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Analysis of Proximate Composition and Aflatoxins of Some Poultry Feeds

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Abstract: In the present study raw samples like bamboo rice, pani varagu, thiri varagu, kampu, saamai and koran thinai used for poultry feeds production were collected from Kolli hills region of Central Tamil Nadu in India. When they were analyzed for proximate composition, high moisture content (11.70%), high protein content (11.34%), high crude fiber (10.16%), crude fat (4.69%) and high total ash content (5.12%) were reported in koran thinai, bamboo rice, thiri varagu, kampu and pani varagu, respectively. Aflatoxins were detected in kampu (220 ppb of AFB1 and 45 ppb AFB2) and in saamai (15 ppb AFB1) only and absent in other samples. Generally aflatoxins production increased in autoclaved samples compared to non autoclaved samples. Significantly kampu showed high level of AFB1 (900 ppb) followed by pani varagu (630 ppb). From this study it is clear that the aflatoxins presence in the raw food samples must be checked properly as it will cause economic loss of crops to be used as source. So, proper storage and harvesting methods should be adopted to prevent aflatoxins contamination.

Key words: Poultry feed, proximate, aflatoxin, Kolli hills

INTRODUCTION

Aflatoxins are a group of polyketide-derived furanocoumarins which are carcinogenic among the known mycotoxins. They are mainly of four major groups, AFB1, AFB2, AFG1 and AFG2 of which, AFB1 is a potent carcinogen. They are hepatotoxic secondary metabolites produced by some strains of *Aspergillus flavus, A. parasiticus, A. nomius* and *A. tamarii* (Dvorack, 1999). They cause health problem to live stock and human beings by contaminating agricultural commodities (Benneth *et al*., 2003; Bhatnagar *et al*., 1993; Payne, 1998). High amount of aflatoxin is produced in agricultural crops like groundnut, cotton seed, wheat, rice, barley, coconut, corn, dried peas, oat, sweet potato, millet and cassava (Goldblatt, 1969). Due to acute aflatoxin poisoning 1000 people were affected out of this 100 people were died in India in 1974 (Krishnamachari *et al*., 1975; Tandon and Tandon, 1988).

Aflatoxins are mainly produced in poultry feeds by many fungi such as *Aspergillus* species and *Fusarium* species. Nearly 20% *Aspergillus* species exhibit toxigenic activity in poultry feeds. Due to high stability they can be easily destroyed by any methods. In humans and animals they may be implicated in high incidence of hepato-cellular and lung carcinoma (Massey *et al*., 1995). The development of aflatoxins depends on the infection and growth of fungi in grains. Moisture acts as an important factor for the growth of fungi. The minimum moisture level for aflatoxin production at 30°C by *A. flavus* is equal to the moisture content of a product in equilibrium with 83% relative humidity or higher, depending on the nature of the substrate and the duration of storage. For starchy cereal seeds such as maize and wheat, the limiting moisture level for growth of *A. flavus* is about 18.5% whereas in oil seeds such as peanuts it is 8 or 9% (Christen and Kaufmann, 1974).

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Aflatoxins are of industrial importance due to the economic loss resulting from the contaminated crops especially in poultry industry (Pillet, 1998). The AFB1 is always present in varying degrees in poultry which are probably the most sensitive food animals to its toxic effects and small amounts of AFB1 cause reductions in growth rate, feed efficiency, hatchability and increased susceptibility to disease (Coulombe, 1993).

In the present investigation some important poultry feeds produced frequently in Kollihills region of Namakkal district,Tamil Nadu,India were analyzed for aflatoxins. This area was selected as it is characterized with high moisture. In Namakkal area there are many poultry farms which obtain poultry feeds from Kolli hills. The main objectives were to determine moisture, crude protein, fat, fiber and total ash that is proximate analysis of poultry feeds, to detect AFB1 and AFB2 by thin layer chromatography and to find out the effect of autoclaving on the production of AFB1 and AFB2.

MATERIALS AND METHODS

Collection of Samples

Samples like bamboo rice, pani varagu, thiri varagu, kampu, saamai and koran thinai were collected from Kolli hills region during winter season (November-December, 2008) and analyzed for proximate composition and aflatoxins in the Biotechnology Research Centre of KSR College at Tiruchengode, Namakkal Dt, Tamilnadu, India. In Kolli hills, plants which act as the sources of these poultry feeds are cultivated. Kolli hills is a small mountain range located in Central Tamil Nadu in India. The mountains are about 1000 to 1300 m in height and cover an area of approximately 280 km^2. The Kolli hills is a part of the Eastern Ghats, which is a mountain range that runs mostly parallel to the East coast of South India reached by road easily from Namakkal city (50 km) (Fig. 1).

Proximate Analysis

The proximate composition of raw poultry feeds such as moisture, crude protein, crude fibre, crude fat and total ash content were analyzed using the procedures described by (Sundaram *et al*., 2001).

Determination of Moisture

Five grams of poultry feed was weighed in a petridish, placed in a hot air oven at 105-110°C for a minimum of 6 h, cooled in a dessicator. The process of heating and cooling was repeated till a constant weight was obtained. The moisture was removed as vapour.

Fig. 1: Kolli hills region of Tamilnadu in India selected for poultry feed samples collection

Determination of Crude Protein

Crude protein was determined using MicroKjeldajhl's distillation assembly (Hexatec, Maharashatra, India). Two grams sample was digested in H_2SO_4 using $CuSO_4$ $5H_2O$ as catalyst and by adding Na_2SO_4 (anhydrous) or K_2SO_4 to elevate the boiling point converting organically bound N to ammonium sulphate which when heated with excess alkali (40% NaOH), NH_3 is liberated which is distilled into a known excess of standard acid (0.1 N H_2SO_4). The unrelated (unneutralised) acid is back titrated with standard alkali (0.1 N NaOH). From the titre values the nitrogen content and crude protein were calculated multiplying by 6.25.

Determination of Crude Fat

The crude fat (a combination of simple fat, fatty acid, esters, compound fat, neutral fat, sterols, waxes, vitamins (A, D2, E, K), carotene, chlorophyll, etc) soluble in ether was estimated by extracting in ether which was continuously volatilized at 60-80°C condensed and allowed to pass through the thimble containing the sample in a Soxhlet's apparatus (Borosil, Mumbai, India).

Crude Fibre

Crude fibre consisting of cellulose, hemicellulose and lignin etc was estimated by successive digestion of two grams of sample with dilute acid (0.255 N) and alkali (0.313 N).The entire residue transferred into a silica crucible and kept in a hot air oven at 105°C, cooled in a dessicator, weighed and finally concordant values were obtained.

Determination of Total Ash

To find out the total content of mineral matter or total ash i.e., non- combustible portion of the feed, 2 g of sample was weighed accurately in a silica crucible. The sample was ignited on a burner till smoke ceases. The crucible was placed in a muffle furnace and heated to 600°C and kept for 2 h. At this temperature all organic matter was burnt leaving behind minerals. The crucible was removed from the furnace carefully and cooled in a dessicator at room temperature and weighed again.

Aflatoxin Analysis

Aflatoxins were analyzed by thin layer chromatography technique according to the method of Romer (1975).

Sample Preparation

In addition to raw feeds analysis, the effect of autoclaving on aflatoxin production was studied. For this 20 g of finely powdered sample was placed in each 500 mL cortical flask and enough water (about 20 mL) was added to just moisten the powder. The flasks were divided into two sets. Set 1 was autoclaved at 15 pounds pressure for 15 min and set 2 was not autoclaved. Two loopful of 5 to 6 days old culture of *A. flavus* was streaked and grown on Sabouraud's dextorse agar. The inoculated flasks were incubated at 26±1°C for 8 days. At the end of incubation period flasks were sprayed with 95% alcohol, dried overnight at 80°C (Nagaragan *et al*., 1973) and used for aflatoxin analysis.

Detection and Estimation

Raw 10 g of dried poultry feed samples were beat with 40 mL of distilled water for 2 min. Then 60 mL of acetone was added and again beat for 2 min and then the contents were slightly heated up. The contents were filtered and to the 30 mL of filterate approximateky 0.6 g of cupric carbonate in beaker (A) was added. Thirty four milliliter of 0.2 M NaOH and 6 mL of $FeCl_3$ (0.41 M) was taken in beaker (B) and swirled. Then both the contents were mixed and filtered through whatman No.1 filter paper. Forty milliliters of the filterate was taken in 250 mL separating funnel. Then, 0.03% of 40 mL

sulphuric acid and 10 mL of chloro form was added and mixed. The chloroform layer was collected in a 200 mL beaker and again 20 mL of chloroform was added again, mixed thoroughly, allowed to settle and the chloroform layer in the same flask was collected. Another separating funnel having 40 mL of 0.02 M KOH and 1% KCl mixture to this collected 20 mL of chloroform extract was added and mixed it slowly and the bottom layer was collected through anhydrous sodium sulphate bed drop by drop to remove any traces of moisture. The chloroform extract was kept in an oven set at 50°C till it becomes dry. Then aflatoxin film was dried, rediluted with 0.2 mL chloroform and spotted on the TLC plate taking exactly 5, 10, 20 and 40 µL besides the standard spots of 5 and 10 µL. A colour change from blue colour fluorescence band to pale yellow colour confirms the presence of aflatoxin. The results were interpreted by visually comparing with standards purchased from Hi-media Co., Mumbai, India and the concentration was ascertained at 364 nm in a UV viewing cabinet.

All the determinations that is for proximate composition and aflatoxins analysis were carried out in triplicates and the date obtained were expressed as Mean±SD.

RESULTS AND DISCUSSION

All the samples were analyzed for moisture, crude protein, crude fiber, crude fat and total ash content. High amount of moisture (11.70%) was observed in koran thinai whereas low amount (9.38%) in bamboo rice. In case of crude protein high amount (11.34%) was resulted in bamboo rice, low amount (6.55%) in thiri varagu. High amount (10.16%) of crude fibre was observed in thiri varagu whereas bamboo rice showed low (0.79%) fiber content. Kampu showed high amount (4.69%) of crude fat whereas bamboo rice showed low amount (1.01%). The total ash content of pani varagu was high (5.12%), while bamboo rice showed low amount (1.63%) (Table 1).

Then aflatoxins were quantified for all the samples using TLC. Out of six samples only two samples were positive for aflatoxin presence. Kambu had 220 ppb of AFB_1 and 45 ppb of AFB_2 whereas saamai had only 15 ppb of AFB_1 (Table 2).

Samples were inoculated with two loopful of standard strain of *Aspergillus flavus* to produce aflatoxin and the effect of autoclaving on the production of aflatoxin was studied. The results clearly show that the autoclaved samples showed high level of AFB1 and AFB2 than non autoclaved samples. In autoclaved and non autoclaved samples, kambu showed high levels of aflatoxins B1 (900 and 600 pbb, respectively), while Saamai showed high levels of B2 (330 and 210 ppb, respectively). The data shows on average there was three fold increase in toxin production in autoclaved samples compared to non autoclaved samples (Table 3).

Table 1: Proximate analysis of poultry feeds

Poultry feeds	Moisture	Crude protein	Crude fiber	Crude fat	Total ash
	(%)				
Kambu	11.02±0.01	08.52±0.04	01.75±0.03	04.69±0.03	01.99±0.01
Thirivaragu	10.97±0.02	06.55±0.02	10.16±0.02	03.19±0.01	03.63±0.03
Bamboo rice	09.38±0.02	11.34±0.04	00.79±0.04	01.01±0.02	01.63±0.04
Pani varagu	11.06±0.03	08.94±0.01	08.80±0.01	03.47±0.02	05.12±0.02
Saamai	11.29±0.02	08.74±0.03	07.08±0.03	04.64±0.01	03.19±0.02
Koran thinai	11.70±0.01	10.69±0.02	06.04±0.02	04.62±0.03	04.13±0.03

The results are presented as Mean±SD

Table 2: Quantification of aflatoxins in raw poultry feeds by TLC

Samples	AFB1 (ppb)	AFB2 (ppb)
Kambu	220±1.00	45±1.53
Thirivaragu	-	-
Bamboo rice	-	-
Pani vargu	-	-
Saamai	15±2.08	-
Koran thinai	-	-

The results are presented as Mean±SD. -: Negative

Table 3: Aflatoxin quantification in *A.flavus* inoculated poultry feeds by TLC

Samples	Conditions	AFB1 (pbp)	AFB2 (pbp)
Kambu	Autoclaved	900±1.00	140±2.52
	Non autoclaved	600±1.53	60±2.00
Thirivaragu	Autoclaved	310±2.65	95±1.53
	Non autoclaved	190±2.08	30±3.05
Bamboo rice	Autoclaved	480±3.21	130±3.06
	Non autoclaved	150±2.52	40±2.52
Pani varagu	Autoclaved	630±2.511	80±3.21
	Non autoclaved	220±2.00	80±3.61
Saamai	Autoclaved	450±4.04	330±1.53
	Non autoclaved	300±3.51	210±3.21
Koran thinai	Autoclaved	420±4.04	120±1.00
	Non autoclaved	280±2.00	70±4.51

The results are presented as Mean±SD

The proximate composition influences the contamination of aflatoxins in raw poultry food samples. Owen *et al*. (2008) have reported proximate composition of heat treated poultry litter and obtained the values for crude protein (20%),crude fiber (10.4%) and ash (18.5%).The total ash content of pani varagu was high (5.12%) but bamboo rice showed low amount (1.63%).The variations in the values may be related to the anatomical structure of the plant sources. Similar results for ash content have been reported for legumes (Elegbede, 1998).

No aflatoxins were detected in four of the samples in our studies except kampu and saamai. It may be correlated to their high fat content compared to other samples. Similarly aflatoxin was not detected in soyabean meal or broken rice analyzed by Purwoko *et al*. (1991). Even though they were absent in raw samples after milling aflatoxin may be produced during storage (Mazen *et al*., 1990). Present results confirm the importance of ingredients before incorporating them into mixed feeds as reported by Thirumala-Devi *et al*. (2002) in some important Indian poultry feeds. Escobar and Regueiro (2002) have reported aflatoxin B1 with the biggest percentage in sorghum and peanut, 83.3 and 40.4%, respectively in the analyzed food stuffs.

Aspergillus flavus isolates produce only AFB_1 and AFB_2, while *Aspergillus parasiticus* and *Aspergillus nominus* produce AFB_1, AFB_2, AFG_1 and AFG_2. The chemical composition of the feeds determines the growth of the different fungal species. Also seasonal and geographical factors and the conditions required for the cultivation of the crops influence the aflatoxin contamination. (Jewers *et al*., 1986). In Kambu the AFB1 level is less than 250 ppb. Poultry diets containing above this level, when fed to poultry predispose them to attack by bacteria and viruses. Aflatoxin is a hepatotoxin causing an excessive build up of biliary ducts (Adav and Godinwar, 1997). The maximum tolerated aflatoxin in the food samples should be 20 $\mu g\ kg^{-1}$ (Smith and Moss, 1985).

Various methods have been tried to decontaminate aflatoxin contaminated commodities such as groundnut, cotton seed, palm kernel cake/meal and maize. These include physical methods (sorting, irradiation techniques, heating), chemical methods (acids, bases, oxidizing agents), biological methods (microbiological) and solvent extraction (Coker, 1986). Ammonia gas appears to be the most promising approach as it is capable of reducing the aflatoxin level in situ by more than 95% and is applicable to a variety of contaminated commodities using batch and continuous processing methods (Coker *et al*., 1985).

The result revealed that aflatoxin production was increased in autoclaved samples compared to non autoclaved. Similar results have been reported with *Aspergillus tamarii* which produces B group of aflatoxin and cyclopiazonic acid (Tetsuhisa *et al*., 1996). It may be related to the denaturation of any compound which may be inhibitory to aflatoxin synthesis in normal conditions and the necessary compound may be available as reported in soyabeans.Phytic acid inhibits aflatoxin formation by combining with zinc. During autoclaving zinc was liberated from phytic acid, zinc was known to have a pronounced simulatory effect on aflatoxin production. The destruction of phytic acid by heat results

in the availability of zinc for aflatoxin synthesis. Here also aflatoxin production increased in autoclaved samples. Soybeans produce aflatoxin but extent of production depends on the variety of soybean and the toxigenic potential of the isolates used (Nagaragan *et al*., 1973). *Aspergillus flavus* strain NRRL 2999 produces aflatoxins on the solid substrate rice. Optimal yields more than 1mg of AFB1 per gram of starting material obtained in 5 days at 28°C have also been reported by Mateles and Adye (1965). So, our poultry feed raw samples, kambu and saamai also act as good substrate for the growth of aflatoxin producing fungi. So proper harvesting and storage method have to be implemented to prevent aflatoxin contamination.

CONCLUSION

As the cost of feeding the animals and birds comes to nearly 70 to 75% of the production cost of the products, assessing the quality of raw materials to regular testing is very important. To inhibit the aflatoxin production the concerned gene should be inactivated in fungi. Some bacterial isolates which have detoxification effect on aflatoxin are also economically important. Bamboo rice, pani varagu, thiri varagu, kampu, saamai and koran thinai have been mostly used as poultry feeds in our local area as these are easily available and cost effective. So, much attention in the processing procedure should be given.

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