Anti-nutritional factors, the potential risks of toxicity and methods to alleviate them

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INTRODUCTION

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 (e.g., inactivation of some nutrients, diminution of the digestive process or metabolic utilization of feed) which exert effects contrary to optimum nutrition. Being an ANF is not an intrinsic characteristic of a compound but depends upon the digestive process of the ingesting animal. Trypsin inhibitors, which are ANFs for monogastric animals, do not exert adverse effects in ruminants because they are degraded in the rumen (Cheeke and Shull, 1985).

The utility of the leaves, pods and edible twigs of shrubs and trees as animal feed is limited by the presence of ANFs. The *raison d'être* of ANFs in plants seems to be as a way of storing nutrient or as a means of defending their structure and reproductive elements (Harborne, 1989). In fact, plants contain thousand of compounds which, depending upon the situations, can have beneficial or deleterious effects on organisms consuming them. These compounds, with the exception of nutrients, are referred to as 'allelochemicals' (Rosenthal and Janzen, 1979). ANFs may be regarded as a class of these compounds, which are generally not lethal. They diminish animal productivity but may also cause toxicity during periods of scarcity or confinement when the feed rich in these substances is consumed by animals in large quantities.

The ANFs which have been implicated in limiting the utilization of shrub and tree forages include non-protein amino acids, glycosides, phytohemagglutinins, polyphenolics, alkaloids, triterpenes and oxalic acid (Table 1).

TABLE 1. **Anti-nutritional factors in the leaves of tree and shrubs documented as being used in livestock feeding.**

Anti-nutritional substances	Species
1. Non-protein Amino acids	
Mimosine	*Leucaena leucocephala*
Indospecine	*Indigofera spicta*
2. Glycosides	
(A) Cyanogens	*Acacia giraffae*
	A. cunninghamii
	A. sieberiana
	Bambusa bambos
	Barteria fistulosa
	Manihot esculenta
(B) Saponins	*Albizia stipulata*
	Bassia latifolia
	Sesbania sesban
3. Phytohemagglutinins	
	Bauhinia purpurea
Ricin	*Ricinus communis*
Robin	*Robinia pseudoacacia*
4. Polyphenolic compounds	
(A) Tannins	All vascular plants
(B) Lignins	All vascular plants
5. Alkaloids	
N-methyl-B-phen ethylamine	*Acacia berlandieri*
Sesbanine	*Sesbania vesicaria*
	S. drummondii
	S. punicea
6. Triterpenes	
Azadirachtin	*Azadirachta indica*
Limonin	*Azadirachta indica*
7. Oxalate	*Acacia aneura*

MIMOSINE

Mimosine, a non-protein amino acid structurally similar to tyrosine, occurs in a few species of *Mimosa* and all species of closely allied genus *Leucaena*. Concern has arisen because of the importance of *L. leucocephala*, in which the level of mimosine in the leaf is about 2-6% and varies with seasons and maturity.

In non-ruminant animals, mimosine causes poor growth, alopecia, eye cataracts and reproductive problems. Levels of *Leucaena* meal above 5-10% of the diet for swine, poultry and rabbits generally result in poor animal performance. The 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 (Hegarty, 1978).

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, a metabolite of mimosine in the rumen (Jones and Hegarty, 1984). Toxic signs like skin lesions also resemble Zn deficiency. Reduction in calving percentage due to *Leucaena* feeding has also been noted (Jones *et al*., 1989).

A solution to the mimosine problem could be the development of low mimosine cultivars. However, low mimosine types are found to be unproductive and of low vigour. The other approach is to feed *Leucaena* mixed with other feeds. Hiremath (1981) suggested that use of *Leucaena* fodder may be restricted to 30% of the green forage in the case of cattle and buffalo, and 50% for goats. The effect of *Leucaena* and mimosine can be reduced by heat treatment (Tangendijaja *et al*., 1990), by supplementation with amino acids (Rosenthal and Janzen, 1979) or with metal ions such as Fe^{2+}, Al^{3+} (D'Mello and Acamovic, 1989) and Zn^{+2} (Jones *et al*., 1978).

CYANOGENS

Cyanogens are glycosides of a sugar, or sugars, and cyanide containing aglycone. Table 1 provides a few examples of fodder trees and shrubs containing cyanogens.

Cyanogens can be hydrolysed by enzymes to release HCN which is volatile gas. 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 CN toxicity than non-ruminants. The HCN is absorbed and is rapidly detoxified in the liver by the enzyme rhodanese 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.

The lethal dose of HCN for cattle and sheep is 2.0-4.0mg per kg body weight. The lethal dose for cyanogens would be 10-20 times greater because the HCN comprises 5-10% of their molecular weight (Conn, 1979). For poisoning, forage containing this amount of cyanogens would have to be consumed within a few minutes and simultaneous HCN production would have to be rapid. Recorded accounts of livestock poisoning by cyanogenic plants show that such situations do arise.

Cyanide can cause goitrogenic effects due to thiocyanate produced during detoxification. Poor animal performance due to *A. sieberiana* pod feeding has been attributed to cyanogens (Tanner *et al.*, 1990). Cyanogens have also been suspected to have teratogenic effects (Keeler, 1984).

Post-harvest wilting of cyanogenic leaves may reduce the risk of cyanide toxicity. Animals suffering from cyanide must be immediately treated by injecting a suitable dose of sodium nitrate and sodium thiosulphate.

SAPONINS

Saponins are glycosides containing a polycyclic aglycone moiety of either C_{27} steroid or C_{30} triterpenoid (collectively termed as sapogenins) attached to a carbohydrate. They are widely distributed in the plant kingdom. Table 1 includes some of the fodder trees in which they may have nutritional significance.

Saponins are characterised by a bitter taste and foaming properties. Erythrocytes lyse in saponin solution and so these compounds are toxic when injected intravenously. The anti-nutritional effects of saponins have been mainly studied using alfalfa saponins. In non-ruminants (chicks and pigs), retardation of growth rate, due primarily to reduction in feed intake, is probably the major concern (Cheeke and Shull, 1985). Such effects have also been noted when *Sesbania sesban* leaf meal (saponin 0.71%) was incorporated in a chick diet (Shqueir *et al.*,1989).

In ruminants, saponins were implicated in causing bloat. However, later studies indicate that they are not involved in the bloat syndrome. Furthermore, because saponins may also undergo bacterial degradation in the rumen, they may not retard the growth of ruminants. Nevertheless, recent studies indicate that they inhibit microbial fermentation and synthesis in the rumen (Lu and Jorgensen,1987).

In ruminants, some reports of toxicity due to dietary saponins have also appeared. Sharma *et al.* (1969) observed that 4-7 weeks of *ad lib.* feeding of *Albizia stipulata* gave rise to toxic manifestations in sheep. The toxicity of broombreed (*Gutierrezia sarothrae*), a resinous shrub, is believed to be due to its saponin content. Symptoms include listlessness, anorexia, weight loss and gastroenteritis (Molyneux *et al.*, 1980). Joshi *et al.* (1989) observed that mowrin, a saponin in *Bassia latifolia* seed cake, was not toxic to calves when consumed orally at as high a level as 94g/day. These results indicate that saponins from different plant species have varied biological effects probably due to structural differences in their sapogenin fractions.

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 (Joshi *et al.*, 1989).

PHYTOHEMAGGLUTININS

Phytohemagglutinins, otherwise referred to as lectins, are proteins which agglutinate red blood cells. They have been shown to occur in some important fodder trees (Table 1). 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 (Liener, 1985). 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, lassitude, weakness and posterior paralysis in cattle (Cheeke and Shull, 1985).

Ricin occurs in castor beans (*Ricinus communis*) which have been reported to cause poisoning in all class of livestock. Due to ricin, de-oiled castor seed cake (CP 35%) is seldom used as a livestock feed. However, the mature leaves of *R. communis* have been found suitable for feeding to sheep (Behl *et al.*, 1986); hence precautions against bean contamination are necessary. Castor bean meal can be detoxified by autoclaving at 20 psi for 60 minutes for incorporation in sheep diets (Rao *et al.*,1988).

TANNINS

Tannins are water soluble phenolic compounds with a molecular weight greater than 500 and with the ability to precipitate proteins from aqueous solution. They occur almost in all vascular plants. Hydrolysable tannins and condensed tannins (proanthocyanidins) are two different groups of these compounds. Generally tree and shrub leaves contain both types of tannins. The two types differ in their nutritional and toxic effects. The condensed tannins have a more profound digestibility-reducing effect than hydrolysable tannins, whereas the latter may cause varied toxic manifestations due to hydrolysis in rumen. Sheep ingesting 0.9g hydrolysable tannins kg/body weight showed signs of toxicity in 15 days. Animals like mule deer, rats and mice have been shown to secrete proline-rich proteins in saliva which constitute the first line of defence against ingested tannins. Nevertheless, deleterious effects and episodes of toxicity suggest the inadequacy of defence against high quantities of dietary tannins (Kumar and Vaithiyanathan, 1990).

The anti-nutritional effects of the tannins present in tree leaves are summarised in Table 2. The mechanism of dietary effects of tannins may be understood by their ability to form complex with proteins. Tannins may form a less digestible complex with dietary proteins and may bind and inhibit the endogenous protein, such as digestive enzymes (Kumar and Singh, 1984). Tannin-protein complexes involve both hydrogen-bonding and hydrophobic interactions; the precipitation of the protein-tannin complex depends upon pH, ionic strength and molecular size of tannins. Both the protein precipitation and incorporation of tannin phenolics into the precipitate increase with the increase in molecular size of tannins (Kumar and Horigome, 1986). However, when the molecular weight is very large (>5000), the tannins become insoluble and loose their protein precipitating capacity. Hence the measurement of the phenolic profile in terms of total phenols, condensed tannins, their protein precipitating capacity and degree of polymerization becomes imperative to asses the role of tannins in ruminant nutrition (Kumar, 1983; Lowry, 1990). In tree leaves tannins are present in NDF and ADF in significant amounts which are tightly bound to the cell wall and cell protein and seem to be involved in decreasing digestibility (Reed *et al.*, 1990). Hence, there is a need to account for these tannins in estimating the nutritive value of tree leaves.

In ruminants, dietary condensed tannins (2-3%) have been shown to impart beneficial effects because they reduce the wasteful protein degradation in the rumen by the formation of a protein-tannin complex (Barry, 1987). The complex appears to dissociate post-ruminally at a low pH where, presumably, the protein becomes available for digestion. However, free condensed tannins would probably be available to form a complex with digestive enzymes such as pepsin and also with the protein of gut wall. Condensed tannins of *Prosopis cineraria* precipitate pepsin at pH 2.0 and the net effect of the presence of condensed tannins may therefore be negligible.

TABLE 2. Some examples of anti-nutritional effects of tannins in shrub and tree forages.

Fodder Tree/Shrub	Predominant Tannin*	Animal	Nutritional Effect	Reference
Acacia aneura[a]	CT	Sheep	Reduction in N digestibility decreased wool yield and growth, decreased S absorption	Pritchard *et al.* (1988)
A. cyanophylla	CT	Sheep	Reduced feed intake, negative N digestibility, loss in weight	Reed *et al.* (1990)
A. nilotica (pods)	CT	Sheep	Low growth rate, reduced N and NDF digestibility	Tanner *et al.* (1990)
A. sieberiana[b] (Pods)	HT	Sheep	Low growth rate, reduced N and NDF digestibility	- do -
Albizia chinensis[d]	CT	Goat	Reduced *in sacco* N digestibility	Ahn *et al.* (1989)
Leucaena leucocephala[c]	CT	Poultry	Poor N retention, low apparent metabolisable energy value	D'Mello and Acamovic (1989)
Manihot esculenta[b]	CT	*In vitro*	Inhibits digestibility	Rickard (1986)
Prosopis cineraria	CT	Sheep	Reduction in feed intake protein, digestibility, decreased wool yield & growth, decreased iron absorption	CSWRI (1989)
Robinia pseudoacacia[e]	CT	Rat	Reduced protein digestibility	Horigome *et al.* (1988)
		Rabbit	Reduced feed intake & growth, cecotrophy increased protein digestibility	Raharjo *et al.* (1990)
Terminalia oblongata	HT	Sheep	Reduction in feed intake, toxicity but no effect upon digestibility	McSweeney *et al.* (1988)
Ziziphus nummularia	CT	Sheep	Reduction in feed intake protein and DM digestibility; decreased wool yield and weight loss	Kumar and Vaithiyanathan (1990)

* CT- Condensed Tannins; HT- Hydrolysable Tannins
In addition to tannins, the other reported deleterious compounds in above top feed are marked with superscripts as: a. oxalate b. cyanogens c. mimosine d. saponins e. lectins.

A number of methods have been tried to overcome the deleterious effects of tannins (Kumar and Singh, 1984). Alkali treatments include ferrous sulphate and polyethylene glycol-4000 (PEG-4000); the last was found to be effective for deactivation of tannins in tree leaves (CSWRI, 1987-89). However, using PEG-4000 in routine feeding may not be economic. Three months feeding of *P. cineraria* leaves and *Cenchrus* spp. (50:50) with 1% urea has been found to maintain adult sheep. In such a feeding system, urea not only provides extra nitrogen to the animals but also deactivates the leaf tannins (Russell and Lolley, 1989). Therefore, the use of urea to overcome the deleterious effects of tannin may be of practical value and should be studied.

OTHER ANTI-NUTRITIONAL FACTORS

Apart from the ANFs discussed in preceding sections, shrub and tree forage may contain alkaloids, terpenoids, oxalate, indospecine, lignins (Table 1) and certain other ANFs. Alkaloids such as N-methyl-a phenethylamine cause locomotor ataxia of the hindquarters in sheep. Sesbaine causes haemorrhagic diarrhoea. The terpenoids azadirachtin and limonin impart a bitter taste and the leaves of *Azadirachta indica* are therefore not relished by cattle. Oxalate in the leaves of *Acacia aneura* may limit the Ca availability and a negative correlation between digestibility and lignin content in tropical browse has been observed (Bamualin *et al.*, 1980).

STUDYING THE ANFs: A CHALLENGE

As previously noted, most of the ANFs belong to a group of related compounds with similar mode of actions. There are about 8,000 polyphenols, 270 non-protein amino acids, 32 cyanogens, 10,000 alkaloids and several saponins which have been reported to occur in various plant species. Studying such a vast number of compounds is bound to provide major challenges.

1. **Detection**: This can be approached either by evaluating animal performance or by chemical analysis. Certain ANFs can be detected through chemical analysis but it is not easy to look for all possible allelochemicals

in a single plant. Furthermore, lesser known fodder trees and shrubs may contain unknown ANFs and their presence would only be revealed through feeding trials, not by chemical analysis directed at known compounds.

2. **Quantification**: There is wide variation in the reported concentration of ANFs in the same plant species. This may be either real, because of the changes occurring due to environmental conditions, or may arise because of lack of standardization of methods between laboratories, as well as their destruction in assays.

3. **Assessment of biological effects:** It is often observed that sensitivity to ANFs varies between species of animals, different ages and physiological stages. Furthermore, the leaves of a particular tree or shrub may contain different group of ANFs and it becomes difficult to separate their biological effects.

METHODS TO ALLEVIATE: AN OVERVIEW

The uncertainty of quantification and the imperfectly understood biological effects of ANFs impede the development of methods to alleviate their effects. The simplest approach of dilution, i.e., feeding allelochemical containing leaves in mixtures with other feeds, may certainly reduce the risk of toxicity but simultaneous nutritional benefits may not accrue. Moreover the required degree of dilution is difficult to recommend because of uncertain quantification.

Several studies indicate that tannin-rich leaves, in combination with concentrate rations, could be fed to animals without any adverse effect (Raghavan, 1990). This happens because animals consume protein in excess of their requirement from the concentrate and therefore, the anti-nutritional effects of tannins were masked. Moreover, these studies do not show the utilization of tree leaf proteins for which they are mostly fed.

The utility of management practices involving lopping/harvesting of tree leaves at times when the concentration of ANFs are lowest, (Vaithiyanathan and Singh, 1989) is limited because pattern of changes in concentration of various allelochemicals may not be same. For a particular ANF, the effect of season also varies between plant species. It has also been noted that, as leaves mature, both the ANF and nutrient contents decrease (Singh,1982).

Another approach of supplementation, e.g., polyethylene glycol-4000 with tannin-rich leaves, may be suitable during acute shortage to avoid livestock losses. These cannot be used routinely because of prohibitive costs. However, metal ions and urea supplementation could be recommended to farmers after thoroughly assessing their alleviating effects against highest possible reported concentrations of allelochemicals.

Many ANFs are heat labile. Hence simple heating or autoclaving has been found useful in removing the effects of allelochemicals. This practice can be used by the feed industry but not by farmers. Unfortunately, heating would substantially increase the cost due to the energy involved both in the treatment and transport. The efficacy of wilting in reducing the risk of cyanide toxicity needs to be tested before it is recommended to farmers. Simple washing with water removes the soluble allelochemicals but nutrients also leach out.

Since ANFs have a major role in plant defence, selecting for low ANFs lines may have undesirable effects on the plant.

Alleviation By Rumen Microbial Activity

Ruminant animals have a symbiotic relationship with rumen microorganisms. The rumen environment (slightly acidic pH:E^0 = -0.35V; 10^{10} microbes/ml) provides many reductive and hydrolytic reactions which, in the majority of cases, decrease the biological activity of the allelochemicals before their absorption from the tract.

Rumen bacteria and fungi capable of degrading lignin have been isolated. Anaerobic degradation of flavonoid and hydrolysable tannins by mixed rumen microbes has also been demonstrated. Such rumen microbes are present in small numbers and their growth rate is slow. Anaerobic microbial degradation of condensed tannins has also been demonstrated.

Dietary oxalate can be degraded by rumen microbes into CO_2 and formic acid. Ruminants adapted to diets with high oxalate content can tolerate oxalate levels that are lethal to non-adapted animals. Moreover, it has been shown that the transfer of rumen fluid from animals in Hawai to Australian ruminants resulted in complete elimination of the toxic effects of mimosine and the bacteria involved in such effects have been identified (Allison *et al.*, 1990). Evidence also exists that rumen microbes can be genetically manipulated (Russell and Wilson, 1988).

These findings imply that future research may be drawn towards identification of the various anaerobic and rumen microbes capable of dissimilating ANFs, testing the survivability of organisms in the rumen and investigating whether the dissimilation is plasmid encoded, so that genetic manipulation of rumen bacteria can be effected for the useful fermentation of ANFs.

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