PRELIMINARY STUDY ON THE EFFECTS OF LABORATORY STORAGE CONDITIONS FOR AFLATOXINS TESTING

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ABSTRACT

This study was conducted to observe the effects of different laboratory storage conditions for aflatoxins testing. Aflatoxins testing were carried out by screening method using ELISA technique. Three types of storage conditions of feeds in the laboratory for aflatoxins testing have been conducted. Three storage conditions of different types of feeds were chill at 4°C, light room temperature (mean = 28.9°C) with humidity mean 62% and dark room temperature (mean 26.8°C) with humidity mean 55% for a duration of 14 days. Twenty feeds sample that were sent to Veterinary Public Health Laboratory (VPHL) were randomly selected in this experiment. It involves formulated feeds, grains and by products feeds. In this experiment, the screenings of total aflatoxins were done at 0 day and after 14 days of different storage conditions. In this study, 65% of selected samples screened were not contaminated on 0 day whereas 25% of samples were detected < 20ppb and 10% of samples were detected >20ppb on 0 day. After 14 days, the results showed that dark environment (room temperature) increase the levels of aflatoxins. The increasing of aflatoxins in dark environment (room temperature) were 33.3% compared in light environment (room temperature) 11.1% and chill (4°C) was 4.4%. In light environment (room temperature, mean = 28.9°C), 55.6% of contaminated samples showed decreased level of aflotoxin compared in chill (4°C) and dark environment (22.2% respectively). As a conclusion, aflatoxin produced more easily in the dark than in light condition. To ensure the aflatoxin levels do not continue to increase during storage in the laboratory, temperature and humidity of laboratory should be in minimum range for the growth of mycotoxins. It is due to ensure testing reports are reliable to customer.

Keywords: aflatoxins; storage conditions; ELISA

INTRODUCTION

Mycotoxins are toxic secondary metabolites produced by fungi growing on crops in the field, during handling and in storage. They enter the animal production system via feed (concentrate, silage or forage) or via bedding. Mycotoxins negatively affect animal performance, animal health and product quality. Thus mycotoxin control is crucial for production economics, animal welfare, and product quality and food safety reasons (Zinidine *et al.,* 2007). In general, most fungi need at least 1-2% oxygen and usually grow at temperatures between 20 and 30°C. It is important to note that if the grain is at high temperature at harvest, it can maintain that high temperature for several days or weeks after harvest unless the storage facility has cooling capabilities. As temperature and moisture levels are key factors for fungal growth and subsequent mycotoxin production, the climate plays a key role in the occurrence of mycotoxins.

MATERIAL AND METHODS

Sample collection

Twenty feeds sample from different sources that were sent to VPHL were randomly selected in this experiment. There were formulated feeds, grains and feeds by products.

Sample preparation

All samples were grounded and homogenized using Ultra Centrifugal Miller, Retsch, sieve size 5mm. The homogenized samples then divided into four portions. One portion was used as a control, one portion kept in chilled, one portion was kept at room temperature in dark environment and the remaining portion was kept at room temperature in light environment for duration of 14 days.

Moisture analysis

All samples were oven dried at 4 hours permanent at 103°C. The moisture content of each sample is expressed as the percentage, by weight, of the dry sample.

Aflatoxins analysis

All samples were extracted using 70% of methanol, filtered and diluted before proceed to ELISA Test (Aflatoxin Total, ELISA Kit), RBiopharm. The aflatoxins total was measured photometrically at 450nm using ELISA reader, Anthos 2020.

RESULTS AND DISCUSSION

In this study, 65% of selected samples screened were not contaminated on 0 day (control) whereas 25% of samples were detected < 20ppb and 10% of samples were detected >20ppb on 0 day. After 14 days, they showed that the levels of aflatoxins had increased in the dark environment (room temperature). The increasing level of aflatoxins in dark environment (room temperature=26.8°C; humidity 55%) is 33.3% compared in light environment (room temperature= 28.9°C; humidity=62%) 11.1% and in chill (4°C) was 4.4%. Several authors have described the chemical changes of aflatoxins under the influence of light. According to Joffe and Lisker (1969), *Aspergillus flavus* can produce more aflatoxin in the dark than in the light. They also mentioned that the formation of aflatoxins was optimal in the acid range. In this study, the temperature of dark environment was 26.8°C, which is an ideal condition for mould development. *Aspergillus flavus* easily produces aflatoxins at approximately 25°C. In light environment (room temperature, mean = 28.9°C), 55.6% of contaminated samples showed decrease level of aflatoxin compared in chill (4°C) and dark environment (22.2%) respectively. According to Schindler *et al.* (1967), temperature, pH value, and light are important factors influencing the production of aflatoxins by *Aspergillus flavus*. Moisture analysis are also included in this study to determine whether the sample moisture affect the growth of aflatoxins during storage. From the data, all samples have moisture content around 5-12%. This range does not cause the fungi growth. Normally, in storage conditions fungi grow at 13-18% moisture. The relationship between the moisture content of stored feeds and the relative humidity of the air profoundly affects mold growth and feeds spoilage.The optimal relative humidity for *Aspergillus flavus* growth is 85-100%. In this study, the relative humidity was around 55-62%. This range does not cause the fungi growth.

Table 1: The effects of aflatoxins before and after 14days in three storage condition

	Samples	Moisture content (%)	Aflatoxins Total (ppb)			
			0 day	After 14 days		
				Chill (4°C)	Dark environment (room temperature: 26.8°C;humidity:55%)	Light environment (room temperature: 28.9°C;humidity:62%)
1	PKC B	10.80	ND	>20	<20	ND
2	Puppy dog feed	10.05	ND	ND	ND	ND
3	Adult dog feed	9.80	ND	ND	ND	ND
4	Soybean hull pellet A	9.96	ND	<20	<20	ND
5	Broiler starter A	10.14	ND	ND	ND	ND
6	Broiler starter B	11.31	<20	<20	ND	ND
7	Cattle pellet A	8.40	<20	<20	<20	ND
8	Cattle pellet B	8.43	<20	<20	ND	ND
9	Kacang dal	7.0	ND	ND	ND	ND
10	Dairy Cattle Pellet A	5.86	>20	<20	>20	<20
11	PKP	6.56	ND	<20	<20	ND
12	Dairy Cattle Pellet B	5.65	<20	<20	<20	<20
13	PKP	6.57	ND	ND	ND	ND
14	Soybean hull pellet B	7.32	ND	ND	ND	ND
15	Grower pellet	9.08	<20	>20	<20	>20
16	PKC B	10.82	>20	<20	>20	<20
17	Broiler starter C	10.50	ND	ND	ND	ND
18	Rice	11.98	ND	ND	ND	ND
19	Maize A	11.99	ND	ND	ND	ND
20	Maize B	12.39	ND	ND	ND	ND

ND= Not Detected

CONCLUSION

As a conclusion, the finding of this study was aflatoxin produced more easily in the dark than in light condition. To ensure the aflatoxin levels do not continue to increase during storage in the laboratory, temperature and humidity of laboratory should be in minimum range for the growth of mycotoxins. This is to ensure testing reports are reliable to customers. Since aflatoxins growth may be affected by several factors, it is recommended that a more comprehensive study be conducted involving more samples with using confirmatory method, liquid chromatography.

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