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AFLATOXINS DETOXIFICATION IN SUNFLOWER MEAL BY AMMONIATION

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Ammonia has been found to reduce efficiently naturally occurring aflatoxins present in sunflower meal on pilot plant scale. One percent of ammonia detoxified 96.53 ± 2.52% of aflatoxins in 30 min at 100 ± 1°C under 10 ± 0.5 psi of steam pressure. The levels of naturally occurring aflatoxins in sunflower meal ranged from 113 µg/kg to 3322 µg/kg. Not much variation was observed in the detoxification process with the increase in aflatoxins content. Resultant detoxified product was found to be free of ammoniacal smell with an average ammoniacal nitrogen of 0.1% and average moisture content of 9.37%. The aflatoxins detoxified sunflower meal showed no toxic effect towards egg production in layer when incorporated in the rations at an inclusion rate from 8.5 to 15%, as a vegetable source of protein. The pilot-plant process demonstrated the effective way of destroying aflatoxins in sunflower meal.

Key words: Aflatoxins, Detoxification, Ammonia, Sunflower meal, Egg production.

Introduction

Aflatoxins are the chemically related secondary metabolites of some strains of *Aspergillus flavus* and *A. parasiticus* and are the most potent toxins which are known to be highly carcinogenic, teratogenic, mutagenic and hepatotoxic [1]. Prevention is preferable for the control of aflatoxins contamination of agricultural commodities but it is practically difficult to achieve this objective, particularly, in the underdeveloped and developing countries. Alternatively, aflatoxin contaminated agricultural commodities have been made useful by physical removal of infected component (seed or kernel) or inactivation of aflatoxins by biological or chemical methods [2-4].Various methods are available for detoxification of aflatoxin, of which ammoniation is the choice of many due to its almost nontoxic post-treatment remnants [5-7]. Major breakdown products, including aflatoxin B_1, were found to be 2,000-20,000 times less mutagenic than that observed with nonammoniated aflatoxin B_1 [8].

Sunflower meal is one of the most important ingredient and an excellent vegetable source of protein supplement in poultry and animal feeds in Pakistan. The sub-tropical climate, poor method of storage and transportation increase the proliferation of mold growth with subsequent contamination by aflatoxin. Pakistan is producing a large quantity of sunflower with an annual production of 34,649 tonnes [9]. But, the inclusion rate of the by-products like sunflower cake and meal is restricted to few percent in poultry and animal feeds in the country due to high incidence of aflatoxins contamination where the level as high as 4000 µg/kg has been observed (unpublished data). Even at such high levels of aflatoxins contamination, the sunflower by-products are eventually sold at cheaper rate or go waste.

The sunflower meal is an important source of vegetable protein in Pakistan. The present study was undertaken to produce a feasible process on pilot plant scale for the detoxification of aflatoxins by ammoniation with a minimum possible detrimental effect on the nutritive value of sunflower meal. The process for detoxification of aflatoxins by ammoniation has been patented in Pakistan [10].

Materials and Methods

The pilot plant for the detoxification of aflatoxins and ammonia 99.99% pure were kind gifts from Natural Resources Institute, Chatham, UK. Chemicals and reagents used in the analysis of aflatoxins were of analytical grade. Sunflower meal, naturally contaminated with aflatoxins, was purchased from local wholesale grain market. The pilot plant consisted of double walled stainless steel reaction vessel of approx. 1 cubic meter. Depending upon the bulk density of sunflower meal, the optimum load detoxified at any given time was 250 kg. Reaction vessel was provided with steam jackets for preheating and for maintaining of constant temperature. Ammonia and steam were introduced quantitatively into the reaction vessel after passing through respective regulators and scale meters. Reaction vessel containing sunflower meal was preheated 60-65°C prior to steam pressurization. Steam was introduced gently into the vessel by opening the steam inlet control valve to raise the temperature to 100 ± 1°C and pressure to 0.7 bar (10psi) within 10 min. The water requirement of 15-20% for ammoniation reaction was maintained by the steam. Prior to injection of ammonia, the steam pressure in the vessel was reduced to 4.5 psi to prevent backpressure and to maintain ammonia inlet pressure above the vessel pressure. Ammonia was gently introduced quantita-

tively through a flowmeter at a suitable driving pressure (0.5 psi) to give required total percentage of 1.0% ammonia for the detoxification of aflatoxins in the reaction vessel in 5 min. After the reaction time of 30 min, excess ammonia was removed under the influx of pressurized steam and by fully opening the in-built vent-valve and exhaust system of reaction vessel. The detoxified sunflower meal was collected and spread on floor in the open air to cool.

Representative samples of 50 kg sunflower meal prior to and after detoxification process were collected at the respective collection points made for this purpose at the inlet and outlet conveyers, respectively. The sample size was finally reduced to 1 kg on rotary sample divider for the analysis of aflatoxins in triplicate. Remaining 40 kg returned to the respective batch. Water-based slurry of 1 kg each of the sample was used [11]. Aflatoxins were determined by Romer clean-up [12] (section 49.2.05, Official Method 975.36) followed by AOAC quantification method [12] (section 49.2.03 Official Method 971.22) of comparisons of standards by thin layer chromatography and aflatoxins were confirmed with the spray of 50% sulphuric acid and making derivatives of trifluoroacetic acid [13]. Residual ammoniacal nitrogen was determined by the micro Kjeldhal as described in the Methods of the AOAC [12] (section 7.038) on the Tecator Kjeltec digestion and distillation units.

TABLE 1. MOISTURE AND AFLATOXIN CONTENT OF SUNFLOWER MEAL BEFORE AND AFTER AMMONIATION.

Batch weight in Kg	Aflatoxin content*µg/kg Before ammoniation B_1	B_2	After ammoniation Total	B_1	B_2	% Detoxification	Moisture (%) Before	After
250	511	52	563	1.6	nd	99.7	10.2	9.3
246	658	nd	658	43	nd	93.4	8.6	9.0
250	572	64	636	2.8	nd	99.6	10.2	9.1
247	669	nd	669	33.5	nd	95	8.6	9.0
250	890	73	963	78	9	90	11.0	10.0
255	915	68	983	71.5	7	92	11.0	10.1
250	816	102	918	nd	nd	99.9	9.8	10.0
250	621	32	653	26	nd	96	8.8	9.0
250	263	10	273	18	4	93	9.7	9.5
250	291	11	302	16	nd	94.7	10.0	10.0
240	137	nd	137	4	nd	97.1	10.8	10.7
245	127	nd	127	2	nd	98.4	8.6	9.5
250	259	6	265	nd	nd	99.9	9.0	8.9
250	105	8	113	2	nd	98.2	9.7	9.5
245	771	52	823	29	nd	96.5	10.0	9.8
250	964	27	991	35	nd	96.7	10.2	9.5
250	315	12	327	11	nd	96.6	10.7	9.6
260	491	nd	491	8	nd	98.4	10.0	9.8
250	567	62	629	22	nd	96.5	9.0	9.1
275	212	nd	212	6	nd	97.2	11.0	10.8
248	3004	318	3322	122	6	96.0	10.5	9.5
250	2456	201	2657	46	3	98.0	9.6	9.4
247	1659	43	1702	57	nd	96.6	10.8	9.6
250	1011	nd	1011	18	nd	98.2	9.2	8.9
245	1273	57	1330	28	nd	97.8	7.3	7.1
250	1806	133	1939	39	nd	98.0	6.9	7.0
Avg=250.11	813.96	51.19	865.15	27.66	1.11	96.53	9.66	9.37
σ^{n}= 6.08	709.01	71.28	772.42	28.56	2.47	2.33	1.06	0.83
σ^{n-1} 6.20	723.05	72.69	787.72	29.12	2.52	2.38	1.08	0.84
n=26	26	26	26	26	26	26	26	26

* = Results of triplicate analysis., nd = Not detected.

Results and Discussion

Ammoniation of naturally aflatoxins contaminated sunflower meal at 100 ± 1°C and pressure of 10 psi resulted on an average 96.53 ± 2.52% detoxification of aflatoxins in half an hour (Table 1). Twenty six batches with an average weight of 250 kg each of sunflower meal were detoxified. The levels of aflatoxins (B_1 + B_2) in sunflower meal ranged from 113 µg/kg (mini) to 3322 µg/kg (maxi). Destruction of aflatoxins by ammonia was independent of the levels of aflatoxins in the sunflower meal and no variation was observed in the effectiveness of the detoxification process. The pilot plant study for the detoxification of aflatoxins by ammoniation under steam pressure and high temperature is an efficient method for inactivation of aflatoxin in a short span of reaction time. The process does not leave any ammoniacal smell with an average ammoniacal nitrogen of 0.1% in the detoxified sunflower meal. The moisture content remained constant for pre-and post treated material. On the basis of data as demonstrated by the present experiments and from many other workers [5-7, 14] the ammoniation is the most practical approach for inactivation of aflatoxins. The present study demonstrates the efficiency of technology and can be used for the preparation of poultry/animal feeds with negligible or low levels of aflatoxins. Feeding trials on layer with an inclusion rate upto 15% ammoniated sunflower meal showed no detrimental effects with detoxified feed and the egg production was 88% while with the ration containing 443 ppb (µg/kg) of aflatoxin B_1, it was 74%. In the first 6 weeks, the egg production and the weight/size of the eggs was lower in the detoxified feed than the control or the toxic feed but in the later part of the egg production the size/weight increased significantly [15]. It has been shown by many investigators that poultry/animal responses to feed, containing ammoniated ingredients, is not different than normal untreated feeds [4,5]. Preparation made from de-oiled meal of sunflower have been successfully used as a protein rich supplement to preschool children [6].

Last 20 years of research on detoxification and biological testing have ample supporting evidence for ammoniation to be the most appropriate technology available [4-7,14]. Ammoniation of agricultural commodities for animal/poultry feed has the approval of many nations [5,6,14]. The present study supports the effectiveness of detoxification process for aflatoxins by ammoniation for sunflower meal and the subsequent use as an ingredient in the preparation of poultry and animal feed.

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