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Abundance of Fungal Flora in Relation to Moisture Content and Storage Period in Different Types of Poultry Feed Ingredients

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Abstract: Six types of poultry feed ingredients viz. maize, rice bran, sesame oil cake, soybean oil cake, wheat husk and oyster shell were used for determination of the presence of fungal flora. Eight genera of fungi have been isolated and identified. The genus *Aspergillus* was the highest in frequency followed by *Penicillium*, *Rhizopus*, *Fusarium*, *Paecilomyces*, *Scopulariopsis*, *Alternaria* and *Candida*. Sesame oil cake was the highest contaminated (43.15%) and oyster shell was the lowest (3.06%). Fungal contamination of poultry feed ingredients increased with the increase in their moisture content and storage period.

Key words: Poultry feed ingredients, fungal flora, moisture content, storage period

Introduction

Poultry is now a very important and widespread agricultural industry in the tropics (Sastry *et al.*, 1983). Poultry provides protective food in human nutrition in the form of egg, meat and employment opportunities at various levels (Das, 1994). Poultry's feeding is one of the most important branches of poultry science. Since feed cost alone accounts for 60-65 % of the total farm expenses (Banerjee, 1998). Nutritionally balanced diets are provided during phases of productive life-in eggers, chicks, growers and layer stages, in broilers, starter and finishing stages (Gopalakrishana and Lal, 1985). Poultry disease and other problems are caused by poultry feed (Jones, 1987). Fungi are heterotrophic microorganisms, which are the major cause of deterioration and spoilage in stored crops. It can enter in animal food chain by direct or indirect contamination. In direct contamination, the food material supports the toxigenic fungal growth (Jones, 1987). For inadequate drying, processing, storage and handling facilities at pre and post harvest period the cereals support mould and toxin formation (Chauhan and Roy, 1996). This investigation was, therefore, undertaken to find the fungal flora present in poultry feed ingredients and the role of moisture content and storage period of the poultry feed ingredients.

Materials and Methods

Six types of poultry feed ingredients viz. maize, rice bran, sesame oil cake, soybean oil cake, wheat husk and oyster shell were collected from different poultry farms of Rajshahi from May 1999 to August 2000. Ten samples of each ingredient were collected separately, packed and brought to Plant Pathology Laboratory, Department of Botany, University of Rajshahi.

A small amount of collected samples was placed on a clean dry slide, moistened with cotton blue and then covered with a clean cover-slip. After keeping the slide at 30 ± 2 °C for few minutes, the preparation was examined microscopically for the presence or absence of hyphae, spore or conidia of fungi.

The fungi were isolated from different types of poultry feed ingredients on 2% PDA medium and bacterial contamination was avoided by incorporating the 50% lactic acid in the medium. A small amount of collected poultry feed ingredients sprinkled on PDA medium in petridishes and kept separately. Petridishes were incubated for 7-10 days at 30 ± 2 °C and examined periodically for the developments of fungal colony on the plates. Identification of the fungi was made grossly by their cultural characteristics and microscopically by their

spores, fruiting bodies and conidiophores. Identification up to genus of each colony was recorded and identification up to species level was tried, wherever possible with the help of standard mycological books and manuals (Gilman, 1957; Anisworth, 1971; Raper and Fennell, 1965; Barnett, 1962; Booth, 1971; Subramanian, 1971; Ellis, 1971 and Alexopoulos and Mims, 1979). Sterile mycelium sub-culture that failed to sporulate at the end of three months was designated as non- identified. Hundred gm of each sample of poultry feed ingredients was taken and kept at 60 °C in an oven for 24hrs. The dried samples were weighed again and calculated the moisture content employing the following formula:

$$\text{Percentage of moisture content} = \frac{W_1 - W_2}{W_1} \times 100$$

where, w_1 = weight before drying
 w_2 = weight after drying

Results and Discussion

All the ingredients of poultry feed were found to be contaminant and 7115 fungal colonies were counted from different poultry feed ingredients (Table 1). Among them, 7106 were identified (Table 1). The identified fungi assigned to eight genera (*Aspergillus*, *Penicillium*, *Rhizopus*, *Fusarium*, *Paecilomyces*, *Scopulariopsis*, *Alternaria* and *Candida*). Fungal colonies (1382) were found in maize samples. Among them, 1379 colonies were identified and six genera of fungi were found. *Aspergillus flavus* (43.77%), *Penicillium* (21.63%), *Aspergillus niger* (20.55%) and *Aspergillus candidus* (3.76%) were dominating fungi. In rice bran samples, 1044 fungal colonies were found from which 1041 colonies were identified and four genera were found. *Aspergillus flavus* (42.81%), *Aspergillus niger* (19.06%), *Penicillium* spp. (17.33%), *Rhizopus* spp. (5.94%) and *Aspergillus fumigatus* (4.98%) were dominating fungi. Two hundred and thirty eight fungal colonies were found in soybean oil cake samples, all colonies were identified and four genera were found. *Aspergillus flavus* (51.26%), *Rhizopus* spp. (23.52%), *Aspergillus niger* (9.66%) and *Aspergillus nidulans* (5.88%) were the dominating fungi. 3070 fungal colonies were found in sesame oil cake samples, among them, 3 colonies could not be identified and five genera were found. *Aspergillus flavus* (71.1%), *Aspergillus niger* (20.16%), *Rhizopus* spp. (3.74%) and *Aspergillus*

Table 1: Number of fungal colonies and their abundance on various components of poultry feed during storage period.

Name of the fungus	Name of the poultry feed ingredients											Total of all samples		Abundance of fungi (%)	
	Maize		Rice bran		Soybean oil cake		Sesame oil cake		Wheat husk		Oyster shell		No. of Colony		Abundance %
	No. of Colony	Abundance %	No. of Colony	Abundance %	No. of Colony	Abundance %	No. of Colony	Abundance %	No. of Colony	Abundance %	No. of Colony	Abundance %			
<i>Aspergillus flavus</i>	605	43.77	447	42.81	122	51.26	2183	71.1	852	73.26	170	77.98	4379	61.56	86.33
<i>Aspergillus niger</i>	284	20.55	199	19.06	23	9.66	619	20.16	145	12.46	33	15.14	1303	18.31	
<i>Aspergillus fumigatus</i>	41	2.96	52	4.98	9	3.78	6	0.19	6	0.51	0	0	114	1.6	
<i>Aspergillus nidulans</i>	10	0.72	10	0.95	0	0	0	0	17	1.46	0	0	37	0.52	
<i>Aspergillus terreus</i>	31	2.24	32	3.06	14	5.88	0	0	26	2.23	0	0	103	1.45	
<i>Aspergillus ochraceus</i>	14	1.01	0	0	0	0	0	0	0	0	0	0	14	0.2	
<i>Aspergillus wentii</i>	2	0.14	0	0	0	0	3	0.09	0	0	0	0	5	0.07	
<i>Aspergillus versicolor</i>	4	0.29	5	0.48	0	0	6	0.19	0	0	0	0	15	0.21	
<i>Aspergillus candidus</i>	52	3.76	41	3.92	0	0	64	2.08	6	0.51	9	4.13	172	2.42	
<i>Rhizopus spp.</i>	8	0.58	62	5.94	56	23.52	115	3.74	18	1.54	0	0	259	3.64	3.64
<i>Penicillium spp.</i>	299	21.63	182	17.33	0	0	60	1.95	51	4.38	0	0	592	8.32	8.32
<i>Alternaria spp.</i>	0	0	0	0	0	0	11	0.36	0	0	0	0	11	0.15	0.15
<i>Fusarium spp.</i>	21	1.52	6	0.57	6	2.52	0	0	10	0.86	0	0	43	0.6	0.6
<i>Scopulariopsis spp.</i>	5	0.36	0	0	8	3.36	0	0	15	1.29	6	2.75	34	0.48	0.48
<i>Candida spp.</i>	3	0.21	0	0	0	0	0	0	6	0.51	0	0	9	0.13	0.13
<i>Paecilomyces spp.</i>	0	0	5	0.48	0	0	0	0	11	0.94	0	0	16	0.22	0.22
Non identified	3	0.21	3	0.28	0	0	3	0.09	0	0	0	0	9	0.13	0.13
Total	1382		1044		238		3070		1163		218		7115		

Table 2: Effect of moisture content and storage period on the incidence of fungi on different poultry feed ingredients.

Name of the component	No. of Sample	Moisture content (%)	Storage period and number of colony					Total of all samples	Abundance (%)	Expected F.	Table value F.		S/NS
			1 st culture period	2 nd culture period	3 rd culture period	4 th culture period	Total no. of colony				5%	1%	
Maize	1	10.98	34	36	38	46	154	1382	19.42	54.46	2.26	4.6	**
	2	9.5	31	33	33	39	136						
	3	10.5	34	35	39	44	152						
	4	11.2	37	36	39	47	159						
	5	8	21	28	31	34	114						
	6	8.5	28	29	27	36	120						
	7	10.9	29	32	33	38	132						
	8	9.5	32	35	35	39	141						
	9	8.5	27	32	33	39	131						
	10	8.5	30	33	40	40	143						
Rice bran	1	9.62	16	25	27	33	101	1044	14.88	157.85	2.26	4.6	**
	2	10	17	22	27	33	99						
	3	9.92	19	25	30	41	115						
	4	8.75	17	24	26	40	107						
	5	10.2	15	23	27	39	104						
	6	8.5	18	24	28	40	110						
	7	7.9	15	19	28	31	93						
	8	10.4	17	21	23	33	94						
	9	10.5	17	27	28	41	113						
	10	9.6	16	24	32	36	108						

Table 2: Continued

Name of the component	No. of Sample	Moisture content (%)	Storage period and number of colony					Total of all sample	Abundance (%)	Expected F.	Table value F.		S/NS
			1 st culture period	2 nd culture period	3 rd culture period	4 th culture period	Total no. of colony				5%	1%	
Soybean oil cake	1	15.2	5	6	8	10	29	238	3.34	11.27	2.26	4.6	**
	2	14.5	4	3	4	7	18						
	3	13.2	4	8	6	7	25						
	4	14.3	5	4	6	5	20						
	5	11.3	6	3	5	8	22						
	6	10.2	2	3	6	7	18						
	7	13.5	4	6	6	10	26						
	8	12.3	5	6	7	7	25						
	9	13.4	5	8	8	7	28						
	10	12.6	3	7	8	9	27						
Sesame oil cake	1	9.32	70	77	83	91	321	3070	43.15	135.87	2.26	4.6	**
	2	9.6	66	76	85	98	325						
	3	8.5	61	63	70	87	281						
	4	9.6	65	70	74	88	297						
	5	8	58	70	74	79	281						
	6	7.9	57	62	75	82	276						
	7	7.5	61	63	74	82	280						
	8	10.8	72	82	97	99	350						
	9	10.5	74	82	88	99	343						
	10	9.3	66	74	85	91	316						
Wheat husk	1	9.2	38	43	52	56	189	1163	16.34	610.75	2.26	4.6	**
	2	7.2	26	28	34	40	128						
	3	7.8	27	29	31	43	130						
	4	6.5	19	27	28	30	104						
	5	6	16	24	26	30	96						
	6	5.9	17	21	23	29	90						
	7	8.2	22	25	23	30	100						
	8	7.3	18	23	23	30	94						
	9	6.7	20	26	27	33	106						
	10	7.1	22	29	35	40	126						
Oyster shell	1	1.62	10	5	9	11	35	218	3.06	4.45	2.26	4.6	**
	2	1.4	5	9	7	8	29						
	3	1.2	5	6	6	5	22						
	4	1.5	4	6	4	8	22						
	5	0.82	4	3	3	5	15						
	6	1.2	4	5	3	5	17						
	7	1.52	6	7	6	8	27						
	8	0.92	3	2	5	6	16						
	9	0.72	2	3	5	5	15						
	10	0.85	4	5	6	5	20						

** Expected value of F. (54.46, 157.85, 11.27, 135.87, 610.75 and 4.45) > Table value of F. at 1 and 5% (4.6 and 2.26). F value at 1 and 5% level of probability is significant.

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candidus (2.08%) were dominating fungi. 1163 fungal colonies were found and identified in wheat husk samples, and seven genera were found. *Aspergillus flavus* (73.26%), *Aspergillus niger* (12.46%), *Rhizopus* spp. (4.38%), *Aspergillus terreus* (2.23%) and *Penicillium* spp. (1.54%) were the dominating ones. Two hundred and eighteen colonies were found in oyster shell samples, all were identified and two genera were found. *Aspergillus flavus* (77.98%), *Aspergillus niger* (15.14%), *Aspergillus candidus* (4.13%) and *Scopulariopsis* spp. (2.75%) were found. Frequency of *Aspergillus* spp. was the highest (86.33%) and *Candida* spp. was the lowest (0.13%) in all the tested samples (Table 1). Sesame oil cake was the highest (43.15%), and oyster shell was the lowest (3.06%) contaminated poultry feed ingredients (Table 2). The fungi were present intermediary in all the tested samples. Begum *et al.* (1995) studied 32 poultry feed samples and found highly contaminated with fungi (range 2 times $10\text{-}26.3 \times 10^{-3}$ cfu/g). They isolated seventeen different genera/species, *Aspergillus niger*, *Aspergillus flavus* and *Rhizopus* species were present in all the samples. The other fungi recorded were *Fusarium* spp., *Alternaria* spp., *Curvularia* spp. and *Monilia* spp. Moharram *et al.* (1989) reported 73 species of mesophilic fungi, mostly *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus fumigatus* in Egyptian samples of soybean meal, ground maize, cotton seed cake, wheat bran and fishmeal. Cook *et al.* (1991) found that *Fusarium moniliforme* was the predominant fungal species in unpolluted feed and maize samples. Bhattacharjee *et al.* (1995) collected and screened samples of cattle and poultry feeds containing wheat and rice bran and 18 species were isolated from the feed samples, *Aspergillus niger* and *Rhizopus* spp. being dominant.

The moisture content of maize was measured to be 8-11.2% and no. of fungal colonies 114-159 were counted (Table 2). In case of rice bran, moisture content ranged from 7.9-10.5% and no. of fungal colonies counted were 93-115. In soybean oil cake, moisture content ranged from 10.2-15.2% and no. of fungal colonies counted were 18-29. In sesame oil cake, moisture content ranged from 7.5-10.8% and no. of fungal colonies counted were 276-350. In wheat husk, moisture content ranged from 5.9-9.2% and no. of fungal colonies counted were 90-189. In oyster shell, moisture content ranged from 0.72-1.62% and no. of fungal colonies counted were 15-35.

In general, the number of fungal colonies increased with the increase in moisture content and storage period (Table 2). But in case of rice bran and sesame oil cake the result was confusing. The highest colonies (3070) were isolated from sesame oil cake and the lowest colonies (218) from oyster shell. Statistical analysis showed that with the increase in storage period, the fungal contamination also increased. Expected F. value, (54.46, 157.85, 11.27, 135.87, 610.75 and 4.45) is greater than table value of F. at 1 and 5% (4.6 and 2.26) level of probability. It indicates that the increase in fungal contamination is highly significant, except in oyster shell. Adebajo (1992) collected 5 types of poultry feed in Nigeria, and spoilage moulds were isolated including aflatoxin producing strains of *Aspergillus flavus* and *Aspergillus oryzae* and reported that high moisture content of the feeds promoted mould deterioration. Fungal contamination of poultry feed ingredients increased with duration of storage period in general, except oyster shell (in this experiment). Keskin and Ugur (1991) reported number of fungi on poultry feeds and feed raw materials, which increased with the duration of storage.

From present observation it is revealed that all the poultry feed ingredients are contaminated by fungi which depends upon the nature of food, moisture content and storage period. Some of the poultry feed ingredients are more favourable for particular group of fungi.

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