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ANTINUTRITIONAL FACTORS IN FEED INGREDIENTS AND THEIR EFFECTS IN FINFISH

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One of the important criteria in selecting feed ingredients for manufacturing complete and supplemental feeds relate to the presence of antinutritional factors, which can significantly reduce the nutritional value of the feeds. These anti-nutritive substances are often referred to as 'toxic factors' because of the deleterious effects they produce when eaten by animals. However, most of these produce sub-lethal effects such as reduced growth, poor feed conversion, hormonal changes and occasional organ damage.

Antinutritive substances can be broadly grouped into (1) those which are generated in the natural feedstuffs by the normal metabolism of the species from which the material originates; (2) the artificial antagonists such as preservatives, chemical additives, toxic compounds introduced as a result of different manufacturing processes, pesticides, herbicides and heavy metals and (3) natural contaminants such as mould, fungus and bacteria leading to the production of microbial toxins.

In the first group there are three main classes of substances: (1) Substances depressing digestion or metabolic utilization of proteins - protease inhibitors, lectins (haemagglutinins), saponins and certain polyphenolic compounds; (2) Substances reducing the solubility or interfering with the utilization of mineral elements - phytic acid, oxalic acid, glucosinolates and gossypol; (3) Substances inactivating or increasing the requirements of certain vitamins - Anti-vitamins A, D, E and K and anti-B vitamins - thiamine, nicotinic acid, pyridoxine and cyanocobalamin.

GROUP I

SUBSTANCES DEPRESSING DIGESTION OR METABOLIC UTILIZATION OF PROTEINS

(a) Proteast inhibitors:

They are distributed widely in plants especially among the legumes. In soyabeans two main groups are found, each of which is a mixture of proteins - the Kunitz and Bowman - Birk inhibitors. The Kunitz inhibitors have specificity towards trypsin, whereas, Bowman - Birk inhibitors inhibit both trypsin and chymotrypsin. Growth depressing effect was due to the slow release of methionine by proteolytic enzymes in the presence of trypsin inhibitors. In some species the release of all the amino acids have been affected by the inhibitors.

Growth inhibition has been reported for fingerling channel catfish (Robinson *et al.*, 1981) and rainbow trout (Sandholm *et al.*, 1976). Raw soyabeans and defatted soyaflour besides, inhibiting growth and reduce protein digestibility, depress metabolizable energy and fat absorption, cause pancreatic hypertrophy, stimulate hyper and hyposecretion of pancreatic enzymes, and reduce amino acid, vitamin and mineral availability (Rackis, 1974).

Autoclaving or heat processing of raw soyabeans destroys the trypsin inhibitors (Ham and Sandstedt, 1944). Extrusion cooking and infra-red cooking or micronization also is effective.

(b) Haemagglutinins (lectins):

These are found in both plant and animal tissues (Stockert et al., 1974). Mostly found in legumes. They are protein in nature and have high affinity for certain sugar molecules. Soyabean haemagglutinin is readily inactivated by pepsin and thus in animals with true stomach, the haemagglutinating fraction appears to be inactivated before feed enters the intestine. For stomachless fish and species which have low peptic activity haemagglutinin containing feedstuffs should be pretreated before usage.

Although very resistant to dry heat, they can be destroyed by cooking, autoclaving and micronization.

(c) Saponins:

They occur in a wide variety of plants and have three important characteristics: a bitter taste, foaming in aqueous solutions and haemolysis of red blood cells. On hydrolysis they yield sapogenins which are either steroids or triterpenoids and sugars. Their primary biological effect is an interaction with cellular and membranal compounds. They form complexes with cholesterol and has a blood-lowering effect in chicks. Dietary cholesterol has also been shown to reduce the growth - depressing effect of saponins. Cheeke and Oldfield (1970) showed that alfalfa saponins inhibit *in vitro* succinate oxidation by rat liver enzymes and also inhibit digestive enzyme secretion (Ishayya and Birk, 1965).

Their presence in feeds have been shown to affect food intake in chicks due to the bitter taste and affect the egg production. Their haemolysing effect on red blood cells is due to an interaction with cholesterol in the erythrocyte membrane.

(d) Polyphenolic compounds:

The most important are the tannins, which are polyphenolic substances with a molecular weight greater than 500. All cereals contain tannins; certain strains of sorghum contain upto 5%. Sal seed cake contains as high as 20% tannin and rapeseed meal is another rich source. Growth depression has been reported in rats and chicks due to the reduced protein and dry matter digestibility. Tannins seems to interfere with activity of trypsin and -amylase.

Chlorogenic acid is a polyphenolic compound which occurs in sunflower seed meal (about 1.2%). Has growth depressing effect and affect the feed utilization due to its inhibiting effect on the activity of proteinase, amylase and lipase. However, these effects can be counteracted by compounds with methyl donors - for example, methionine and choline (Singeleton and Kratzer, 1969).

SUBSTANCES REDUCING THE SOLUBILITY OR INTERFERING WITH THE UTILIZATION OF MINERAL ELEMENTS

(a) Phytic acid (phytate):

Plant phosphorus is found in the form of phytic acid, which is a cyclic compound containing six phosphate groups. Phytin forms a protein - phytic complex with zinc, manganese, copper, molybdenum, calcium, magnesium and lowers the dietary availability of these essential elements (Rackis, 1974). The addition of 0.5 percent phytic acid to purified diets fed to rainbow trout resulted in a 10 per cent reduction in growth and feed conversion (Spinelli et al., 1982). Protein digestibility and zinc availability has been significantly affected by phytin. High levels of vitamin D has been shown to improve the utilization of phytate phosphorus.

(b) Oxalic acid:

Certain plants like beet, spinach and seeds of sesame contain oxalic acid, and its main antinutritional effect is through complexing with calcium. Growth depression and reduction in calcium retention have been reported in animals.

(c) Glucosinolates (thioglucosides):

These are responsible for the pungent flavours found in condiments, horse-raddish, mustard and rapeseed meal. Their main biological effect is to depress the synthesis of thyroid hormones. The thyroid depressing effect is due to their reducing the incorporation of iodine into the precursors of thyroxine as well as interfering with its secretion. In young chicken 0.15 percent causes depression of growth. Hyperplasia and hypertrophy of the thyroid. Liver haemorrhage and enlargement of the liver and kidneys also reported in some mammals.

(d) Gossypol pigments:

These are found exclusively in the pigment glands of cotton seed. They are present in both oil and meal and exist in the free form or as a gossypol - protein complex. Whole seeds contain about 1.09-1.53 g/100 g, of which 0.19 g/100 g exists in free form. Decorticated seeds contain 0.15 g/100 g in free form. Dietary gossypol causes depressed growth and decreased utilization of feed for weight gain.

In fish anorexia and lipid deposition in the liver have been reported. (Wood and Yasutke, 1956). Roem <u>et al</u>. (1967) reported adverse effects on the growth of rainbow trout fed levels of 1000 ppm or higher but 250 ppm had little detrimental effect. Sinnhuber <u>et al</u>. (1968) found that dietary gossypol along with aflatoxin B to act as cocarcinogen in rainbow trout. In channel catfish fingerlings growth inhibition occurred when cotton seed meal greater than 17.4 percent was included in the diet (Dorsa <u>et al</u>., 1982). However, in glandless cotton seed gossypol is absent.

In higher animals reduction in haemoglobin, has been reported. Other physiological effects include reduced appetite and loss of body weight, reduced oxygen-carrying capacity of blood and adverse effect on certain liver enzymes (Chubb, 1982).

SUBSTANCES INACTIVATING OR INCREASING THE REQUIREMENT OF CERTAIN VITAMINS

This group consists of natural organic compounds which can either destroy certain vitamins or combine with them to form unabsorbable complexes or interfere with their digestive or metabolic utilization.

Anti-thiamine - has been reported in rice bran, mustard seed, cotton seed and the greatest source is raw fish. Most of the freshwater fish mussels **and clams** contain high levels of thiaminase, whereas a saltwater fish contain relatively less quantities. Thiaminase can be inactivated by heat.

Anti-nicotinic acid - Niacinogen present in maize has been shown to combine with nicotinic acid thereby making it resistant to enzyme digestion, leading to pellegra

Pyridoxine antagonist – Linseed meal contains 0.002 to 0.005% of a pyridoxine antagonist, 1 – amino – D – proline, which occurs naturally in combination with glutamic acid as a peptide called linatine. This substance can be extracted with water and can be destroyed by autoclaving.

Anti-vitamin B_{12} – Raw soyabeans by autoclaving shown to contain a heat-labile substance which accentuates the requirement for vitamin B_{12} in rats, but the causal factor is not yet identified.

Anti-Vitamin A – Raw soybeans contain the enzyme lipoxygenase which destroys carotene and lowers the levels of vitamin A. This enzyme is destroyed by heating for 15 min. with steam under atmospheric pressure.

Anti-vitamin D – Protein isolated from unheated soyabean has been shown to produce rickets in turkeys. Autoclaving this protein eliminated this rachitogenic effect.

Anti-vitamin E – Found in kidney beans and cause muscular dystrophy in chicks and lambs. It can be destroyed partially by autoclaving.

Alkaloids: Pyrrolizidine alkaloids are toxins found in many plants. At 100 ppm in the diet caused severe growth depression and mortality in rainbow trout (Hendricks et al., 1981). When fed at 2 ppm these toxins caused severe hepatic lesions.

Cyanogens: Cyanide in trace amounts is fairly widespread in the form of glucosides, and relatively high levels can be found in certain grasses, pulses and root-crops. Tapioca contains relatively high levels (53 mg/100 g to while collecting plankton from the wild and fed to larvae of fish and shellfish care must be taken to exclude toxin containing algae.

Toxic algae, such as Gonyaulax sp. and Gymnodinium breve are avidly eaten by some molluscs that store toxins in their tissues (Sparks, 1972). Incorporating the contaminated molluscs into feeds for finfishes or crustaceans could result in toxicity.

In addition to algae, metabolites or streptomycetes and actinomycetes have been shown to cause muddy off-flavour in channel catfish in the U.S. (Lovell, 1979).

GROUP II

ANTINUTRIENTS RESULTING FROM NATURAL CONTAMINATION OF FEEDSTUFFS (MAINLY MICROBIAL IN NATURE

Animal by-products and fish meals are often contaminated with Salmonella. There are approximately 1200 serotypes of Salmonella of which 80 serotypes have been associated with diseases in animals. Their effect on fish and crustaceans need elucidation.

Raw materials and to a lesser extent finished feeds contain considerable number of bacteria, yeasts and moulds. Whenever these have a moisture content greater than the accepted 'dry level' the potential for bacteria, yeast and mould to propagate exists and can result in poor performance of the animals. This can arise from three distinctly different causes.

(i) The microbes may be pathogenic and cause an infection ex:bacterium - Pathogenic serotypes of Salmonella, the yeast Candida spp. and fungus Aspergillus sp.

(ii) Microbial action can alter the nutritional status of the product. Mould growth can cause fustiness which results in reduced palatability. Microbial action can destroy or make unavailable to the animal certain vitamins-- for example the B complex vitamins or the fat-soluble vitamins A, E and D.

(iii) Mould growth can result in the production of mycotoxins, which can cause a wide range of pathological and physiological effects in fish.

Mycotoxins can be produced from any stage from growing crop to the formulated feed as indicated in Fig. A list of feed ingredients with demonstrated natural contamination by aflatoxin are given in Table . If proper production and storage conditions are maintained mycotoxins will not be produced in the formulated feed.

In order to control the mycotoxin accumulation, removal of damaged and discoloured particles is suggested to reduce the overall concentration in finished feeds. Another way of reducing the animals exposure is to reduce the percentage incorporation of the raw materials into the formulated feed with reference to safe-concentrations. In oilcakes aflatoxin can be destroyed by ammoniation and monomethylamine treatment. The best way to prevent problems with aflatoxins and other mold - produced mycotoxins is to store feedstuffs under conditions of low humidity and temperature.

Symptoms of mycotoxin depend upon the amount of toxin in the feed, the period for which the feed is ingested, the nutritional status of the feed and the susceptibility of the animal. There are four important groups of mycotoxins, aflatoxins, ochratoxin A, zearalenone and the tricothecenes.

Aflatoxins:

These are the best documented mycotoxins. There are mainly four compounds named B_1, B_2, G_1 and G_2. Aflatoxin is a potent liver toxin and a carcinogen with aflatoxin B_1 being the most toxic compound. In trouts high incidence of hepatoma was reported by Wolf and Jackson (1963) when fed in a cotton seed meal diet and the causative agent was found to be aflatoxin B_1 (Halver, 1967). In trout hepatoma occurred in a dosage as low as 0.5 ppb of aflatoxin (Ashley et al., 1965; Sinnhuber et al., 1965). Rainbow trout are extremely sensitive. But brook trout and Coho salmon are less sensitive to aflatoxin ingestion (Wolf and Jackson, 1967; Halver et al., 1969). Warmwater fishes also seem to be less sensitive. Ashley (1966) and Friedman and Shibko (1972) reported that channel catfish fingerlings showed a relatively low response when fed aflatoxin in doses up to 100 mg/kg body weight. Thus there seems to be significant differences in susceptibility of fish to aflatoxin. This calls for intensive research on the effect of aflatoxins on cultivated finfish and crustaceans of India.

The carcinogenicity of aflatoxins has been affected by several other dietary factors. Cyclopropenoic fatty acids and gossypol present in cotton seed meals have been shown to serve as cocarcinogens with aflatoxin in rainbow trout (Lee et al., 1968; Sinnhuber et al., 1968). The presence of dieldrin, a pesticide, in a diet with 6 ppb aflatoxin B, increased the incidence of hepatocellular carcinomas (Hendricks et al., 1979).

Ochratoxin:

Produced by species of the genera Aspergillus and Penicillium. Ochratoxin A affects the proximal kidney tubules causing nephropatty. It is teratogenic to many animals. This is mostly reported from temperate countries. Recently rice bran and soyabean meal from tropics also have been found to contain this toxin. Decreased growth and egg production and diarrhaea have been reported as symptoms in poultry.

Zearalenone:

In an oestrogenic mycotoxin produced by several Fusarium species and occurs in many agricultural commodities, affect the reproductive system.

Tricothecenes:

Also produced by Fusarium fungi and four compounds have been known to be associated with animal disease, deoxynivalenol, nivalenol, T-2 toxin and diacetoxy-scirpinol.

Wu and Sonekha (1978) reported mycotoxin - producing fungi associated with dry shrimp. Friedman and Shibko (1972) identified 27 mycotoxin - producing fungi in dry shrimp. T-2 toxin proved lethal to rainbow trout at levels of 6 mg/kg body weight (Marasas et al., 1967). Feed refusal and vomiting syridromes have been observed in fish.

GROUP III

CHEMICAL CONTAMINANTS

The most common contaminants are organo-chloride pesticides, DDT, DDE, dieldrin, endrin and industrial chemicals such as polychlorinated biphenyls (PCB), phthalate esters and hexachlorobenzene (HCB) and heavy metals.

Heavy metals:

The toxicity of dietary heavy metals will depend upon the level of those metals in the water as well as the hardness of the water (Spear and Pierce, 1978; Carrol et al., 1979) and its pH (Reed et al., 1980). The levels of metal chelators in the diet also affect the heavy metal toxicity.

The U.S. Food and Drug Administration recommend a safe level of 0.5 ppm of dietary mercury. Selenium has been found to have protective effect against the toxicity of methyl mercury (Friedman et al., 1978). However, selenium itself has been found to be toxic to rainbow trout at a dosage of 13 µg/g of food (Hilton et al., 1980). Chronic poisoning in animals occur in dosages ranging 2 to 20 mg/g (Lo and Sindi, 1980).

Cadmium is a potentially toxic metal for fish and shellfish (Smith et al., 1976). Seaweeds and molluscs have been found to accumulate cadmium in levels as high as 20,000 to 1,000,000 times to that of seawater (McLeese, 1980). The inclusion of these items in feeds should be based on prior knowledge of their cadmium levels.

Ashley (1972) noted that Coho Salmon tolerated copper at 1 mg/g dry diet but with retarded growth and impaired pigmentation. Low levels of dietary silver (0.5 mg/kg dry weight) has been shown to protect juvenile lobsters (Homarus americanus) from growth depression and mortality associated with high levels (greater than 16 mg/kg dry weight) of copper (Chou et al., 1982).

Polychlorinated biphenyls (PCBs)

These organic chemicals are widely used as plasticizers and in hydraulic fluids. PCBs are poorly biodegraded, tend to accumulate in lipids and bioconcentrated. A PCB dosage of 14.5 mg/kg body weight resulted in 100 percent mortality of coho salmon after 260 days (Mayer et al., 1977). Sublethal effects of PCB in fish include liver enlargement, alterations in liver structure, induction of hepatic microsomal enzymes, increased thyroid activity (NRC, 1981). The maximum permitted concentration in finished feeds is 0.2 ppm.

Pesticides and Herbicides:

Pesticides and herbicides tend to bioaccumulate or bioconcentrate (NRC, 1981). Their toxicity is usually greatest in young fish, causing dysplasia or sterility of gonads, weakness, nervous disorders, loss of appetite and death (Ashley, 1972). Crustaceans are particularly highly sensitive to most pesticides.