Special Communication

AFLATOXIN-THE DEADLY POISON

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If you are eating groundnuts, you may have noticed a couple of fungus-ridden discoloured kernels. You eventually throw them away, but if these are evenly mixed such as in the case of pea-nut butter and served to you, you are taking a deadly poison. The unsual kernels may be contaminated with aflatoxin of which only about 20 microgram (one millionth of a gram) can cause the death of a duckling. In fact all animals and humans are affected by it. The young ones are more susceptible to it than adults.

What are Aflatoxins?

Aflatoxins belong to a group of mycotoxins which are produced by certain species of fungi. Mycotoxin is derived from the Greek *mykos* meaning fungus and Latin *toxicum* denoting poison. Fungi that elaborate toxins from food or feed are commonly called moulds. Although there are more than 100,000 species of fungi and hundreds of toxins are formed by them, the actual number of mycotoxins are relatively few. Aflatoxins are produced by moulds of certain species of the genus Aspergillus (*A. flavus and A. parasiticus*). Some other moulds are reported to produce aflatoxins but results have not yet been confirmed.

Aflatoxins were first discovered in groundnuts, but later it was known that aflatoxins contaminate a number of agricultural commodities, including cottonseed, corn, wheat, rice, sorghum and soybeans. The last named seems to be least susceptible to *A. flavus* growth. However, it may be pointed out that the presence of *A. flavus* series organisms does not necessarily indicate aflatoxins unless confirmed by chemical or biological tests.

A number of aflatoxins have been discovered and their chemical structures identified. The most important of these are Aflatoxins B_2, and G_2, the letters deriving from the colour of the fluorescence, blue or green, produced when exposed to ultraviolet (uv) light. When aflatoxin contaminated feed is consumed by milch animals, it is excreted into milk in the form of aflatoxin or Mn.

The Toxic Effects of Aflatoxins

Aflatoxins vary in their toxicity. The lethal dose of the four aflatoxins when given orally to a day-old duckling (50 g in weight) is for B_1 18 2 ug, G_1 39.2 ug, B_2 84.8 ug and G_2 172.5 ug. It shows that aflatoxin B_1 is the most G_2 toxic of all, almost 10 times as toxic as G_2. Unfortunately aflatoxin B_1 is more abundant than the other toxins.

The toxicity of aflatoxin has been demonstrated in insects, the turkey, goat, swaine, guinea pigs and cattle. Even lower forms, such as protozoa, are not immune to its toxicity. A day-old duckling is the most sensitive of all animals to aflatoxin and therefore employed for biological testing of the material. Sheep is more resistant to aflatoxin injury. In green plants it causes chlorophyl deficiency and the inhibition of seed germination.

In vertebrate animals the effects of feeding aflatoxin results liver damage. With long-term feeding of low levels of aflatoxin, malignant liver tumours develop. The only organ where aflatoxin is retained is the liver; the remainder of the aflatoxin is either excreted in urine or faeces or decomposed. In calves, levels of 100-300 parts per billion (ppb) of aflatoxin caused no apparent abnormalities but levels of 700-1000 ppb when fed for 4-6 months caused appreciable growth retardation, decreased feed efficiency and increased liver and kidney weights.

When the feed containing aflatoxin is given to milch animals, the milk contains aflatoxin M_1 and M_2. Like other aflatoxins they also induce liver damage in birds. The toxicity of aflatoxin M_1 and M_2 is 16 and 61.4 ug respectively as compared to 12 ur/duckling of B_1.

The findings that aflatoxins are extremely potent carcinogen has caused world-wide concern. Some of the studies carried out in India and Thailand have implicated aflatoxin in causing childhood cirrhosis. Similarly autopsy specimen of Thai children who died from acute encephalopathy and fatty degeneration of viscera showed varying levels of aflatoxins in the liver, viscera, brain, kidney and urine.

Aflatoxins have also been found to inhibit vitamin A utilization and interfere in protein synthesis. Children of the developing countries, including Pakistan, are known to suffer from vitamin A and protein deficiency diseases and therefore consumption of aflatoxin-contaminated food may aggravate the present situation.

Tolerance Limits of Aflatoxin

The question now arises: What are the tolerance limits of aflatoxins in various agricultural commodities? Due to insufficient data, it has not been possible so far to do so, but a level of 50 ppb or below is considered low in toxicity in animal feeds. The joint FAO/WHO Expert committee on Nutrition has recommended that the

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level of aflatoxin in protein supplements of which 100 g are eaten per day should not exceed 30 ppb. It may be noted that the groundnut meal involved in the turkey debacle in UK contained about 7000 ppb which is an extremely high figure. However, it will be safe to accept the FAO/WHO recommendation, though certain experts feel that for poultry a lesser figure (5-10 ppb) would be more desirable.

Control of Aflatoxin

The problem of the prevention and control of aflatoxin has attracted World-wide interest and a lot of work has been done and is currently in progress particularly in the United States. As already stated, aflatoxin is produced by moulds and they require for growth certain levels of temperature and humidity. Generally temperatures above 20° C and relative humidity of over 90% will provide favourable conditions for growth. These are very common in tropical countries. The moisture content of the material upon which the mould grows is also important. It should be below 8%. So the best control measure is to dry the material to below the critical moisture level before it is stored. It is important to dry all the material uniformly. If areas of higher moisture level exist, moulds will grow with the development of hot pockets; the moulded areas will enlarge subsequently owing to the release of additional moisture from the growth of moulds. Grains always carry mould spores that will germinate as soon as favourable conditions are provided to them.

When first lifted, groundnuts contain over 30% moisture, which provides an ideal environment for mould growth. The groundnut shell provides a good deal of protection, but unfortunately shell damage due to insect attack or mishandling is very frequent. Therefore, the first precaution to be taken is to dry the nuts soon after lifting. In the USA special dryers for groundnuts and other commodities are in frequent use to obviate damage due to mould growth.

Electronic sorting of foodgrains is now generally employed in developed countries to separate discoloured grains (which is mainly due to mould attack) from the normal ones. Other methods of physical separation, i.e. by means of cyclones, flotation etc. are also employed.

It is difficult to eliminate aflatoxin once it is developed in the material, but it is possible to check growth by various means. For example, it has been found that when carbon dioxide concentration is increased from 0.03% in the, air to less than 20%, mould growth was reduced. In 100% carbon dioxide, no growth occurred. Similarly reduction of oxygen from 5 to 10% checked mould growth. Recently treatment of contaminated material with ammonia solution has been found to reduce aflatoxin.

Work Done in Pakistan

About eight years ago when we were planning the utilisation of groundnuts as a source of protein for human feeding, apparently encouraged by the success of Indian Multipurpose Food, which constituted 75% by weight of groundnut flour, we examined a few samples of groundnut meal for aflatoxin contamination. To our surprise all samples contained aflatoxin and one of these sent to us by a reputed Oil Mill contained 1500 ppb. These results discouraged us from undertaking research on groundnut meal produced by our oil mills. This finding was reported by the author in the CENTO Panel Meeting on Human Nutrition held in Teheran in 1969. Prof. Kamaluddin Ahmad of Dacca University reported aflatoxin in varying degrees in all agricultural commodities.

Our interest in aflatoxin work was revived by the visit of Dr. Campbell and Dr. Rodericks of the Food and Drug Administration, USA in 1974 and on their advice immediate steps were taken to examine cereals and legumes used in the preparation of weaning food formulae developed in our laboratory. Since the best-quality grains are purchased for this purpose, aflatoxin B_1 has not yet been detected so far and only traces of G_1 were observed sometimes. In summer 1976 we received reports from various quarters of aflatoxin injury in poultry. Examination of samples of ingredients meant for poultry feed showed presence of aflatoxin. Corn seemed to be worst affected showing afllatoxin level ranging from 100-200 ppb. Wheat and wheat flour were found to contain very low level, less than 6 ppb but seeds of a leguminous weed associated with wheat contained 200 ppb. Other poultry feed ingredients such as blood meal, fish and meat meals, were also found to be contaminated. Aflatoxins of both B and G groups were observed though B_1 was noted to be predominant.

We are surprised to find reports of aflatoxin injury only recently. Surely the problems must have always existed, but it became apparent only due to unprecedented rains in 1976. Floods all over the country disrupted movement of all agricultural commodities and produced conditions-high ambient temperature and humidity-for optimal growth of. *Aflavus.* Although wheat examined by us contained very low levels of

aflatoxin, it is probable that higher levels could have been detected in low-grade wheat particularly those grown and stored in areas of high rainfall. Since wheat is the staple diet of the people of Pakistan, Government should take special precautionary measures to obviate danger of consumption of aflatoxin-contaminated wheat flour. Perhaps it may be advisable to analyse the wheat for aflatoxins before it is issued to flour mills.

We have now developed sufficient expertise for the evaluation of aflatoxins in different types of foods and feeds and both private and government agencies are approaching us for aflatoxin analysis. It is also planned to train workers in this field. For this purpose the first course in aflatoxin analysis, sponsored by the CENTO Scientific Co-ordinating Board, was arranged at the PCSIR Laboratories, Lahore in 1977.

Research Needs

Hitherto no organised work has been undertaken on the survey of our agricultural commodities with respect to aflatoxin. It is necessary to conduct such a survey of foods and feeding stuffs grown and stored under various environmental conditions. If areas of high aflatoxin contamination are identified, it would be desirable to check mortality due to liver damage in young children, if it is caused by intake of aflatoxin containing food. Most important area of research will be to devise ways and means of controlling production of mycotoxins in agricultural product.

A Word of Caution

Mycotoxins have been recently discovered and therefore there is at present world-wide interest in this field and a vast amount of literature has been accumulated during the last decade. There is now a growing tendency to incriminate aflatoxins particularly in animal feed. However, one should not lose sight of the fact that there may be other toxic factors such as saponins, glucosides, trypsin inhibitors, toxic pigment (gossypol) trace elements like lead and mercury, and pathogens etc. Even residues of insecticides may limit growth and cause mortality in animals. So in our zeal for investigating aflatoxins we should not forget to work for other common and well documented toxicants with which the feed may be possibly contaminated.