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DEPARTMENT OF ANIMAL NUTRITION

ANTINUTRITIONAL FACTOR
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Antinutritional Factors- may be defined as those substances present in the diet which by themselves or their metabolic products arising in the system interfere with the feed utilization, reduce production or affects the health of the animal. These anti-nutritive substances are often referred to as "toxic factors' because of the deleterious effects they produce when eaten by animals.

Toxic substances of natural origin can be classified based on their chemical properties and on the basis of their effect on utilization of nutrients.

A. According to their Chemical Properties :

Group I: Proteins-	1. Protease inhibitor
	2. Haemagglutinins (Lectins)
Group II : Glycosides	1. Saponins
	2.Cyanogens
	3. Glucosinolates (Goitrogens) or Thioglucosides
Group III : Phenols	1. Gossypol
	2. Tannins
Group IV: Miscellaneous	1. Antimetals
	2. Antivitamins

B. On the Basis of Nutrients they Affect Directly or Indirectly-

1. Substances depressing digestion or metabolic utilization of proteins.

a. Protease inhibitor (Trypsin and Chymotrypsin inhibitor)

b. Haemagglutinins (Lectins)

c. Saponins

d. Polyphenolic components

2. Substances reducing solubility or interfering with the utilization of minerals-

a. Phytic acid

b. Oxalic acid

c. Glucosinolates (Thioglucosides)

d. Gossypol

3. Substances increasing the requirements of certain vitamins.

a. Anti-vitamin A, D, E, K.

b. Anti-vitamin B_1, B_6, B_{12} and nicotinic acid.

4. Substances with a negative effect on the digestion of carbohydrates

a. Amylase inhibitors

b. Phenolic compounds

c. Flatulence factors.

ά -Amylase inhibitors: It has been reported that **ά** -amylase inhibitor is responsible for the impaired digestion of starch from kidney beans.

Flatulence factors: Flatulence factors are related to oligosaccharides which are fermented by intestinal bacteria in the large intestine. These oligosaccharides are not broken down in the small intestine because of a lack of appropriate enzyme, and subsequently enter the large intestine and broken down by **bacterial ά-1, 6-galactosidase.** The monomers of these sugars are converted into VFA, carbon dioxide, hydrogen, methane, resulting in flatulence, diarrhoea, nausea, cramps and discomfort.

5. Substances that stimulate the immune system (antigenic proteins):

Antigenic proteins in feed or raw materials are macromolecular proteins or glycoproteins capable of inducing a humoral immune response when fed to animals. In this case humoral immune response means the synthesis of specific polyclonal antibodies secreted in body fluids, such as blood, for eliminating the antigenic protein. The humoral immune response, together with the cellular immune response, forms the specific immune system, which is one of the defence mechanisms of man and animals to keep the integrity of the body. As a result of the specific immune response man and animals can become immune to the antigen.

When the antigen is an infectious microbial agent immunity is a desired effect. In the case of a feed antigen it is questionable whether an immune response attractive. In case of feed antigens, man and animals are continuously exposed. This continuous exposure increases the chance that the immune response develops into an acute hypersensitivity reaction (causing tissue damage) or into a chronic hypersensitivity reaction. Chronic hypersensitivity is common in case of feed antigens. Antigenic globulins of soybean are **glycinin and ß-conglycinin.**

Brief Description of Antinutritional or Toxic Factors-

Group - I Proteins

1. Protease inhibitors

E.g. Legume seeds: Soybean, kidney bean, mung bean.

Protease inhibitors are two types:

a. Kunitz inhibitor (inhibits only trypsin) and **b**. Bowman - birk inhibitor (inhibits trypsin and chymotrypsin). The inhibitory substances are mostly heat labile and thus proper heat treatment inactivates the protease inhibitors. The important factors controlling trypsin inhibitor destruction are: 1. temperature 2. duration of heating 3. particle size and 4. moisture level. The trypsin inhibitor activity of solvent extracted SBM was destroyed by exposure to steam for 60 minutes, or by autoclving under the following conditions. 5 psi for 45 min, 10 psi for 30 min and 15 psi for 20 min. duration.

2. Haemagglutinins(Lectins)- Soybean, Castor bean (ricin) and other legume seeds contain haemagglutinins. Although resistant to destruction by dry heat, lectins are destroyed by the same conditions as those used to inactivate protease inhibitors i.e. by steam.

Group - II Glycosides-

1. **Saponins:** legume forages saponins lower the surface tension of ruminal contents leading to accumulation of gas in the digesta. This condition is known as "bloat". This is also known as tympany/tympanitis. It is characterised by the distension of the rumen due to accumulation of gas (CO_2 and CH_4). The negative effect of saponins might be because of their well-known effect as a surface-active component on the biological membrane by which the permeability of the intestinal mucosal cells is increased and the active nutrient transport hindered.

2. Cyanogens: Sorghum and Sudan fodder, linseed may develop toxic levels of HCN in the new growth that follows either a period of drought, or a period of heavy trampling or physical damage by frost, etc. Heavy nitrate fertilisation followed by an abundant irrigation or rainfall may increase the potential of HCN poisoning of these crops. Excess cyanide ion can quickly produce anoxia of the central nervous system inactivating the cytochrome oxidase system and death can result within a few seconds. injected intravenously with 3 g sodium nitrate and 15 g sodium thiosulphate in 200 ml H_2O for cattle; for sheep 1 g sodium nitrate and 2.5 g sodium thiosulphate in 50 ml H_2O

There are three distinct glycosides-

	Glycoside	**Plant source**
1.	Amygdalin	Bitter Almonds
2.	Dhurrin	Sorghum vulgare and other immature grasses
3.	Linamarin or phaseolunatin	Linseed, cassava, Java beans (*Phaseolus lunatus*)

3. Glucosinolates: Most plants of crucifera family (cabbage, turnips, rutabaga, rapeseed and mustard green) contain these substances. Their main biological effect is to depress the synthesis of the thyroid hormone (Thyroxine, T_4) and Triiodothyronine (T_3), thus producing goitre, although the latter is not caused by the glucosinolates per se but by their products of hydrolysis. Ruminants appear to be less susceptible to the toxic effect of glucosinolates compared to pigs and poultry. This is probably the result of the glucosinolates being relatively unhydrolysed in the rumen.

Group-III Phenols-

1. Gossypol: In genus Gossypium, gossypol is present in pigment glands of leaves, stems, roots and seeds. It is highly toxic to monogastric animals. Pigs and rabbits are more sensitive than poultry. Horses are resistant. Ruminants are more resistant due to the formation of stable complexes with soluble protein in rumen which are resistant to enzymatic breakdown. Whole cotton seed contain a total of 1.09 to 1.53%, of which free form is 0.19%. The physiological effects of free gossypol are reduced appetite, loss of body weight, accumulation of fluid in the body cavities, cardiac irregularity, reduced oxygen carrying capacity of the blood (reduced haemoglobin content) and an adverse effect on certain liver enzymes. Although heat treatment as in the commercial production of cottonseed meal decreases the content of free gossypol, the availability of lysine is reduced because of the interaction of the aldehyde groups of gossypol with the amino group of lysine.

2. Tannin:- It is polyphenolic substance with molecular weight greater than 500.
Tannins are of two types-

A. Hydrolysable tannins: These can be readily hydrolysed by water, acids, bases or enzymes and **yield gallotannins** and **ellagitannins.**

B. Condensed tannins: These are Flavonoids-polymers of **flavonol**. Both hydrolysable and condensed tannins are widely distributed in nature. **Chlorogenine** is a polyphenolic compound present in **sunflower seed meal.** Tannins may, therefore, have a beneficial effect (increasing bypass protein or decreasing ammonia loss) or a detrimental effect (depressing palatability, decreasing rumen ammonia, decreasing post-ruminal protein absorption) on protein availability.

Detannification of Feedstuffs or Methods of detannification: The methods available for removal/ inactivation of tannins can be divided into two main categories:

1. Physical treatments: Soaking and cooking decrease the tannin content. However, these treatments, cause a substantial loss of dry matter between 20 to 70%. Anaerobic storage of moist sorghum grains for two and nine days resulted in 40% and 92% reduction in tannins, respectively.

2. Chemical treatments: Addition of tannin complexing agents like polyethylene glycol (**PEG**) and polyvinylpyrrolidone (**PVP**) prevent formation of complexes between tannin and protein as well as break the already formed complex thus liberating protein. Alkalies, formaldehyde, organic solvents like acetone, acids, oxidizing agents ($KMnO_4$, Potassium dichromate, alkaline H_2O_2), ferrous sulphate reduce the tannin content.

3. Simple methods based on post-harvest technology, treatment with low-cost chemicals, biological treatments, and supplementation with tannin-complexing agents are developed to enhance the feeding value of tannin-containing feeds.

4. H.P.S. Makkar did series of experiments on chemistry and estimation of tannins and on several detannification methods. Oak leaves are rich in tannins and young oak leaves cause toxicity. Although consumption of mature oak leaves does not normally lead to the death of animals, their consumption impairs production. The detoxification (by inactivation or removal of tannins) methods employed in case of oak leaves may also be followed to any feed resource rich in tannins.

1. **Wood ash:** Wood ash is a good source of alkali. A 10% of solution of oak wood ash decreased the content of total phenols, condensed tannins and protein precipitation capacity by 66, 80 and 75% in oak leaves.

2. **Chopping and storage: The 'chopping of leaves and then storage'** has been found practical application at the farmer's level. instead of feeding the leaves on the same day as they are lopped, they need only be chopped and stored for about 5-10 days before feeding. The higher extent of inactivation of tannins by chopping of leaves could be due to oxidation of tannins by **phenol oxidases** present in leaves, as chopping is expected to increase the availability of tannins to the enzyme. In addition, inactivation of tannins during storage was due to their polymerization to higher 'inert polymers.

3. Drying: Drying of mature oak leaves under different conditions (60° C for 48h, 90°C for 24h, shade drying for 1 day, 2 days and 3 days and sun drying for 24h, 48h) had no effect on the levels of total phenols, condensed tannins, protein precipitation capacity, degree of polymerization, specific activity of tannins and bound condensed tannins. On the other hand, drying conditions (90°C for 24h) decreased tannin content in cassava and leucaena leaves. One of the reasons for this

difference was found to be different level of moisture in these leaves. Cassava and leucaena leaves had about 65% moisture whereas oak leaves had 40%. Increase of moisture of oak leaves followed by the heat treatment decreased tannin levels.

4. **Solid state fermentation**: Biodegradation of tannins using white- rot fungi (*Sporotricum pulverulentum, Ceriporiopsis subvermispora* and *Cyathus steroreus*) is a promising approach.

5. **Tannin complexing agents:** Polyethylene glycol, a non-nutritive synthetic polymer having high affinity for condensed tannins, makes them inert by forming PEG-tannin complex. Polyvinylpyrrolidone (PVP) is also used as tannin-complexing agent. These tannin-complexing agents are also considered to break 'the already formed tannin-protein complexes' since their affinity for tannins is higher than for proteins. **The affinity of PVPs for tannins was lower than of PEG**. The **PEG 6000** may be preferred for inactivation of tannins in feedstuffs as its binding to tannins was highest at near neutral pH values.

Group-IV-

1. **Antimetals: Substances depressing the utilization of minerals**.

- **Phytic acid:-** Phytic acid is an ester formed by combination of the **6 alcoholic groups of inositol with 6 molecules of hexa- phosphoric acid. Hence its name inositol hexa-phosphoric acid**. Phytic acid concentration is more in the rind (pericarp + aleurone layer) and the embryo than the core (endosperm). Phytic acid depresses the utilization of several mineral elements such as Ca, Mg, Fe, Zn, etc. It forms insoluble compounds which are eliminated in the faeces.

- **Oxalic acid:-** The greater part of oxalic acid in plants is present in the form of soluble oxalates (potassium, sodium and ammonium oxalates) and only 10-20% appears as insoluble calcium and magnesium oxalate especially within the cells. Ageing as well as over ripening of vegetables is accompanied by an increase in the proportion of calcium oxalate. Oxalate poisoning in cattle and sheep are characterised by rapid and laboured respiration, depression, weakness, coma and death.

2. Antivitamins:- These are organic compounds which either destroy certain vitamins or combine and form unabsorbable complexes or interfere with digestive and/or metabolic functions.

a. Antivitamin A: **Raw soybean** contains enzyme lipoxygenase which can be destroyed by heating 5 min with steam at atmospheric pressure. **Lipoxygenase** catalyses oxidation of carotene, the precursor of vitamin A.

b. Antivitamin E: **Lipoxygenase** destroys vitamin E also. Present **in kidney bean**. Diets with raw kidney beans produced muscular dystrophy in chicks and lambs by reducing plasma vitamin E. Autoclaving destroys the factor.

c. Antivitamin K: Eating mould infected sweet clover cause fatal haemorrhagic condition in cattle. This is known as "Sweet clover disease". **Dicoumarol** present in sweet clover is responsible for this. Dicoumarol reduce prothrombin levels in blood and affects blood clotting .

d. Antivitamin D: **Rachitogenic** activity of isolated **soya** protein (unheated) has been found with chicks and pigs. Autoclaving eliminates this rachitogenic activity.

e. Anti-pyridoxine: An antagonist of pyridoxine from **linseed** has been identified as **1-amino-D-proline**. It occurs naturally in combination with glutamic acid as a peptide and it is called linatine. Nutritive value of linseed meal for chicks can be considerably improved after water treatment and autoclaving.

f. Antiniacin: An antagonist of niacin**, niacytin** is found in maize, wheat bran, etc. which cause perosis and growth depression.

g. Antithiamine: The enzyme **thiaminase** present in bracken fern (*Pteridium aquilinum*) acts as antithiamine factor by destroying it.

Nitrate Poisoning-

Nitrate per se is not toxic. Nitrate poisoning or '**Oat hay poisoning**' in cattle is due to the consumption of nitrates present in some grasses and other crops; examples include sorghum, sudan grass, maize, lucerne, wheat, barley, soybean. The nitrates are reduced to nitrites in the rumen.

When high levels of **nitrites** accumulate in the GI tract, they are absorbed into the bloodstream. Nitrites oxidize the ferrous iron of haemoglobin to the ferric iron of methaemoglobin which does not transport oxygen. In severe cases, the blood becomes almost **chocolate brown** and there is a brownish discolouration of nonpigmented areas of the skin and mucous membranes. The pulse is rapid and breathing is laboured. Death may result because of anoxia. Nonruminants can tolerate nitrate but ruminants don’t because the bacteria in the rumen convert nitrate to nitrite.

Sources of nitrate/nitrite: Water contaminated with animal or industrial wastes, feeds containing high levels of nitrate. Cornstalks and oat hay were two of the feeds first reported to occasionally contain high level of nitrate. Hay or straw containing more than 2.2% potassium nitrate is toxic.

Nitrite poisoning- There is considerable variation between species in their susceptibility to nitrite poisoning**. Pigs are the most susceptible, then, in order, cattle, sheep and horses.** Non ruminates, such as horses and pigs, have no mechanism for converting nitrate to nitrite in their digestive tract, so they are not susceptible to **nitrite poisoning from excessive intake of nitrates**. However, they are highly susceptible **to poisoning from nitrite intake** (for instance in mouldy hay) because they cannot convert the nitrite to ammonia. Sheep are more efficient at converting nitrite to ammonia; that is why probably, they are less susceptible to nitrite poisoning than cattle.

Treatment- Treatment involves conversion of methaemoglobin into oxyhaemoglobin by administration of suitable reducing agents such **as methylene blue, thionin or ascorbic acid.** Acute nitrate toxicity is treated with intravenous administration of methylene blue solution. Methylene blue apparently accepts electrons for NADPH reductase in the blood and acclerates the reconversion of **methaemoglobin to functional haemogobin.** The recommended dosage of methylene blue is **2 to 4 mg/kg to 15 mg/kg body weight** (in severe methaemoglobinemia), intravenously in **a one percent** solution. The dosage should be repeated if clinical signs reoccur. Oral doses of mineral oil (one litre for adult cattle) or saline cathartics (sodium sulphate 500 g per adult cattle) are recommended as supportive treatment, which help in removing toxic materials from gastrointestinal tract. To be at safer side, the fodder cut in the early part of regrowth may be fed mixed with other dry roughages to neutralize the nitrate concentration.

Mimosine Toxicity-

- **Subabul green forage** contain a toxic amino acid, mimosine at 2-5% in the leaves on DMB. When fresh leaves are masticated and mostly on rumen microbial degradation, a goitrogen **3-hydroxy - 4 (1H) - pyridone (3, 4DHP)** is autocatalytically formed from the mimosine. The DHP is toxic and symptoms of toxicity include alopecia, excessive salivation, enlarged thyroid glands, low serum thyroxine (T_4), low serum triiodothyronine (T_3), oesophageal lesions, poor appetite, weight loss and death. Abortion of pregnant animals, death of calves are also reported. Some animals developed cataract. Mimosine toxicity is observed in ruminants if subabul constitutes more than about **30%** of the total diet dry matter intake and **5 to 7%** in pigs and rabbits. Subabul **is toxic to poultry** and other monogastric animals. The maximum quantity of subabul leaf meal acceptable to layers was 2.5% and 5% to broilers.
- Sundrying has been reported to reduce the mimosine content to the extent of 10%. Drying at high temperature, ensiling and addition of ferrous sulphate reduce the mimosine content of subabul. **Ferrous sulphate** binds mimosine, hinders in its absorption and promotes its excretion through faeces; this is more useful for monogastric species.

Secondary Plant Compounds (SPC)-

- **Plants contain lignin, cutin, suberin, biogenic silica** and a far more diverse and active chemical defence arsenal of secondary plant compounds. They physically impede digestive enzymes or microorganisms and their effects can range from feeding deterrence to toxicity. They have been labeled "secondary" because few have primary metabolic functions within the plant and at one time they were viewed as end products of other metabolic systems.

- Secondary Plant Compounds are anti herbivory chemicals. Although SPC are invariably viewed as antinutrients, some may have beneficial roles in animal nutrition. The **three** most prevalent groups of SPC are soluble **phenolics, alkaloids and terpenoids.** Soluble plant phenolics include flavonoids, isoflavonoids (phytoestrogens) and hydrolysable and condensed tannins.

S. No.	Plant	Anti- Nutritional factor	Detoxification
1.	Soyabean	Trypsin inhibitor Anti vit A Anti vit D	Autoclaving
2.	Castor bean	Ricin	By Moist Heat
3.	Groundnut cake (GNC)	Aflatoxin	Treated with NH_3 or NH_4OH
4.	Lucerne (Alfa-Alfa)	Saponin	Minearl oil, Simethicone (Bloatosil)
5.	Subabool	Mimosine	Iron Supplement or $Feso_4$
6.	Cotton seed cake	Gossypol	Iron Supplement or $Feso_4$
7.	(a) Jowar (Sorghum), sudan grass (b) Linseed, cassava (Tapoica) (c) Bitter alomond	Cyanide poisoning (a) Dhurin (b) Linamarin (c) Amygdalin	$NaNO_2$ (Sodium Nitrite) + Na- thiosulphate

8.	**Brassicaceae Family plants Mustard, Rapeseed, Cabbage, Cauliflower, Turnip**	**Glucosinolate (Mainly as goitrogens & causing goitre)**	**Iodine supplement**
9.	**Oat hay poisoning**	**Nitrate poisoning**	**1% Methylene blue Solution.**
10.	**Mollases, Napier grass, bajra**	**Oxalate**	**$CaCO_3$ Supplement**
11.	**Neem**	**Nimbin, Nimbidin, Azadirachtin**	
12.	**Top feeds, Khejri leaves, Babul seeds, salssed meal**	**Tannin**	
13.	**Whaet**	**Arabinoxylans**	
14.	**Barley, Oats**	**ß- Glucans**	
15.	**Ray kidney bean**	**Antivitamin E**	
16.	**Maize, Whaet bran**	**Niacin**	
17.	**Raw fish, Bracken fern**	**Thaiminase (Anti Vit B_1)**	
18.	**Raw egg white (raw albumin)**	**Avidin (egg white injury factor)**	
19.	**Sweet clover poisoning, warfarin, sulfa drug**	**Dicoumarol (antivitamin K)**	