



SCREENING OF DISTILLER'S DRIED GRAINS WITH SOLUBLES (DDGS) FOR TOTAL AFLATOXINS USING THIN LAYER CHROMATOGRAPHY (TLC)

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ABSTRACT

Distillers Dried Grains with Solubles (DDGS), a by-product of alcohol industry are an important source of domestic animal feed due to high nutritive value. The presence of mycotoxins in DDGS limited its use as animal feed. A total number of 115 DDGS feeds samples were collected and analysed for total Aflatoxin (*i.e.* Aflatoxin B₁, B₂, G₁ & G₂) by Thin Layer Chromatography (TLC) as per AOAC method. The average moisture percentage of DDGS ranges from 8-12%. The results showed that the number of contaminated samples in DDGS is highest with Aflatoxin B₁ (27.82 %, range of 10-40 ppb) followed by Aflatoxin B₂ (20.86 %, 5-20 ppb) and both Aflatoxin G₁ & G₂ (5%, range of 5 ppb each). Because of the confirmed threat of toxic compounds, it is recommended that every batch of DDGS reaching farmsteads is examined in reference laboratories for the presence of mycotoxins.

KEY WORDS: Aflatoxin, DDGS, Thin Layer Chromatography.

INTRODUCTION

Distillers Dried Grains with Solubles (DDGS) is a by-product of the alcohol industry produced by the fermentation process of some cereal grains such as maize, barley, wheat, sorghum and rye in dry mill ethanol plants (Aines *et al.*, 1986 and Abd El-Hack, 2015). DDGS contain high levels of protein, fiber, minerals and vitamins, are an important source of animal feed and may be used to enrich human foods (Khatibi *et al.*, 2014). The presence of mycotoxins in DDGS limited its utility to use as animal feed. The increased level of a given mycotoxin in DDGS was reported to be approximately 3 times as high as the level in the grain that could limit its application in the animal feed industry (Zhang & Caupert, 2012). This poses a risk to animal health and consequently human health due to metabolites of mycotoxins that can be transferred. The tremendous growth in the fuel-ethanol industry has been accompanied by concomitant growth in the production of DDGS, simultaneously the potential for using DDGS as animal feed is great.

Currently more than 300 mycotoxins are known. Among them, Aflatoxin B₁ is the most potent natural carcinogen classified as class I by IARC and is associated with both toxicity and carcinogenicity in human and animal populations.

Thin layer chromatography (TLC), a method still broadly used for quantitative and semi-quantitative measurements of mycotoxins with detection by fluorodensitometry or visual procedures. TLC based on silica gel, F₂₅₄ fluorescent silica gel or silica gel impregnated with organic acid has been reported to be applied for detection of common mycotoxins (aflatoxins, citrinin, fumonisin)

(Lin *et al.*, 1998). Thus the present study was designed to rapidly screen the presence of Aflatoxin B₁, B₂, G₁ & G₂ in Distillers Dried Grains with Solubles (DDGS) using thin layer chromatography (TLC).

MATERIALS & METHODS

A total number of 115 Distillers Dried Grains with Soluble (DDGS) feeds samples were collected randomly from different parts of Chennai, Tamil Nadu. Extraction of Aflatoxin B₁, B₂, G₁ & G₂ was done as per AOAC method with screening and quantification by TLC.

Determination of moisture content

About 500 gms of samples were collected and were finally ground (20 mesh particle size) using explosion proof laboratory blender. Then, the moisture content of each of the sample was determined as per the method described by Ubwa *et al.*, 2012.

Extraction of aflatoxin

Sample analysis was carried out by taking a known quantity (25g) of the powdered sample in a 250 ml flask and treating with 19 ml distilled water and 106 ml acetone. This mixture was shaken for 30 minutes at 200 rpm on a shaker. It was then filtered through Whatman paper (No.1). To the 75 ml of filtrate, 1.5g of cupric carbonate was added. Another solution of 85 ml of 0.2N NaOH and 15 ml of 0.4M FeCl₃ was prepared. This solution was mixed with filtrate containing cupric carbonate thoroughly and then filtered through Whatman No. 1 filter paper. Transfer the 100 ml filtrate into a 500 ml separating funnel and add 100 ml of 0.03% H₂SO₄ and 25ml of chloroform. Shake the mixture vigorously releasing the fumes or gases

and allow for separation. Then the lower layer was transferred to a 100 ml separating funnel and treated with 40 ml 1% KCl in 0.02M KOH solution by gentle shaking and allowing for separation. The lower layer was collected in a vial, by passing through anhydrous sodium sulphate bed. The extract was evaporated in a hot plate under fume hood. Finally the dried extract was re-dissolved in 0.2 ml of chloroform and used for TLC (Ramesh *et al.*, 2013 & 2014).

Sample assay

The dissolved residue was then spotted on to a silica gel F₂₅₄ 10 × 20 cm TLC plates of about 0.5 mm thickness as 5 µl drops using micro-syringes. The standard solution of aflatoxin was also spotted on to the same plate as drops of 1, 3, 5 µl. These standards were calibrated and checked for its purity by UV Spectrophotometer (AOAC, 2000). Samples of chloroform extracts and standard solution of aflatoxins were spotted in 1 cm bands. The spotted plate was placed vertically in the development tank containing chloroform: acetone (9:1, v/v) mobile phase upto 80 mm from lower edge of plate and covered properly. It took 20 to 30 min for the solvent to reach the stop line (9 cm) from the base line. After each development, the plate was then removed and dried with a hair drier. This process was repeated for all the samples and the developed plates were viewed under a long wavelength UV lamp (366 nm). The aflatoxin B and G when present fluoresced blue and greenish blue, respectively. The fluorescence intensities of aflatoxin spots of sample were compared with those standard spots. The sample spot, which matches one of the standard spots, was selected. Standard was also used to compare the colour and R_f / R_i value of unknown sample streak on the plate. The amount of aflatoxin B₁, B₂, G₁ & G₂ was estimated. For further confirmation TLC plates were sprayed with 20% H₂SO₄ and heated 10 min at 110° C, the fluorescence intensity increased. Method validation was performed in blank samples spiked with aflatoxin standards at 5µg kg⁻¹ (Sarithchandra and Muralimanohar, 2013).

Reagents

Aflatoxin B₁, B₂, G₁ and G₂ standards were procured from Sigma Aldrich. All the chemical and solvents used for analysis were of analytical grade (Emerck).

RESULTS & DISCUSSION

Our study showed that the average moisture percentage of DDGS is 8-12%. Moisture is also important because it influences microbial growth and thus affects shelf life during storage. Bhadra *et al.* (2009) obtained the result of moisture content with 4.32 - 8.89 (% , db), but Rosentrater (2006) obtained a higher value with 13.2 - 21.2 (% , db). In addition, the moisture content data in this study are very similar to the finding of Spiels *et al.* (2002); Kingsly *et al.* (2010); Zhang & Rosentrater (2013). The reasons for the differences in moisture percentage are probably because of the method of producing DDGS at the ethanol plants.

The total aflatoxin in DDGS samples were detected by TLC in the present study. Sherma (2000) and Fuch *et al.* (2011) reported that TLC is simple to operate, availability of many sensitive and selective reagents for detection and confirmation without interference of the mobile phase, ability to repeat detection and quantification and cost

effective analysis, because many samples can be analyzed on a single plate with low solvent usage, and the time that TLC employs to analyze the sample is less than LC method. The results showed that maximum number of DDGS samples is contaminated with aflatoxin B₁, with the percentage of 27.82 % and within 10-40 ppb concentration range, followed by aflatoxin B₂ (20.86 %; 5-20 ppb), while both aflatoxin G₁ and G₂ have 5% of contamination with concentration range of 5 ppb each. Out of 115 DDGS samples, aflatoxin B₁ were detected in 32 samples, aflatoxin B₂ in 24 samples, aflatoxin G₁ and G₂ in 5 samples each. Most of the DDGS samples contaminated contains 10 ppb concentration of aflatoxin B₁, on an average of 15.93 ppb. While aflatoxin B₂ contaminated DDGS samples have an average of 9.16 ppb. The range of the above mentioned aflatoxins in DDGS feed samples are presented in Table 1. The aflatoxin contamination level of DDGS samples in our studies were below the guidelines provided by US FDA or European Union in animal feed.

Garcia *et al.* (2008) reported that all the mycotoxins except DON examined in both coproducts, DDGS and WDG, were well below the FDA recommendations for each mycotoxin in animal feed. The report by Rodrigues (2008) showed that 99% of the 103 DDGS samples that they studied contained at least one detectable mycotoxin, with 8% containing detectable aflatoxins. Among the 103 DDGS samples, 67% were from the United States and 33% were from Asia. Zhang *et al.* (2009) conducted an extensive literature review of published studies and evaluated samples from three large data sets of DDGS samples to determine the extent and level of mycotoxin contamination among U.S. DDGS sources. Concentrations of all mycotoxins in DDGS were generally below the FDA action levels for all mycotoxins. Similar finding was also reported by Caupert *et al.* (2011). In contrast to the above finding, Rodrigues and Chin (2011) reported that 2% (AF) and 8% (DON) of DDGS samples analysed in their survey showed levels exceeding FDA's limits. Kathirvelan *et al.* (2014) reported that out of a total number of 20 DDGS samples analysed, 16 samples contained traces of aflatoxin B₁ and four samples contained 50-100 ppb using TLC. The higher percentage of positivity in their finding when compares with our present studies might be due to differences in sources of DDGS or the seasonal variation in sample collection. The source of the DDGS collected in our present study is from rice and wheat. It has been reported that sample with high aflatoxin, had high level of moisture (13-15%) at the time of collection of raw material sample. The environmental condition which includes the warm and humid storage conditions favor the aflatoxin development in feed ingredients. Like many grain-based feed ingredients, DDGS may contain amounts of mycotoxins that can negatively affect animal performance. Mycotoxins can be present in DDGS if the grain delivered to an ethanol plant is contaminated with them and are not destroyed during the ethanol production. Mycotoxin regulations have been established in more than 100 countries (Van Egmond *et al.*, 2007), and the maximum acceptable limits vary greatly from country to country. In the case of lots intended for industrial purposes (*e.g.*, bioethanol or biopolymer production), neither maximum limits nor guidance levels have been

established. The European Union has set a maximum level of aflatoxin in agriculture commodities with aflatoxine B₁ of 4 µg kg⁻¹ (Binder, 2007). The U.S. FDA has established maximum tolerable levels for aflatoxins in feed ingredients for various types of animal feeds which are presented in table 2 (Zhang *et al.*, 2009).

Feeding of DDGS to livestock and poultry will be a promising strategy to reduce the feed cost on production with simultaneous maintenance of nutritive value of feed.

One of the great advantages of DDGS is the possibility of storing it even for a year but WDGS (Wet Distillers Grains with Solubles) can be stored for a week only. The main disadvantage in feeding DDGS to livestock and poultry is the presence of mycotoxins. Because of the confirmed threat of toxic compounds, it is recommended that every batch of DDGS reaching farmsteads is examined in reference laboratories (with the use of chromatographic techniques) for the presence of mycotoxins.

TABLE 1. Aflatoxin B₁, B₂, G₁ & G₂ contamination in DDGS feed samples by TLC

| Mycotoxin | No. of sample tested | No. of contaminated samples | % of contamination | Concentration range (ppb) | | Average of positive (ppb) |
|--------------------------|----------------------|-----------------------------|--------------------|---------------------------|------|---------------------------|
| | | | | Low | High | |
| Aflatoxin B ₁ | 115 | 32 | 27.82 | 10 | 40 | 15.93 |
| Aflatoxin B ₂ | 115 | 24 | 20.86 | 5 | 20 | 9.16 |
| Aflatoxin G ₁ | 115 | 5 | 4.7 | 0 | 5 | 5 |
| Aflatoxin G ₂ | 115 | 5 | 4.7 | 0 | 5 | 5 |

TABLE 2. FDA action levels for aflatoxin in complete feeds and feed ingredients (Zhang *et al.*, 2009)

| Animals | Action Levels (ppb) |
|--|---------------------|
| Finishing beef (i.e., feedlot) cattle | 300 |
| Finishing swine (> 100 pounds) | 200 |
| Breeding beef cattle, breeding swine or mature poultry | 100 |
| Immature animals, dairy cattle or intended use unknown | 20 |

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