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Fungal Toxins in Grains, Oilseeds and Other Agricultural Commodities Used in Feed And Food - A Review of Literature

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FUNGAL TOXINS IN GRAINS, OILSEEDS AND OTHER AGRICULTURAL COMMODITIES USED IN FEED AND FOOD - A REVIEW OF LITERATURE

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The bacteria and the molds are well known for the toxins that they produce. Of course, it is only a very small percent of the bacteria and molds that have the capacity to synthesize substances that are poisonous to man and other animals. The essential requirements for growth of a given organism and concomitant production of toxin are:

(1) Suitable nutrients, including especially source of energy, source of carbon, source of nitrogen, and presence or absence of oxygen.
(2) Suitable moisture level.
(3) Suitable temperature range.
(4) Mass inoculum of given organism.

When farm commodities have abnormally high moisture at harvest or subsequently become unusually moist, they become vulnerable to microbiological attack. Molds in general are able to grow at a lower moisture content than are bacteria, and we may ascribe the frequency of reports of mold poisoning in feeds, as compared to bacterial poisoning, to this fact. Dr. Joseph Forgacs, who studied fungal toxicity for many years, says "The critical moisture level necessary for germination of many fungal spores is approximately 14.5 percent. This corresponds to a relative humidity of 74-75 percent in the ambient air." Control of moisture offers the best means of controlling mold growth.

Temperature is not as important as moisture in the growth of mold on farm commodities. A temperature of 25 or 30° C. is often optimal but many molds proliferate at higher and lower temperatures. Higher temperature in the tropical and semi-tropical areas doubtless boosts the effect of moisture in accounting for abundant mold growth. Fungal toxicosis is referred to as not uncommon in southern United States.

How does inoculation of the feed or food material take place? It appears that contact with soil is the usual means of introduction of the toxin-producing mold. Nevertheless, one must remember that fungal

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spores are hardy and may be air-borne for great distances. It may be assumed that practically all of the mold growth and production of toxin take place during harvest or subsequently during storage of the commodity. (Ergot is a notable exception.)

One fact to be observed is that a given mold may grow -- and may produce toxin -- on different agricultural materials. For example, Aspergillus flavus can grow and produce toxin on peanut meal, on corn and on a wheat product. The molds found on stored corn pretty well duplicate those found on stored wheat. If a vigorous fermentation were required to produce a demonstrable amount of toxin, then poisoning might be expected to develop more often in highly nutritious oilseed meal than in hay, e.g. However, hay and forage material have furnished their quota of poisoning episodes. When there is a concatenation of required favorable factors for growth and activity of a given toxin-producing mold, poisoning can be brought about at different times, at different places, and with different commodity substrates.

One theory of the action of antibiotics in feed is that they counteract widely prevailing low-grade inimical bacterial infections in farm animals. We would not assume that molds are at large producing toxin in feeds and foods. Rather, we would consider the phenomenon as one of sporadic outbreak, at the same time acknowledging that doubtless not all of the culprits or all of the feed and food materials subject to their contamination have thus far been recognized.

The following review of the literature on fungal toxins in agricultural commodities is not exhaustive, but is intended rather to supply a perspective of the field without neglecting major factors of major contributions. Attention is restricted to toxins; the area of fungal infection (e.g. infection of the lungs with Aspergillus fumigatus) is disregarded.

Fungal Poison in Oilseeds

The great burst of scientific interest in and public concern with Aspergillus flavus and aflatoxin was stimulated in 1960 by the loss of an estimated 100,000 turkey poults in England in consequence of their being fed toxic peanut meal. Intensive investigation at the Tropical Products Institute in London, Central Vetinary Laboratory at Weybridge, Pest Infestation Laboratory at Slough, and the Microbiological Research Establishment at Porton as well as the Unilever Laboratories in both England and the Netherlands revealed the following salient points:

1. The toxin was produced by a mold, Aspergillus flavus.
2. The toxin was extremely potent, a lethal dose for a duckling being 20-50 micrograms.

3. The toxin to the extent of 0.5 p.p.m. was found in a great many specimens of peanut meal (30%). It is found in the oil of toxic nuts but this is removed by alkali washing in refining. The toxin could presumably find its way from toxic nuts into peanut butter. No toxin was found in selected high grade nuts.
4. The toxin is most easily detected by fluorescence under ultra-violet light after extraction with methanol or chloroform. It can be chromatographed on paper strips or thin layer preparations of alumina or silica gel. Negative tests exclude presence of toxin but positive tests must be confirmed by tests preferably on ducklings which are quite sensitive to the poison.
5. Two crystalling products have been obtained:

   Aflatoxin "B" $C_{17}H_{12}O_6$
   mp. 270°
   a_D - 562°
   LD_{50} (ducklings) 20 micrograms
   Blue fluorescence (UV)
   Mixture of 2 or more compounds

   Aflatoxin "G" $C_{17}H_{12}O_7$
   mp. 250°
   a_D - 533°
   LD_{50} (ducklings) 60 micrograms
   Green fluorescence (UV)
6. The symptoms of Turkey-X disease in turkey poults are: internal hemorrhage, convulsions, staggering, acute hepatic necrosis and bile duct proliferation. Not all species react the same or to the same degree, but the general pattern of the disease in all the species examined was that of a toxic hepatosis. Young animals, such as young pigs and young calves, are more susceptible than the adults of the species.
7. Liver tumors or evidence of precancerous lesions have been reported following feeding of toxic peanut meal or the toxin to rats.

Fatal poisoning in Indonesia by improperly prepared tempeh should be mentioned for the reason that the soybeans and mold culture ordinarily employed are not responsible. The toxin called toxoflavin is produced by Pseudomonas cocovenenas, an aerobic bacterium, in tempeh to which copra has been added to extend the soybeans during the fermentation normally carried out by strains of Rhizopus. Poisoning occurs in this product when

unsanitary conditions are involved, and usually where unskilled operators prepare the produce. The poisoning does not occur when only soybeans are involved. The fatal poisoning is called "bongkrek". The toxin was first isolated in 1933 and is highly toxic, having antibiotic activity. Numerous deaths of humans have been attributed to this poison. In 1961, Daves, et al., reported the chemical systhesis of the poison which has been named toxoflavin. It is a yellow crystalline solid and has the formula, $C_7H_7N_5O_2$. Interestingly, the same compound has been isolated as an antibiotic from a Streptomyces. A second poison formed by Pseudomonas cocovenenans is referred to as bogkrekic acid with a formula of $C_{29}H_{40}O_7$. It is said to be a highly unsaturated fatty acid.

In view of the documented evidence on poisoning of farm animals with moldy peanut meal, one raises the question as to whether the same thing can happen with soybean meal. No report of a poisoning outbreak with soybean meal is at hand. In the absence of such evidence, Richardson and associates (1962) at College Station, Texas, purposely caused soybean meal to mold. A large amount was divided into two lots. One lot was stored with 10 percent moisture at ambient temperature for six weeks. The second lot was stored with 19 percent moisture at 31°C (relative humidity of 78 percent) for six weeks. Growth of naturally-occurring fungi began in five to six days in the lot having 19 percent moisture. The predominant fungi are Penicillia and the Aspergillus glaucus group. The moldy lot was dried and fed as was also the unmolded (10 percent moisture) soybean meal. The poults fed the molded diet showed reduced growth and there was some mortality. Examination of the moribund poults showed toxic hepatitis and nephritis.

Work at NU on the production of tempeh showed that there is some substance in soybeans which inhibits the growth of the tempeh-producing mold, Rhizopus oligosporus, but it has not been determined that this inhibitory substance exerts appreciable inhibitation of mold growth in general. Obviously, much more investigation should be made of the possibility of mold poisoning in soybeans and soybean meal.

References

Toxicity associated with certain samples of groundnuts. M.C. Lancaster, F.P. Jenkins, and M. Mc.Philip. Nature 192: 1095-1096 (1961).

Toxicity associated with certain samples of groundnuts (continuation). K. Sargeant, A. Sheridan, J. O'Kelly, and R.B.A. Carnaghan. Nature 192: 1096-1097 (1961).

A method for the detection of aflatoxin in groundnuts and groundnut products. Tropical Products Institute, Department of Scientific and Industrial Research, T.P.I. Report No. 30, 5pp. and 1 fig. (1962)

The detection and estimation of aflatoxin in groundnuts and groundnut materials by a paper chromatographic procedure. Tropical Products Institute, Department of Scientific and Industrial Research, T.P.I. Report No. 36, 5pp. (1962).

Toxicity associated with certain batches of groundnuts. Anonymous. Report of the Interdepartmental Working Party on Groundnut Toxicity Research, Great Britian, IV + 8 pp + 1 pp. appendix A + 8 pp. appendix B (June 1962).

Toxic products in groundnuts. Biological effects. R. Allcroft and R. Carnaghan. Chemistry and Industry, No. 2, 50-53 (January 12, 1963).

Chemistry and origin. K. Sargeant, R.B.A. Carnaghan, and R. Allcroft. Chemistry and Industry, No. 2, 53-55 (January 12, 1963).

Groundnut microflora and toxicity. P.K.C. Austwick, and G. Ayerst. Chemistry and Industry, No. 2, 55-61 (January 12, 1963).

On the origin of the toxicity of toxoflavin. H. Latuasan and W. Berends. Biochem. Biophys. Acta. 52:503-508.

The total synthesis of toxoflavin. G.D. Daves, Jr., R.K. Robins, and C. C. Cheng. Journal of the American Chemical Society 83. 3904-3905 (1961).

Effect of moldy diet and moldy soybean meal on the growth of chicks and poults. L.R. Richardson, S. Wilkes, J. Godwin, and K.R. Pierce. J. Nutrition 78: 301-306 (1062).