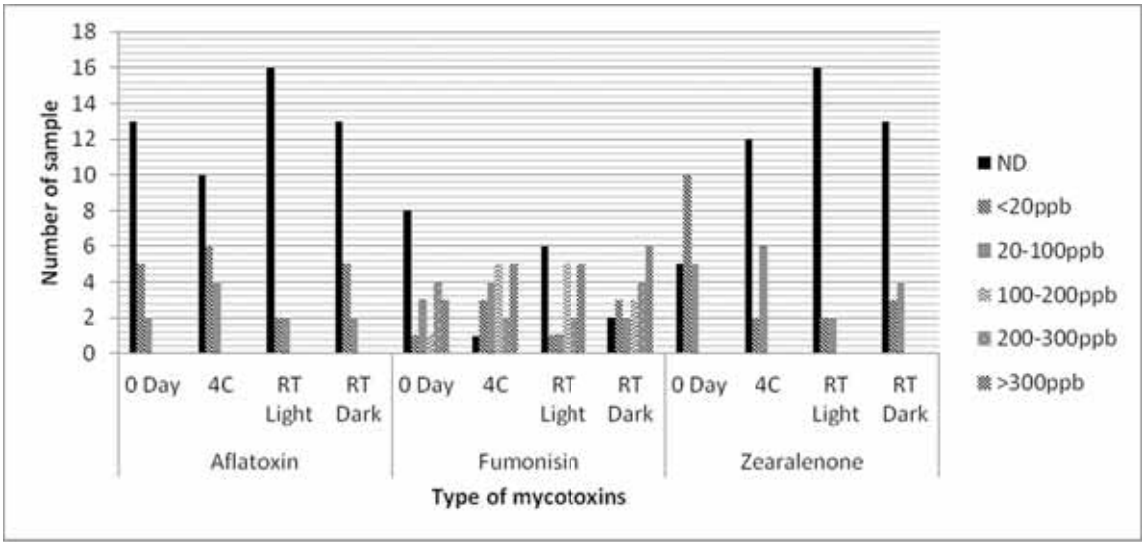

FIGURE 1. Summary of mycotoxin detections after 14 days of different storage conditions

DISCUSSION

Most fungi are aerobic and are found almost everywhere in extremely small quantities due to the minute size of their spores. They consume organic matter wherever humidity and temperature are sufficient. Where conditions are right, fungi proliferate into colonies and mycotoxin levels become high. The reason for the production of mycotoxins is not yet known; they are neither necessary for growth nor for the development of the fungi (Fox EM, Howlett BJ, 2008). Because mycotoxins weaken the receiving host, the fungus may use them as a strategy to improve the environment for further fungal proliferation. The production of toxins depends on the surrounding intrinsic and extrinsic environments and the toxins vary greatly in their severity, depending on the organism infected and its susceptibility, metabolism, and defense mechanisms (Hussein HS, Brasel JM, 2001). In this study, the average room temperature of 'light' storage and 'dark' storage were 28.9 °C and 26.9 °C, and the mean relative humidity were 62.5% and 55.7%. The temperatures were assumed to be optimum for growth of certain aflatoxins. The relative humidity was relatively high due to the rainy season. The percentages of inconsistency of concentration detected were 11.7% for aflatoxin total as occurred in PKC, broiler pellet, and cattle pellet (Table 2); 20% for fumonisin as occurred in puppy dog food, broiler pellet, and maize (Table 3); and 50% for zearalenone as occurred in PKP, DCP, and soybean hull pellet (Table 4) when compared to the concentrations at Day 0 respectively. The inconsistent pattern of growth of each type of the mycotoxin in the sample of different storage conditions could have been effected by improper sampling procedures (Figure 1). Moisture is the most important factor in determining how rapidly molds will grow in feeds. In this study, it is not known whether there is a correlation between moisture and crude protein contents in the samples used. In a separate study conducted by this laboratory, a number of 218 samples of various animal feeds were tested for correlation between moisture content (mean = 6.2%, SD=2.68) and protein content (mean = 19.4%, SD = 12.94) showed that there was no significant correlation ($p>0.5$). The focus of this discussion is to emphasize on the aspect of sampling and handling procedures because it is imperative that these procedures are accurate, since the results are the basis for deciding whether or not to use a given lot of feed or feed ingredient. Mycotoxins are not evenly distributed in grain or mixed feeds, taking a feed or grain sample which will give a meaningful result in mycotoxin analyses is difficult . It is said that nearly 90% of the error associated with mycotoxin assays can be attributed to how the original sample was collected (Whitaker TB, Slate AB, 1995). Study on effects of water activity and types of animal feeds on specific mycotoxin growth will be further conducted in the

future to identify the variability associated with mycotoxin analysis.

CONCLUSION

The sampling, sample preparation, and analytical steps of the mycotoxin test procedure contribute to the variability of mycotoxin test results. To achieve a more precise estimate of the true lot concentration, the total variability of the test procedure should be reduced i.e. by decreasing the variability associated with each step involved.

REFERENCES

1. Cullen JM, Newberne PM (1994). Acute hepatotoxicity of aflatoxin, in The Toxicology of aflatoxin (D.L. Eaton & JD Goopman Eds): 3-26
2. Food and Agriculture Organization (1995). Worldwide Regulations for Mycotoxins 1995. FAO Food and Nutrition Paper 64. FAO Vialedella Terme di Caracalla, 00100, Rome , Italy. :43
3. Food and Arriculture Organization. Animal Production and Health Manual. Quality Assurance for Animal Feed Analysis Laboratories, 2011.
4. Fox EM, Howlett BJ (2008). "Secondary metabolism: regulation and role in fungal biology". Curr. Opin. Microbiol. 11 (6): 481–7.
5. Hussein HS, Brasel JM (2001). "Toxicity, metabolism, and impact of mycotoxins on humans and Ecology 167 (2): 101–34.
6. Whitaker TB, Slate AB and Johansson A.S. (1995). Sampling feeds for mycotoxin analysis. US Department of Agriculture, Agriculture Research Service, North Carolina State University, Raleigh, North Carolina, USA.