Animal Nutrition UG Course Topic Name

"Harmful Natural Constituents and Common Feed Adulterants"

Harmful natural constituent is a substance which under practical circumstances can impair some aspect of animal metabolism and produce adverse biological or economic effects in animal production. Virtually, everything is toxic, including oxygen, water and all nutrients, if given in a large enough doses. Thus, the term "toxicant" refers only to those substances which might normally be encountered at toxic levels. Natural toxins may reduce the availability or usability of nutritious feedstuffs, or may necessitates the use of costly feed processing techniques to eliminate their effects.

The anti-nutritional factors (ANFs) may be defined as those substances generated in natural feed stuffs by the normal metabolism of species and by different mechanisms which exert effects contrary to optimum nutrition.

Natural toxicants can be classified on the basis of their chemical structure. With some exceptions most are distributed among the following broad categories: alkaloids, glycosides, proteins, amino acids and derivatives, carbohydrate, lipids, glycoproteins, glycolipids, metal binding substances, resins, phenolics etc. In addition to these, it is convenient to consider mycotoxins as a specific category.

Classification of Harmful Natural Toxicants by chemical structure:

1. <u>Alkaloids</u>:

Alkaloids (alkali-like) are compounds that contain nitrogen, usually in a heterocyclic ring. They are usually bitter and toxic in nature. These compounds are of particular interest since many of them have poisonous properties. In plants, their presence is restricted to a few orders of the dicotyledons. A number of the more important alkaloids, with their sources. The alkaloid in ragwort, for example, attacks the liver and much of this organ can be destroyed before symptoms appear. Another nutritionally significant source of alkaloids is the fungus ergot, which grows on cereal grains.

Some important alkaloids occurring in plants

Name	Source
Coniine	Hemlock
Nicotine	Tobacco
Ricinine	Castor plant seeds

Atropine	Deadly nightshade
Cocaine	Leaves of coca plant
Jacobine	Ragwort
Quinine	Cinchona bark
Strychnine	Seeds of *Nuxvomica*
Morphine	Dried latex of opium poppy
Solanine	Unripe potatoes and potato sprouts

2. <u>Glycosides</u>:

It contains glycan (carbohydrate moiety) and a non-carbohydrate moiety (aglycone) joined with an ether bond. It is usually bitter substances. They are classified on the basis of the structure and properties of the aglycone.

i. Cyanogenic glycosides:

- They yield hydrocyanic acid (prussic acid) when hydrolysed. It is hydrolysed by Beta-glycosides to release HCN, glucose and benzaldehyde.
- However, the glycosides occur in vacuoles in plant cell and enzymes are found in the cytosol. Damage to the plant results in the enzymes and glycoside coming together and producing HCN.
- The hydrolytic reaction can take place in the rumen by microbial activity. Hence ruminants are more susceptible to cyanogen toxicity than non-ruminants.
- The HCN is absorbed and is rapidly detoxified in the liver by the enzyme rhodanase which converts CN to thiocyanate (SCN).
- Excess cyanide ion inhibits the cytochrome oxidase. This stops ATP formation; tissues suffer energy deprivation and death follows rapidly.
- Drying of cyanogenic leaves may reduce the risk of cyanide toxicity. Cattle are more susceptible to HCN poisoning than sheep, horse and pig. Immature green Jowar, sudan fodder and linseed may cause HCN toxicity.
- Animals suffering from cyanide poisoning must be immediately treated by injecting intravenous 3 g sodium nitrate and 15 g sodium thiosulphate in 200 ml distilled water for cattle and for sheep 1 g sodium nitrate and 2.5 g sodium thiosulphate in 50 ml distilled water.

- Some glycosides such as Amygdaline (Almond), Dhurrin (Jowar and other immature grasses), Linamarin (Linseed, cassava, pulses).

ii. Goitrogenic glycosides:

- It decreases production of the thyroid hormones (Thyroxine, T4 & Triiodothyronine, T3) by inhibiting their synthesis by thyroid gland. As a result, the thyroid enlarges to compensate for reduced thyroxin output, producing goitre.
- Goitrogenic glycosides are commonly found in Brassica spp. such as cabbage, turnip, kale, rapeseed, mustard green etc.
- They are hydrolysed by glucosinolates to Beta-D-glucose, $HSO4^-$ and derivatives of the aglycone (isothiocyanates, nitriles, thiocyanates). Glucosinolates are formerly called thioglucosides.
- Myrosinase is released from plant tissue by crushing (mastication) and is also produced by rumen microorganisms.
- Glucosinolates are always accompanied by the enzyme myrosinase (thioglucosidase) which are capable of hydrolyzing them to thiocynates and isothiocynates to venyloxazolidinethione which is potently goitrogenic causes depressed iodine uptake and liver damage.
- Ruminants are less susceptible than pig and poultry.

Brassica anaemia factor; plants in the *Brassica* genus, such as kale, rape, cabbage, cauliflower and turnips are important livestock feeds. Two types of sulphur containing compounds limit the feeding value of these brassica crops. These are the glucosinolates and an amino acid, S-methylcysteine sulfoxide. The SMCO is a fairly rare amino acid, found only in brassica, garlic, and onion. In the brassica it may occur at levels as high as 4-6% of the dry matter. It leads to RBC hemolysis and anemia. SMCO is probably not the primary haemolytic agent. It is metabolized in the rumen, producing dimethyl disulfide. Dimethyl disulphide is an oxidant that attacks the red cell membrane. It is inactivated by reacting with reduced glutathione (GSH), producing methylmercaptan. The toxic principle in onions is n-propyl disulfide and SMCO. This compound is an oxidant that will cause RBC hemolysis and Heinz-Ehrlich bodies. It is reduced by the glutathione peroxidise system previously described. Garlic contains S-allylcysteine sulfoxide, which is metabolized to allyl disulfide oxide. The reputed cholesterol lowering properties of garlic may be due to a reaction of the disulfide group with the sulfydryl group of CoA, leading to inhibition of lipid synthesis.

iii. Coumarin glycosides:

- Found in sweet clover (Melilotus spp.) as melilotoside. Coumarin is converted by mould growth to dicoumarol an antagonist of vitamin K.
- Sweet clover poisoning, caused by feeding mouldy sweet clover hay, is therefore an induced vitamin K deficiency.

iv. Steroid and Triterpenoid glycosides:

a) Cardiac glycosides: Best known CG is digitonin, contained in foxgloves (digitalis spp.). Physiologically, they are potent stimulators of heart rate and are used medicinally.

b) Saponin:

- Saponins are glycosides containing a polycyclic aglycone moiety of either C27 steroid or C30 triterpenoid (collectively termed as sapogenins) attached to a carbohydrate.
- They are widely distributed in the plant kingdom. Saponins are characterised by a bitter taste and foaming properties.
- Erythrocytes lyse in saponin solution and so these compounds are toxic when injected intravenously. In non-ruminants (chicks and pigs), retardation of growth rate, reduction in feed intake occurs. In ruminants, saponins were implicated in causing bloat.
- The adverse effects of saponins can be overcome by repeated washing with water which makes the feed more palatable by reducing the bitterness associated with saponins.

v. Vicine: Vicine is a glycoside in Fava beans (*Vicia faba*). It causes haemolytic anemia (favism) in people who have a genetic deficiency of glucose-6-PO4-dehydrogenase activity in their RBC. Fava beans are being utilized as a protein supplement for livestock.

vi. Isoflavones: Which are called phytoestrogens, contain a flavones nucleus. Ex- genistein, formononetin and coumestrol. It causes reproduction problems in ruminant especially sheep.

3. <u>Proteins</u>: Several important inhibitors in plants are proteins. In some case, the effect of these are to inhibit the utilization of other proteins by animals.

i. Protease (Trypsin) and Amylase Inhibitor:

- Inhibitors of enzymes, such as trypsin, chymotrypsin, carboxpeptidases, elastase appear in many food products, including legumes, cereals, potatoes, and tomatoes.

- Adverse effects following short- and long-term ingestion of raw soybean meal (the richest source of dietary trypsin inhibitors) by mammals and birds on protein utilization and growth have been attributed to the presence of soybean trypsin inhibitors. Protease inhibitors fall into 2 main categories:

1. **Kunitz inhibitor** that have a molecular weight of about 21.5 kilodalton with 2 disulfide bridges and possess specificity directed mainly against trypsin.

2. **Bowman-Birk inhibitor** that have a molecular weight of about 8 kilodalton with a high proportion of disulfide bonds and the capability of inhibiting chymotrypsin and trypsin at independent binding sites.

- Among common food and feed products, soybeans are the most concentrated source of trypsin inhibitors.
- Protease inhibitors make up 0.2-10.0% of total seed protein of edible dry beans of various species. Levels of trypsin inhibitors (mainly as the Kunitz trypsin inhibitor) in soybeans have been reported to vary from 17-48 mg/g sample or from 37-123 mg/g protein.
- Owing to their protein in nature, protease inhibitors can be inactivated by the heat-processing method, such as extrusion, infrared radiation, micronizing, autoclaving, steam processing, or flaking.

Mode of action of trypsin inhibitors:

- The feeding of raw soybean preparations or isolated inhibitors from soybeans cause an enlargement of the pancreas in susceptible animals, which could be described histologically as hypertrophy, that is, an increase in the size of the acinar cells of the pancreas.
- Concomitant with this increase in the size of the pancreas was an increase in the secretion of digestive enzymes, including trypsin, chymotrypsin, and elastase. This provided support to the hypothesis that the growth depression caused by the trypsin inhibitors was the consequence of an endogenous loss of amino acids in the form of enzymes being secreted by a hyperactive pancreas.
- The pancreatic enzymes, such as trypsin and chymotrypsin, are particularly rich in sulfur-containing amino acids. Therefore, the effect of a hyperactive pancreas would be to divert these amino acids from the synthesis of body tissue protein to the synthesis of these enzymes, which are subsequently lost in the faeces.

- The trypsin inhibitor-induced pancreatic hypertrophy/hyperplasia observed in susceptible animal species has been explained by a negative feedback mechanism in which enzyme secretion is inversely related to the level of trypsin present in the small intestine.
- Therefore, when the level of active trypsin in the gut is depressed due to the presence of the inhibitor, the pancreas would respond in a compensatory fashion by producing more enzymes.
- The mediating agent between trypsin and the pancreas has been reported to be the hormone cholecystokinin (CCK), which is released from the jejunal endocrine cells when the level of trypsin in the small intestine becomes depleted.
- Protein and/or amino acid digestibility have been reported to be negatively affected in animal by high levels of dietary trypsin inhibitors.

Amylase inhibitors occur in beans and have been commercialized as "Starch blockers" to decrease obesity in humans.

ii. Hemagglutinins (lectins):

- Hemagglutinins, otherwise referred to as lectins, are proteins which agglutinate red blood cells. Soyabean lectin strongly binds to mannose of RBC and cause agglutination of cells.
- They have been shown to occur in some important fodder trees. The highest concentrations of lectins are found in seeds but, in the leaves, their concentration is low due to translocation.
- The biological effects of lectins probably result from their affinity for sugars. They may bind to the carbohydrate moieties of cells of the intestinal wall and cause a non-specific interference with nutrient absorption.
- In fodder trees, the lectins of interest are robin and ricin. Robin, a lectin from *Robinia pseudoacacia*, has been reported to cause symptoms of anorexia, weakness and posterior paralysis in cattle.
- The castor bean (*Ricinus communis*) is grown commercially as a source of castor bean oil which is used as an industrial lubricant and for medicinal purposes. The seed press cake, and foliage are poisonous and not used as a livestock feed but the oil is non-toxic.

iii. Enzymes:

- An example of an enzyme toxin is thiaminase, found in bracken fern (*Pteridium aquilinum*) and certain fish such as Carp. Enzyme cleaves the B vitamin thiamine and inactivating it. Such thiamine deficiency (Chastek's paralysis).

- Other enzymes in feeds which produce deleterious effects in livestock includes Lipoxidases in soybean and alfalfa, which degrade fat soluble vitamin.

4. <u>Amino Acids and Amino Acid Derivatives</u>:

i. Amino Acid:

(a) Mimosine (toxic amino acid), a non-protein amino acid structurally similar to tyrosine, occurs in *Leucaena leucocephala* forage plant.

- Concentration of mimosine in the leaf is about 2–6% and varies with seasons and maturity. In non-ruminant animals, mimosine causes poor growth, alopecia (loss of hair), eye cataracts, reproductive problems and is metabolised in the rumen to a goiterogenic compound, producing goitre in ruminants.
- Levels of Leucaena meal above 5–10% of the diet for swine, poultry and rabbits generally result in poor animal performance.
- Mechanism of action of mimosine in producing its effect is not clear but it may act as an amino acid antagonist or may complex with pyridoxal phosphate, leading to disruption of catalytical action of B6-containing enzymes such as trans-aminases, or may complex with metals such as zinc.
- The main symptoms of toxicity in ruminants are poor growth, loss of hair and wool, swollen and raw coronets above the hooves, lameness, mouth and oesophageal lesions, depressed serum thyroxine level and goitre. Some of these symptoms may be due to mimosine and others to 3, 4 dihydroxypyridine (DHP), a metabolite of mimosine in the rumen.

(b) Avidin, a glycoprotein in egg albumin, is an antagonist of B vitamin Biotin. Raw eggs can be used to induce biotin deficiency in experimental animals.

5. <u>Carbohydrates</u>:

There are few toxicity problems due to carbohydrate xylose, a hexose sugar, cause decrease growth and eye cataracts in pigs and poultry. Raffinose are not digested in small intestine and so, promote bacterial growth in the hind gut. These are the flatulence factors in beans. The Beta- glucans in certain barley varieties sometimes cause nutritional problems in poultry.

6. <u>Lipids</u>:

Several fatty acids are toxic. There includes erucic acid in rape seed. Cyclopropenoid fatty acid, such as sterculic and malvalic acids in cottonseed, have toxic properties and cause pink albumins to develop in stored eggs. They are also cocarcinogens, increase the carcinogenicity of aflatoxins. Trans fatty acid is unsaturated fatty acids of naturally occurring fats generally have a cis configuration of their double bonds. Trans isomer may have hypercholesterolemic and carcinogenic effect in human.

7. <u>Metal binding substances</u>:

i. Oxalates: It is a chelating agent which chelates Ca very effectively. Plants with a high oxalate content may produce acute metabolic Ca deficiency (hypo calcemia) when consumed by livestock. Oxalic acid converted to Ca-oxalate.

ii. Phytates: Phytic acid in cereal grains and soyabean meal causes reduced mineral availability, particularly zinc, through the formation of unabsorbable phytates. Organic P (phytin P) is of low availability to non-ruminant animals. Phytate is synthesized in plants by successive phosphorylation of inositol. Breakdown of phytate involves successive dephosphorylation by phytases present in plants, microorganisms, and certain animal tissues. Phytase supplementation improves the digestibilities of proteins, amino acids and apparent metabolizable energy for poultry.

iii. Mimosine: Toxic amino acid in *Leucinea leucocephala* is reputed to have metal binding properties.

8. <u>Resins</u>:

Soluble in numbers of organic solvents, insoluble in water, and donot contain nitrogen. Examples are cicutoxin, a poisonous principle of *Cicuta spp*. (water hemlock). It is one of the most spectacular poisons known, acting directly on the CNS to produce violent convulsion.

9. <u>Phenolic compounds</u>:

Phenolics contain an aromatic ring with one or more hydroxyl groups.

i. Gossypol:

- Gossypol is a phenolic compound found in pigment glands of cotton seed (*Gossypium* spp.).

- In animal feeding, the main concern from a toxicological point of view is with free gossypol. The bound gossypol is physiologically inactive, but because it is bound to protein and particularly lysine, it reduces the biological value of the protein.
- The physiological effects of free gossypol are as follows; olive green yolks in hen's eggs, depress appetite and growth, ascites and tissue edema, cardiac lesions and male infertility may happen.
- Gossypol form complex with metal like iron and cause iron deficiency in livestock. It is prefer to supplement iron source as ferrous sulphate when fed with cotton seed cake containing diet to livestock.

ii. Tannins:

- The term tannin is derived from the use of plant extracts (e.g., oak leaves) to tan leather.
- Tannin have the ability to precipitate protein, forming hydrogen bonds with the protein, with hydrophobic binding also contributing to the stability of the protein-tannin complex.
- Mainly there are two types of tannins, condensed and hydrolysable tannin.
- Condensed tannin is not readly hydrolysed and are often of complex structure, formed from the condensation of flavanols such as catechin and epicatechin.
- Hydrolysable tannins consist of esters of glucose with gallic acid or related compounds. They can readly hydrolysed by hot mineral acid to yield the sugar core. They are astringent and adversely affect feed intake.
- Low to moderate concentrations of condensed tannin precipitate soluble plant proteins and thus protect them against degradation in the rumen, but if the proteins are too firmly bound to the tannins they are not digested in the small intestine.
- By protecting proteins from hydrolysis in the rumen, they increase amino acid absorption from the small intestine.
- They also modify gas production in the rumen, thereby reducing the danger of bloat and possibly reducing methane production.
- Condensed tannins are also claimed to restrict the growth of gut parasites.

10. <u>Mycotoxins</u>:

- Metabolites of fungi (molds) that is toxic to animals.

- There are at least 25 specific disease entities in livestock's that can be attributed to mycotoxins.
- Toxigenic species within the three dominant genera, Aspergillus, Penicillium and Fusarium, occupy a wide range of habitats, sometimes alone, sometimes in competition.
- The most common chronic effects in livestock are decreased growth rate and feed intake.
- Subclinical effects occurring in the liver, kidneys, gastrointestinal tract may be responsible.
- Diagnosis of mycotoxicoses is generally imposible from clinical signs or post-mortem examination or residues in tissues or excrement.
- Some toxic effects are secondary, such as for aflatoxin B1 and T2 toxin which interfere with the immune system, increasing the susceptibility to infectious diseases.
- They are implicated in diverse condition such as acute death in poultry (Turkey X-disease), liver cancer, lupinosis, sweet clover poisoning, facial eczema of sheep, ryegrass staggers and ergotism.

11. <u>Other toxins</u>:

i. Plant Carcinogens: Carcinogenic effects in livestock due to consumption of a poisonous plant is bladder and intestinal cancer in cattle consuming braken fern. Some of the Pyrolizidine alkaloids have carcinogenic properties.

ii. Trimethylamine oxide and formaldehyde: Occurs in the flesh of certain types of marine fish and impair iron absorption when the fish are used in the diet of fur animals. Iron deficiency signs such as reduced growth, anemia and loss of hair pigmentation (achromotrichia) are observed.

iii. Nitrates:

- Nitrate toxicity in ruminant is also known as "Oat hay poisoning".
- Oat hay poisoning is attributed to the relatively large amounts of nitrate present in green oats.
- Quite high levels of nitrate have been reported in herbage given heavy dressings of nitrogenous fertilizers.
- Nitrate per se is relatively non-toxic to animals. The toxic effect in ruminants is caused by the reduction of nitrate to nitrite in the rumen. Nitrite, but not nitrate, oxidises the ferrous iron of haemoglobin to the ferric state, producing a brown

pigment, methaemoglobin, which is incapable of transporting oxygen to the body tissues.

- Toxic signs include trembling, staggering, rapid respiration and death.
- Nonruminants can tolerate nitrate but ruminants do not because the rumen bacteria convert nitrate to nitrite.
- It has been reported that toxicity may occur in animals grazing herbage containing more than 0.7 g nitrate-N/kg DM (2.8 g NO3), although the lethal concentration is much higher than this.
- Acute nitrate toxicity is treated with I/v methylene blue solution (2 to 4 mg/kg to 15 mg/kg BW i/v in 1% solution) or ascorbic acid (reducing agents) which accept electrons for NADPH reductase in blood and accerate the reconversion of methaemoglobin to functional haemoglobin. Oral dose of mineral oil (1 lit. For adult cattle) or 500 g sodium sulphate saline drip per cattle as supportive therapy

Classification of natural toxicants by their occurrence in feeds:

Most feedstuffs and forages used in the feeding of livestock contain potentially deleterious factors. Indeed, it is rare to find feedstuffs in which a deleterious factor cannot be identified. Even the ubiquitous chlorophyll can have adverse effects on animals, causing photosensitization reaction under certain condition. A listing of some of the more common and important toxicants in feedstuffs is provided to illustrate their widespread distribution and to provide ready access to potential deleterious factors in common feeds;

Natural Toxicants in common feedstuffs:

Feedstuffs	**Toxicants**
Grains	
All	Phytates, Mycotoxins
Rye, Triticale	Trypsin inhibitors, Ergot
Milo	Tannins
Tubers	
Potato	Solanum alkaloids
Cassava	Cyanogenic glycosides

Protein supplements	
Soybean	Trypsin inhibitors, Lectins, Goiterogens, Saponins, Phytates, Mycotoxins.
Cottonseed	Gossypol, Tannin, Cyclopropрnoid fatty acid, Mycotoxin
Rapeseed / Mustard oil cake	Glucosinolates, Tannins, Erucic acid, Sinapine
Linseed meal	Linamarin, Linatine
Fava beans	Tripsin inhibitors, Vicine, Lectins
Field beans	Trypsin inhibitors, Lectins
Forages	
Legumes:	
Alfalfa	Saponins, Phytoestrogens, bloating agents
White clover	Cyanogens, Phytoestrogens, bloating agents
Red clover	Slaframine, Phytoestrogens, bloating agents
Sweet clover	Coumarin
Leucaena spp.	Mimosine
Grasses:	
Tropical grass, Paddy straw	Oxalates
Forage Brassicas	Brassica anemia factor

Management of Toxicosis:

Toxicosis in livestock may involve spectacular acute effects or insidious conditions of a chronic nature. Buck et al. (1976) describe a management plan for toxicologic emergencies, as follows:

- Institute the necessary emergency and supportive therapy to keep the animal alive.
- Establish a tentative clinical diagnosis on which to base therapy.
- Institute the appropriate remedial and antidotal procedures.
- Identify the toxic agent as rapidly as possible.
- Determine the source of the toxin.
- Counsel the livestock owner on the hazards of the implicated toxicant, and provide instruction for the avoidance of the problem in the future.

<u>Common adulterants in feed and fodder</u>:

Adulterant can be defined as intentional admixture of a pure substances with some low-quality substances for earning more profit.

Feed ingredients	Adulterants
Groundnut cake	GNC husk, urea, UNCF cakes
Mustard cake	*Argimona maxicana* seed, urea, UNCF cake
Soyabean meal	Urea, hulls
DORB, Wheat bran	Ground rice hulls, saw dust
Fish meal	Common salt, urea
Mineral mixture	Common salt, marble powder, sand, lime stone
Molassses	Water
MBM	Leather meal, blood meal, sand
DCP	Calcite powder, rock phosphate

Feed adulterant can be checked in lab. by different methods:
1. Chemical analysis 2. Bioassay assessment (less common method) 3. Feed microscopy