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Pretreatment and Process to Control Mycotoxin in Sustainable Feed Production Using Extracts of Neem (*Azadirachta indica)*

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***Abstract*— By some recent estimates, mycotoxins affect as much as 25% of the world's food crops each year. These highly toxic, naturally occurring compounds are by products (metabolites) of the growth of fungi or moulds. Mycotoxins can result in significant economic loss for crop producers and broiler producers since it represent an ongoing risk to profitability. Factors that contribute to mycotoxin contamination of food and feed include environmental, socio-economic and food production. Environmental conditions especially high humidity and temperatures favour fungal proliferation resulting in contamination of food and feed. The resulting implications include immuno-suppression, impaired growth, various cancers and death depending on the type, period and amount of exposure. Mycotoxin concerns have grown during the last few decades because of their implications to human and animal health, productivity, economics of their management and trade. This has led to development of maximum tolerated limits for mycotoxins in various countries. This paper highlights the use of naturally derived aflatoxin inhibitors from Azadirachtin compound of neem oil, and tray drying in controlling moisture content to inhibit the aflatoxin contamination in pretreated soy based animal feed. High moisture content found in soy based animal feed increases the risk of fungal growth during storage. Neem extract has shown potential as an effective feed additive to minimize the growth of fungi and possibly reduce the risk of toxic effects from mycotoxin production. Preservation methods by means of tray drying have also shown positive results towards the control of fungal growth.**

***Keywords*— Mycotoxin, Neem, Tray Drying, Animal Feed**

I. INTRODUCTION

Recently, post-harvest products are wasted due to mycotoxin contamination. Mycotoxin is a toxic compound residue created from secondary metabolite of fungal growth in wide array of commodities. Aflatoxins are one of mycotoxins produced by toxigenic strains of Aspergillus flavus and A. parasiticus, and have mutagenic, carcinogenic and teratogenic effects on humans and animals [1]-[2].

Mycotoxins occurrence in the food chain caused by fungal infection of crops, might be consumed directly by humans, or in livestock feed. Factors that promote the risk of mycotoxin contamination are due to ambient temperature, relative humidity, alkalinity, and the mechanical damage of the crops itself [12]-[13]. Therefore, poor agricultural and harvesting practices, improper drying, storage and transport conditions promote fungal growth and increase the risk of mycotoxin production. Some mycotoxins such as aflatoxins, are considered by the US Food and Drug Administration (FDA) to be unavoidable contaminant of food. The goal therefore has been to minimize contamination, by modifying agricultural practices to avoid fungal growth [3].

Soybean processing waste, which is the outer hulls are commonly reused in the production of animal feed. The remaining amount of protein is approximately 15%, which is applicable for sustainable production of livestock feed. However, they might be exposed to mycotoxin contamination without proper handling and storage.

The eradication of these fungi may inhibit the production of mycotoxin as a whole. However this technique does not detoxify mycotoxin readily existed in farm feeds contaminated with fungi. Most of the mycotoxins possess chemical and thermal stability.

Neem plant (Azadirachta indica) is known to have inhibitory effect in aflatoxin production. It has been demonstrated that addition of neem leaf extract at concentrations greater than 10% (v/v) effectively inhibit aflatoxin production [9]. Neem extract interferes with the metabolic pathways of aflatoxin biosynthesis without affecting fungal growth [10].

Tray drying is one of the preservation methods of crops product, which the feedstuffs are spread on trays, and drying process takes place. Water activity of the feedstuffs is reduced, minimizing enzymatic and microbiological reactions. Fungal growth is restricted with controlled water activity in feedstuffs, therefore inhibit the production of mycotoxin and also increase the shelf life of crops product.

This paper presents a review of pretreatment of feedstuffs with additives and their effects on mycotoxin contamination. This paper also reviews the drying process used to control mycotoxin contamination.

II. Mycotoxin Contamination in Animal Feed

Livestock production is an important part of national economy and it plays a significant role in providing high quality foods for human beings. The role of livestock production sector in enhancing agricultural productivity is necessary, and its contribution in eradicating poverty in rural areas is well recognized [4]. Commonly, various stresses like low quality feed, naturally occurring contamination of toxic compounds in feedstuffs, climatic extremes, and poor management presence as threats that can adversely affect the health and performance of animals. Fungi are one of the naturally occurring threats that invade feedstuff and produce toxic compounds, mainly mycotoxins which are detrimental for the health of animals.

The commonly known mycotoxins are aflatoxins, fumonisins, ochratoxins A, trichotecenes, zearalenone, and patulin [5]. Aflatoxins are commonly detected in animal feed due to favorable climatic conditions for aflatoxin production. Aflatoxins are the fungal metabolites produced by some strains of Aspergillus flavus and Aspergillus parasiticus [7]. Aflatoxin is produced at a temperature of 12-40°C and requires 3-18% moisture [11]. The four most common aflatoxins are B1, B2, G1 and G2. One of the enormous incidences of aflatoxin toxicity in animals was the Turkey-X disease occurred in UK in the early 1960s [6]. More than 100,000 turkeys which had been fed with feedstuffs, subsequently found to contain aflatoxin B1, resulting in sudden death.

Mycotoxins attract worldwide attention because of the significant economic losses associated with their impact on human health, animal productivity and trade. Most of crop producers worldwide should be aware of guidance and regulations established on tolerance levels varied by region to mitigate mycotoxin contamination. The Food and Agricultural Organization (FAO) has worked with developing countries to mitigate mycotoxin contamination in foods and feeds [8]. FAO programs included advisory assistance, technical assistance and the implementation of maximum tolerance levels control of mycotoxins.

III. Processing of Soybean Based Feedstuffs

The main basis for the production of animal feed is balanced in terms of material formulation of nutrients and the quality control measures are followed at every stage of processing up to pelletizing.

Agricultural products for use as livestock feed ingredients shall undergo through strict quality control measures to avoid any form of contamination during processing stage. In terms of feed formulation, selective ingredients should be applied to compromise the energy, protein, carbohydrate, vitamin, minerals, and other additional requirements, such as texture, hygroscopic nature, maximum fibre content, and certain additives.

Marine protein sources commonly used is in the form of fish meals and approximately 30-50% (wt/wt of overall composition), depends on desired composition. Plant protein sources such as corn gluten and soybean, also can be added to improve overall nutrient contents. Carbohydrates in the form of cassava starch and corn starch are also added by 20-30% to the composition. The remaining ingredients include fatty meals, vitamins, and fibres.

Initial stage of producing feedstuffs into pellets involves simple mixing, literally by hands, and later to processing by conventional mixing equipment. Flowchart of processing soybean based feedstuffs is demonstrated in Fig. 1.

Pelletization of soybean based feedstuffs is done by continuous die presses, in which the mixed components are forced through small holes in the die, with average pellets diameter of 1-1.5 cm, according to the desired poultry diets.

TABLE I

Overall Composition For Processing Of Soybean Based Feedstuffs

Pellet Ingredients	**Weight Composition (% wt/wt)**
Soybean processing residue (dried and crushed)	40
Local fish meal	15
Corn gluten meal	15
Palm kernel cake	5
Potato starch	15
Neem leaves extract	5
Calcium carbonate	2.5
Palm oil	2.5
Water	Required quantity

IV. Pretreatment and Conditioning To Control Mycotoxin Contamination

A. Proximate analysis of soybean processed residue and pellets composition

An analysis of composition is carried out to develop clear overview on the composition of initial untreated soybean processed residue and processed soybean pellets. The composition is analysed at different processing stages of soybean based feedstuffs.

The resultant composition shown that initial untreated composition of soybean processed residue is low in protein and carbohydrate contents as described in Fig. 2. Soybean based feedstuffs which contains mixed components as described in Table I showed several folds increase in percentage composition of protein and carbohydrate. It is observed that protein content is increased from 14.9% to 53.6%, and carbohydrate content also increased from 5.1% to 18.2%, by adding several ingredients as described.

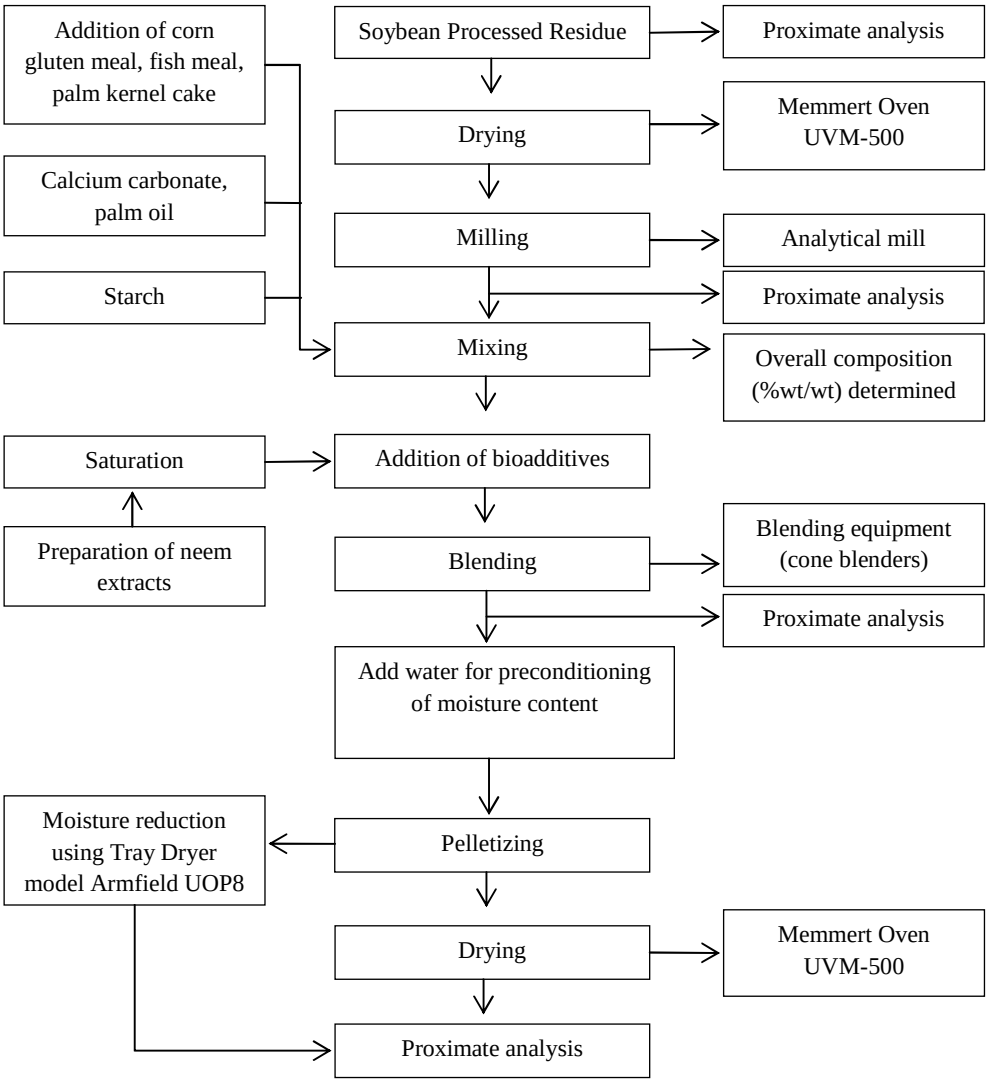

Fig. 1 Process flowchart of producing soybean pellet feedstuffs

The reduction of moisture content of the processed soybean based feedstuffs is done using tray drying. Soybean pellets feedstuffs are placed in trays, arranged vertically in stacking order in the tray dryer. During drying process, moisture is removed by the gradient temperature differences or vapour pressure.

In tray dryer model Armfield UOP 8, moisture content is removed by hot air through forced convection mechanism to low temperature region, wherein the moisture is condensed on cooler surface. Fig. 2 showed moisture content is reduced after materials mixing stage that requires addition of water for preconditioning to increase effectiveness of pelletization process. Prior to pelletization stage, the pellets are placed in the tray dryer to dry the pellets feedstuffs. The drying temperature is controlled below 50°C to prevent deterioration of important nutrients. Above 60°C, the main components such as carbohydrates and protein contents will be degraded.

The pellets are further dried in an oven (Memmert UVM-500) to achieve stable moisture control. However, it is observed that a slight increase in moisture content of the pellets after cooled at room temperature, from 5.16% to 7.41%. This is related to equilibrium moisture content acquired by the feedstuffs pellets at ambient condition.

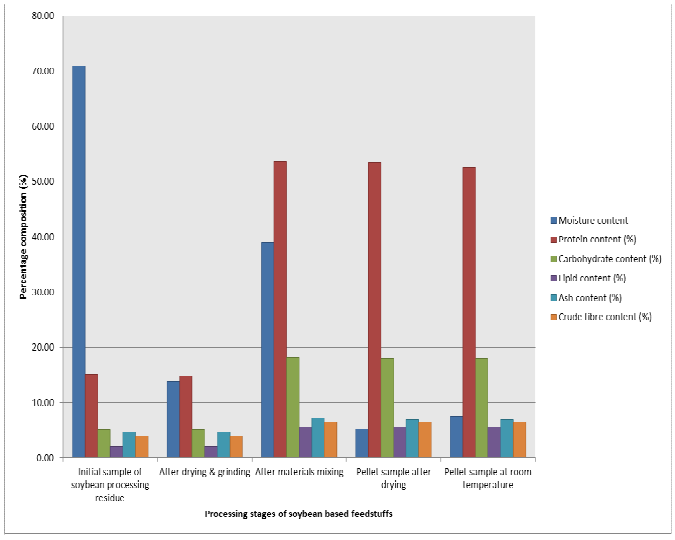

Fig. 2 Analysis of composition at different processing stages of soybean based feedstuffs

The pellets are further dried in an oven (Memmert UVM-500) to achieve stable moisture control. However, it is observed that a slight increase in moisture content of the pellets after cooled at room temperature, from 5.16% to 7.41%. This is related to equilibrium moisture content acquired by the feedstuffs pellets at ambient condition.

B. Additives effects on mycotoxin contamination

Feed additives is defined as substances which improve both feedstuffs in which they are incorporated and livestock production. It is prerequisite that they do not adversely affect either human or animal health, or environment. Feed performance is enhanced by additives like antimicrobial agents, binding agents, and enzymes.

By adding plant extracts from neem tree in processed animal feed, in this case, soybean pellets, therefore reducing the risk in encountering mycotoxin contamination. Neem extract is known to have inhibitory effects on fungi which capable of producing mycotoxin, from aflatoxin species. In this study, neem extract is added at 20% (v/v) in the soybean processed into pellets. Results in Table II showed that fungal growth is not visible in soybean pellets with neem extract additives.

TABLE II

FUNGAL GROWTH COUNT FOR SOYBEAN PROCESSED RESIDUE AND PELLETS SAMPLE

Type of soybean processing samples*	**Fungal growth count**
Control soybean processed residue	50-100
Control soybean processed pellets	50-100
Soybean pellets with additive (20% v/v)	Not visible
Soybean pellets (no additive)	30-50
Soybean pellets dried using tray dryer	Not visible
**Observation after 96 hours*	

To support this result, it is previously demonstrated by Bhatnagar and coworkers that neem extract significantly influence the regulation of the synthesis of the secondary metabolite enzymes involved in aflatoxin biosynthesis [9].

Further work is continued to investigate a possible correlation between aflatoxin biosynthesis and the activities of enzymes which are either directly or indirectly involved in aflatoxin biosynthesis. It is confirmed by monitoring changes in enzymes activities under conditions where aflatoxin biosynthesis is inhibited.

The neem extract at a concentration of 50% (v/v) caused more than 90% inhibition in aflatoxin production [10]. Following these investigation, it is further demonstrated that fungal growth is retarded which is associated to fungi cell wall destruction, according to their morphological findings [14].

C. Drying effects on fungal growth

Feedstuffs are dried as a preservation method from natural decay by depriving moisture necessary for microorganism such as fungi, to remain active.

During storage, mycotoxin production in human food and animal feed can be prevented by inhibiting the mycotoxin producing fungal from growing at the first place. In account to this, factors determining the ideal surrounding for mycotoxin producing fungal to grow are eliminated.

During processing stages of producing soybean pellets feedstuffs, the reduction of moisture content of the pellets is accomplished by 64% (final moisture content - 5%). From Table II, it is shown that fungal growth count in soybean pellets dried using tray dryer is not visible by physical inspection after 96 hours.

In this study, positive correlation between tray drying as preservation method against fungal growth has been achieved. Positive results showed no fungal growth on soybean processed pellets for animal feeds, which is highly hygroscopic in nature.

Drying technique is suitable for controlling moisture in animal feedstuffs, in the form of pellets. However, pellets size is a limiting factor for drying principle, as the surface area for drying process is limited. Thus pellets should be produced in equivalent size as commercial feedstuffs, to wholly meet the requirements for animal feeding diets and drying principle.

V. CONCLUSIONS

High quality animal feed production involves proper handling, storage, and processing, particularly free of contamination (such as mycotoxin) in their processing materials. Quality control measures strictly needed, briefly during sampling of each processing stages to evaluate their levels of contamination in complying with tolerance level of mycotoxin established by authorities.

The strategy to add in bioadditives in animal feed should be practiced by animal feed producers as it is highly effective and sustainable ways to control mycotoxin contamination from fungal growth. This approach is found to be cost-effective and safe in comparison with conventional ways to solve contamination problems, including ammonia treatment, chemical extraction, and irradiation technique.

During storage and distribution of animal feed, proper control of air humidity and ambient temperature of the storage area is necessary to avoid mycotoxin formation. Drying technique like tray drying is highly effective in controlling moisture of animal feedstuffs. Results shown that moisture content in soybean processed pellets have been brought to 5% from initially untreated soybean processing waste of 71% moisture content.

Upon study conducted, agricultural waste like soybean processing residue, could be produced in high quality in terms of nutritional value and shelf life by treated composition of soybean feedstuffs and appropriate moisture control. Neem extract has showed potential to be used as safe bioadditives in animal feed production, and efficient ways to control mycotoxin contamination.

NOMENCLATURE

%v/v	volumetric percentage	cm^3/cm^3
%wt/wt	weight percentage	kg/kg

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