MODULE II

ANTI-NUTRITIONAL FACTORS

Anti-nutrients or anti-nutritional factors (ANFs) may be defined as those substances generated in natural feedstuffs by the normal metabolism of species and by different mechanisms (for example inactivation of some nutrients, diminution of the digestive process or metabolic utilization of feed) which exerts effect contrary to optimum nutrition. Anti-nutritional factors are substances which either by themselves or through their metabolic products interfere with feed utilization and affect the health and production of animal or which act to reduce nutrient intake, digestion, absorption and utilization and may produce other adverse effects.

The main ANFs that interfere with nutrient digestion and absorption are lectins, protease inhibitors, tannins, antigenic proteins, phytic acid, glucosinolates and gossypol. For some ANFs lectins and tannins in particular, their proper characterisation and analysis are the main limitations towards a better understanding of their effects on animals.

ANFs can be classified in various ways. The following classification, based on their effects on the nutritive value of feedstuffs and on biological responses in animals, can be suggested:

i. Factors that have a depressive effect on protein digestion and utilisation (trypsin and chymotrypsin inhibitors, lectins or haemagglutinins, polyphenolic compounds, NSP-s and saponins).

ii. Factors which have a negative effect on the digestion of carbohydrates (amylase inhibitors, polyphenolic compounds, NSP-s, flatulence factors).

iii. Factors which have a negative effect on the digestion and utilisation of minerals (glucosinolates, oxalic acid, phytic acid, gossypol).

iv. Factors which inactivate vitamins or cause an increase in the animal's vitamin requirements (anti-vitamins).

v. Factors that stimulate the immune system (antigenic proteins)

The type and content of ANFs in different types of feedstuffs vary considerably. Moreover, many feedstuffs contain several ANFs, and the amounts of ANF can vary considerably between batches of the same feedstuff. The latter variation can be attributed to the plant's growing conditions as well as to genetics; different varieties can have different levels of ANF. In legume seeds (soya beans, peas and beans) protease inhibitors and lectins are the most important ANFs. However, some varieties of cereal grains, rye and triticale in particular, may also contain moderate levels of trypsin inhibitors. Tannins are mainly present in the coloured-flowering varieties of vicia faba beans, peas, rapeseed (canola), sorghum and some varieties of barley. Glucosinolates and sinapins are important in rapeseed; alkaloids in lupins, and gossypol in cottonseed.
Common types of anti-nutritional factors include:

1. **Tannins:** Tannin is an astringent, bitter plant polyphenolic compound that either binds or precipitates proteins and various other organic compounds including amino acids and alkaloids. Tannins are the most widely occurring antinutritional factors found in plants. These compounds are present in numerous tree and shrub foliages, seeds and agro-industrial by-products. Tannins have a property of binding to protein to form reversible and irreversible complexes due to the existence of a number of phenolic hydroxy groups. Tannins are water soluble phenolic compounds with a molecular weight greater than 500 and hydrolysable tannins and condensed tannins are two different groups of these compounds. The two types differ in their nutritional and toxic effects. The condensed tannins have more profound digestibility-reducing effect than hydrolysable tannins, whereas, the latter may cause varied toxic manifestations due to hydrolysis in rumen.

Tannins are heat stable and they decreased protein digestibility in animals and humans, probably by either making protein partially unavailable or inhibiting digestive enzymes and increasing fecal nitrogen. Tannins are known to be present in food products and to inhibit the activities of trypsin, chemotrypsin, amylase and lipase, decrease the protein quality of foods and interfere with dietary iron absorption. Tannins are known to be responsible for decreased feed intake, growth rate, feed efficiency and protein digestibility in experimental animals. If tannin concentration in the diet becomes too high, microbial enzyme activities including cellulose and intestinal digestion may be depressed.

2. **Saponins:** Saponins are steroid or triterpenoid glycosides that are present in many feedstuffs. They have a bitter taste, can form foams in aqueous solutions and haemolyse red blood cells. They are known to depress growth performance in both poultry and swine. Their antinutritional properties seem related to their ability to form complexes with sterols, in particular those in membranes of animal cells. This appears to result in increased permeability of the intestinal mucosa. Poultry, compared to other monogastrics, are more sensitive to saponins. Significant saponin levels are present in alfalfa meal with minor levels in other legumes such as soya beans, rapeseed and various varieties of peas. In general saponins are of minor concern in monogastric animals because they are present at only low levels in common feedstuffs.

3. **Alkaloids:** Alkaloids are compounds that contain nitrogen in a heterocyclic ring, are generally basic and often have a bitter taste. The word alkaloid simply means 'alkali-like'. Alkaloids are present in many plants whereby they are thought to serve as a chemical defence against herbivory. Lupins in particular, contain high levels of alkaloids, specifically quinolizidine alkaloids. In some instances soya beans and linseed may be contaminated with Datura stramonium. The latter seed contains the alkaloids hyoscyamine and scopolamine. Alkaloids are oxidized in the liver resulting in metabolites, such as dehydrosparteine, which are responsible for the observed toxicity. The level of toxicity is influenced by the structure of the alkaloids. There is a high degree of variation in the ability of different animal species to deal with these compounds. The toxic
effects of alkaloids and their metabolites are primarily mediated through the nervous system, although they are also stimulate copper uptake by liver cells thus leading to copper toxicity. Pigs appear more sensitive to alkaloids than poultry.

4. **Alkaloids**: Alkaloids are one of the largest groups of chemical compounds synthesised by plants and generally found as salts of plant acids such as oxalic, malic, tartaric or citric acid. Alkaloids are small organic molecules, common to about 15 to 20 per cent of all vascular plants, usually comprising several carbon rings with side chains, one or more of the carbon atoms being replaced by a nitrogen. They are synthesized by plants from amino acids. Alkaloids are considered to be anti-nutrients because of their action on the nervous system, disrupting or inappropriately augmenting electrochemical transmission. For instance, consumption of high tropane alkaloids will cause rapid heartbeat, paralysis and in fatal case, lead to death. Uptake of high dose of tryptamine alkaloids will lead to staggering gate and death.

5. **Mycotoxins**: Mycotoxins are those secondary metabolites of fungi that have the capacity to impair animal health and productivity. The diverse effects precipitated by these compounds are conventionally considered under the generic term “mycotoxicosis”, and include distinct syndromes as well as nonspecific conditions. Mycotoxin contamination of forages and cereals frequently occurs in the field following infection of plants with particular pathogenic fungi or with symbiotic endophytes. Contamination may also occur during processing and storage of harvested products and feed whenever environmental conditions are appropriate for spoilage fungi. Moisture content and ambient temperature are key determinants of fungal colonization and mycotoxin production. It is conventional to subdivide toxigenic fungi into “field” (or plantpathogenic) and “storage” (or saprophytic/spoilage) organisms. Claviceps, Neotyphodium, Fusarium and Alternaria are classical representatives of field fungi while Aspergillus and Penicillium exemplify storage organisms. Mycotoxigenic species may be further distinguished on the basis of geographical prevalence, reflecting specific environmental requirements for growth and secondary metabolism. Thus, Aspergillus flavus, A. parasiticus and A. ochraceus readily proliferate under warm, humid conditions, while Penicillium expansum and P. verrucosum are essentially temperate fungi. Consequently, the Aspergillus mycotoxins predominate in plant products emanating from the tropics and other warm regions, while the Penicillium mycotoxins occur widely in temperate foods, particularly cereal grains. Fusarium fungi are more ubiquitous, but even this genus contains toxigenic species that are almost exclusively associated with cereals from warm countries.

6. **Aflatoxins and Gossypol**: This group includes aflatoxin B1, B2, G1 and G2 (AFB1, AFB2, AFG1 and AFG2, respectively). In addition, aflatoxin M1 (AFM1) has been identified in the milk of dairy cows consuming AFB1-contaminated feeds. The aflatoxigenic Aspergilli are generally regarded as storage fungi, proliferating under conditions of relatively high moisture/humidity and temperature. Aflatoxin contamination is, therefore, almost exclusively confined to tropical feeds such as oilseed by-products derived from groundnuts, cottonseed and palm kernel. Aflatoxin contamination of maize is also an important problem in warm humid regions where A. flavus may infect the crop prior to harvest and remain viable during storage. Gossypol pigment in cottonseed occurs free and bound forms. In whole seeds, gossypol exists essentially in the free form, but
variable amounts may bind with protein during processing to yield inactive forms. Free gossypol is the toxic entity and causes organ damage, cardiac failure and death. Cottonseed meal fed to bulls can induce increased sperm abnormalities and decreased sperm production.

7. **Protease Inhibitors:** Protease inhibitors are widely distributed within the plant kingdom, including the seeds of most cultivated legumes and cereals. Protease inhibitors are the most commonly encountered class of anti-nutritional factors of plant origin. Protease inhibitors have the ability to inhibit the activity of proteolytic enzymes within the gastrointestinal tract of animals. Due to their particular protein nature, protease inhibitors may be easily denatured by heat processing although some residual activity may still remain in the commercially produced products. The anti-nutrient activity of protease inhibitors is associated with growth inhibition and pancreatic hypertrophy.

8. **Phytate / Phytic acid:** Phytic acid is the acid form of the anion phytate (myo-inositol hexa phosphate). Phytic acid cannot be hydrolysed by enzymes secreted into the gut by mammals and birds. Phosphorus present in phytic acid has a low bio-availability. Moreover, phytate can form complexes with a variety of minerals, including calcium, copper, cobalt, iron, magnesium, manganese, selenium and zinc, thus reducing the availability of these nutrients. Phytic acid can also form complexes with basic residues of proteins and therefore it may interfere with the activity of endogenous enzymes and digestibilities of nutrients other than minerals.

9. **Lectins:** Lectins, or haemagglutinins, are proteins that are generally present in the form of glycoproteins. They vary considerably in their molecular weight and chemical structure and are characterized by an ability to bind to specific sugars. Glycoproteins in the gut wall contain sugars to which lectins have affinity and, as a result, binding of lectins to epithelial cells occurs. This leads to growth depressions. A prerequisite for the anti-nutritional properties of lectins is resistance to proteolysis. A variety of lectins exist, both within and between different types of plant seed, which have differing effects on the animal. In general, the lectins in common beans are highly toxic, while lectins in peas and faba beans appear to be the least toxic. Furthermore, different animals may respond to the same lectins in different ways; piglets appear to be more sensitive than rats and chickens. Haemagglutination of red blood cells is most commonly used to measure lectin activity. This is based on the ability of lectins to bind to sugars on the surface of the red blood cells. However, this assay appears not to be very specific and it is inaccurate in predicting the effects of lectins on animals. An additional effect of lectins is that they stimulate the proliferation of bacteria in the intestinal lumen. The exact reason for this is not clear, although it may be related to increased nutrient availability to the bacteria and an increase in epithelial cell turnover, which may then increase the number of potential binding sites for bacteria on epithelial cells.

10. **Glucosinolates:** Glucosinolates are present in all cruciferous seeds and plants. Over a hundred glucosinolates have been identified. Of these, less than ten are of concern to animal nutritionists, in particular those present in rapeseeds and rapeseed meal. Glucosinolates can be categorised into two main groups: the aliphatic types and indole types. Their quantities in feedstuffs can be determined by gas chromatography. These ANFs are hydrolysed by the enzyme myrosinase present in cruciferous seeds and plants, although bacteria that are present in the
gastrointestinal tract also contribute to the hydrolysis of glucosinolates. Hydrolysis of glucosinolates yields glucose, various goitrogenic compounds (thiocyanates, isothiocyanates and oxazolidinethione), and nitriles. The thiocyanate ions in particular, inhibit the uptake of iodine by the thyroid gland for the production of triiodothyronine (T3) and thyroxine (T4) leading to lower plasma levels of these compounds. The severity of the effects of glucosinolates or their degradation products in animals is influenced by a number of factors including species, age and the growth status of the animal.

Anti-nutritional factors are eliminated or reduced in feed stuffs through each or combine effects of two main processing ways i.e heat treatment and fermentation.

**BENEFICIAL EFFECTS OF ANTINUTRIENTS**

Excessive degradability of proteins could be reduced by the use of heat and various chemicals. The use of heat is very expensive, thus most often the chemicals, such as formaldehyde, are used. However, the formaldehyde is carcinogenic and, in addition, its incorrect use can lower the absorption of amino acids from intestine. Another way to protect proteins against excessive degradation in the rumen is the use of tannins, which form reversible complexes with proteins. These complexes are not degraded at pH values present in rumen, but they disintegrate at pH values of the abomasum and small intestine. The positive of tannin in animal feeding includes; increased efficiency of protein utilization, reduction of parasite burden, reduction of proteolysis during ensilage, bloat prevention, increase quality of animal products, reduction of n emission into the environment and defaunation rumen. Condensed tannins (CT) have improved live weight gain, wool production and reproductive efficiency in sheep fed temperate forages and reduced the impact of gastro-intestinal parasitism. However, their value is also linked to environmental issues, such as reducing nitrogen pollution from animals grazing lush pastures with a high nitrogen content and lessening methane emissions from rumen fermentation.

Saponins have shown a variety activities such antitumor, cholesterol lowering, immune potentiating, anticancer, antioxidants and to presser lower risk of implicated in coronary heart diseases, and saponins potential as ointment hydrocarbon to shape of first collagen. Potential beneficial effects of protease inhibitors remain unclear, although lower incidences of pancreatic cancer have been observed in populations where the intake of soybean and its products is high. While protease inhibitors have been linked with pancreatic cancer in animal studies, they may also act as anti-carcinogenic agent.