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**Review** Article

# Secondary Metabolites in Forage Crops – A Review

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#### ABSTRACT

India currently faces two constraints in the sector of forage production namely the widened gap between the demand and supply and the availability of inferior quality forage. Amongst these, the former can only boosted by a shift in the cropping systems adopted now and inclusion of more area under forage cultivation. But the latter, quality of the forage available, is largely determined by the presence of secondary metabolites even though they are byproducts of plant metabolism. There are nine important secondary metabolites namely tannins, saponins, cyanogenic glycosides, nitrates, mimosine, oxalates, phytates, protease inhibitors and alkaloids. Their toxic effects can be alleviated by adopting different methods, both easy and complex, which can be thus recommended to increase the quality of forage available in the country.

Key words: Secondary metabolites, Tannins, Saponins, Oxalates, Mimosine.

## **INTRODUCTION**

Livestock sector in India is very important as it accounts for the livelihood of about 20.5 million people and provides 4.11 percentage of total GDP of the nation. But Indian milk productivity is low (1538 kg year<sup>-1</sup>) compared to world average  $(2238 \text{ kg year}^{-1})$ . The livestock population is expected to reach a whopping 286 million adult units in another 30 years. Also the demand for green fodder in the country is expected to become 1012 million tons even though there is a deficit supply of 30 percent now<sup>9</sup>. It is observed that fodder production has the lowest priority in land and other resources allocation in India. This demand- supply gap is not the only matter of concern, so is the quality of available forage.

#### **Quality and Metabolites**

Forage crops are classified as fodder grasses, fodder legumes, tree fodders and fodder cereals<sup>11</sup>. Manasiet al <sup>14</sup>.found that Leucaena leucocephala was superior in palatability to many other tree fodder crops and the respective decreasing order was found to be leucocephala>Bauhinia Leucaena variegata>Grewia optiva>Morusserrata> Areca catechu >Pittosporumf loribundum>Oouginia *oojeinensis*>*Celtis* australis. Similar variations can be expected in other categories of forage crops too, there by questioning the quality of the available forages.

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Primary metabolites are molecules that are essential for growth and development of an organism like carbohydrates, proteins, lipids, nucleic acids, hormones etc. Secondary metabolites are molecules that are not essential for growth and development of an organism and are derived from primary metabolites which help in many functions like defence and stress responses. They are the products of secondary metabolism and are common in tropical forages. They are also called as Anti-Nutritional Factors - ANF or Anti- Quality Factors - AQF as they 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<sup>1</sup>. They have been shown to be highly biologically active and most of these secondary metabolites elicit very harmful biological responses, while some are widely applied in nutrition and as pharmacologically active agents.

# **Classification of secondary metabolites**

Secondary metabolites may be divided into two major categories. They are Proteins (such as lectins and protease inhibitors) which are sensitive to normal processing temperatures and other substances which are stable or resistant to these temperatures and which include, among many others, polyphenolic compounds (mainly condensed tannins), nonprotein amino acids and galactomannan gums. Huisman and Tolman, divided the antinutritional factors into the following groups:

• Factors with a depressive effect on protein digestion and on the utilization of protein, such as protease inhibitors, tannins, saponins and lectins;

• Factors that affect mineral utilization, which include phytates, gossypol pigments, oxalates and glucosinolates;

• Factors that stimulate the immune system and may cause a damaging hypersensitivity reaction, such as antigenic proteins;

• Factors with a negative effect on the digestion of carbohydrates, such as amylase

inhibitors, phenoloc compound and flatulence factors;

• Miscellaneous substances, such as mycotoxins, mimosine, cyanogens, nitrates, alkaloids, photosensitizing agents, phytooestogens and saponins.

# Major secondary metabolites

According to Aletor, there are several antinutritional factors that are very significant in plants and some most common ones with their mechanism of toxicity and impact on animal health and productivity are discussed here under:

Tannins: Astringent, bitter plant poly phenolic compounds that either bind or precipitate proteins to form reversible and irreversible complexes there by inhibiting the activities of trypsin, chemotrypsin, amylase and lipase. There are three types of tannins. Phlorotannins are least complex, least known are found in brown algae.Hydrolyzable tannins/ anthocyanidins are susceptible to enzymatic & non enzymatic hydrolysis. They are highly water soluble and can be toxic. Tannins combine with dietery proteins and form less digestible complex in turn causing astringency. This leads to lesser feed intake by the livestock<sup>19</sup>. The major crops with significant tannin content are fodder sorghum, subabul (Leucaena leucocephala), alfalfa (Medicago sativa) and egyptian clover (Trifolium sp.).

Ally and Kunjikutty<sup>2</sup>. studied the percentage chemical composition and tannin contents of locally available tree leaves commonly fed to goats (on dry matter basis) in Kerala and found that *Manilkhra hexandra or "Kirini"* and *Ficus memosa* – "*Athi*" had the highest (6%) and lowest (3.6%) content of total tannins respectively.

**Saponins**: Structurally diverse molecules and consist of non-polar aglycones coupled with one or more monosaccharide molecules. Saponins can affect animal performance and metabolism through: erythrocyte haemolysis, reduction of blood and liver cholesterol, depression of growth rate, bloat (ruminants), inhibition of smooth muscle activity, enzyme inhibition and reduction in nutrient absorption<sup>1</sup>. The structural complexity of

saponins results in a number of physical, chemical, and biological properties, which include sweetness and bitterness, foaming and emulsifying , pharmacological and medicinal, haemolytic properties, as well as antimicrobial, insecticidal activities.

Saponins reduce the uptake of certain nutrients including glucose and cholesterol at the gut through intralumenal physicochemical interaction. Hence, it has been reported to have hypocholesterolemic effects<sup>21</sup>. In chickens saponins have been reported to reduce growth, feed efficiency and interfere the absorption of dietary lipids and vitamins<sup>10</sup>. (A & E) Major forage crops with high saponin content are napier grass and alfalfa (*Medicago sativa*).

Cyanogenic Glucosides: Cyanogens are glycosides of a sugar or sugars and cyanide containing aglycone. It can be hydrolysed by enzymes to release HCN. Excess cyanide ion inhibits the cytochrome oxidase. This stops formation, tissues suffer ATP energy deprivation and death follows rapidly. Prussic acid causes asphyxiation by inhibiting the action of the enzyme that links oxygen with red blood cells **Ruminants** are more susceptible than non-ruminants. HCN level will be high in young seedlings rather than in matured seedlings

The major crop with cynanide content is fodder sorghum (*Sorghum bicolor*)<sup>13</sup>. points out that the fresh fodder is safe to be fed when it has below 100 ppm HCN content while it is toxic to the cattle when it goes beyond 200 ppm.

**Oxalate:** Strong bonds are formed between oxalic acid, and various other minerals, such as Ca, Mg, Na and K giving salts, rendering them inaccessible to the body. Prolonged mobilization of bone mineral results in nutritional secondary hyperparathyroidism or osteodystrophyfibrosa<sup>16</sup>.

Major crops with high oxalate are hybrid napier, guinea grass, buffel grass (*Cenchrusciliaris*)and pangola grass (*Digitaria decumbens*). Smitha *et al*<sup>19</sup>.found that the leaf blade had comparatively higher oxalate content in case of setaria grass (*Setaria sphacelata*), kikuyu grass(*Pennisetum*  *clandestinum*) and napier grass. The oxalate content ranges in BN hybrid, guinea grass and fodder bajra were 2.58 - 5.62 %, 1.05 - 2.40% and 0.63 - 1.98% respectively<sup>17</sup>.

Antony and Thomas<sup>4</sup>, concluded that the common hybrid napier varieties cultivated in Kerala like the Suguna and Supriya has higher content of oxalate content in both leaves and stem and therefore care must be taken while these are used for fodder. Out of all the varieties, IGFRI- 3 or Swethika (3.70%) has the largest content of oxalate content in leaves while it is very high in stem of the common variety, Suguna (4.18%).

**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*.Chauhay and Poosoran<sup>7</sup>, found that Fresh leaves of *Leucaena leucocephala* contained 4.346% mimosine.

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.

Nitrates: When forages have an unusually high concentration of nitrate, the animal cannot complete the conversion and nitrite accumulates. Nitrite is absorbed into the bloodstream directly through the rumen wall and converts haemoglobin (the oxygen in carrying molecule) the blood to methaemoglobin, which cannot carry oxygen. Feeds can be safely fed to the animals only when the content of nitrate is 0- 1000 ppm. Once the level of nitrate in forage reaches 1500-2000 ppm, it is recommended to limit the particular feed to 50 per cent of ration's total dry matter. It has to be noted that as the content of nitrate increases in the forage feed, the more it is limited to be given to the animals. Feeds containing over 4000 ppm of nitrate are potentially toxic and never given as feed to the animals<sup>3</sup>.

Some of the fodder crops such as sudan grass, pearl millet and oats<sup>18</sup>, can

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accumulate nitrate at potentially toxic levels. The nitrate content ranges in BN hybrid, guinea grass and fodder bajra were 790 - 2100 ppm, 624-1285 ppm and 850- 2620 ppm respectively<sup>17</sup>.

**Phytate**: Also known as inositol hexakisphosphate, is a phosphorus containing compound. The presence of phytate in feeds has been associated with reduced mineral absorption due to the structure of phyate which has high density of negatively charged phosphate groups which form very stable complexes with mineral ions causing nonavailability for intestinal absorption<sup>22</sup>. All cereal fodders are rich in phytates.

**Protease inhibitors**: Have the ability to inhibit the activity of proteolytic enzymes within the gastrointestinal tract of animals. The antinutrient activity of protease inhibitors is associated with growth inhibition and pancreatic hypertrophy<sup>8</sup>. They are common in different fodder cereals and fodder legumes.

Alkaloids: Small organic molecules, common to about 15 to 20 percent of all vascular plants, usually comprising several carbon rings with side chains, one or more of the carbon atoms being replaced by nitrogen. They are synthesized by plants from amino acids.Insects and hervibores are usually repulsed by the potential toxicity and bitter taste of alkaloids . 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.

# Methods to alleviate the toxicity

A number of methods are available to reduce the toxicity caused by secondary metabolites in forage crops. Chauhay and Poosoran<sup>7</sup>. carried out an experiment to find the per cent reduction of tannin and mimosine content due to different treatments in Leucaena leucocephala leaves. The fresh leaves contained about 37. 58 per cent tannin and 4.346 per cent mimosine. But when leaves were dried at  $60^{\circ}$  C for 24 hours after harvest the tannin and mimsoine content were 7.15 per cent (80 per cent reduction) and 2.925 per cent (32.6 per cent reduction) respectively. Many other treatments are also carried out and when the dried leaves were soaked in water for 72 hours which then dried at  $60^{\circ}$  C after 48 hours resulted 99 per cent tannin content and 94.7 per cent reduction in mimosine content.

Bahrani and Deghani<sup>5</sup>. found that the forage sorghum in the second cut contained very less prussic acid content (mean = 2.5 percent) when compared to first cut forage (Mean = 15 percent) due to degradation of acid and a higher metabolic activity due to higher temperatures during growth. So second cut fodder is comparatively safer.

Oxalic acid content and its relationship with cutting interval and fertilizer management were also studied in B X N hybrid<sup>12</sup>. It was found that prolonged cutting interval of 75 days resulted in lesser oxalic acid content (1.87 percent) than the crop cut at 45 days interval. Prolonged time helped the oxalic acid to convert to carbonates and bicarbonates. When the effect of fertilizer management was studied it was concluded that as the fertilizer applied increased from 100 percent to 150 percent of RDF, the oxalic acid content increased significantly. Higher RDF resulted more growth, development and metabolic activities which increased production of oxalic acid. From the interaction studies of fertilizer management and cutting interval, the oxalic acid content in BN Hybrid was found to be minimum (1.83 percent) when the crop was applied 100 percentage RDF and cut at prolonged intervals of 75 days.

Singh *et al*<sup>18</sup>,studied the nitrate N concentration on oat fodder as influenced by the level of nitrogenous fertilizer in two and three splits. Higher nitrate N content was found in two split application of N fertilizers compared to three splits at each level. N fertilizer applied in three splits was safer.

Mukherjee and Maiti<sup>15</sup>. proposed simpler methods to manage toxic secondary metabolites in various forage crops. Cyanogenic glycosides in fodder sorghum was said to be managed by adopting practices like harvesting never before 40 – 45 DAS,

harvesting after flowering, providing adequate irrigations and avoiding plants damaged due to frost, diseases or mechanical damages as such stress eventually cause higher synthesis of cyanogenic glycosides. In sudan grass, cyanogenic glycosides can be managed by harvesting only after 60 DAS and by selective harvesting of 1 meter high plants.Oxalic acid content in hybrid napier can be managed by harvesting at longer intervals (45-60) days. Also the harvested fodder must be fed along with other grasses and legumes, fortified with 15 g per day of calcium carbonate. A similar fortification of concentrated feed with dicalcium phosphate (15-20)**g**) is recommended to manage oxalate formation due to intake of setaria grass. In case of oxalic acid management in fodder bajra, the total herbage can be fed with Ca rich legumes or ground nut cake. Fortification with 15 g of dicalcium phosphate may be added to the ratio. The various secondary metabolites are found to deteriorate the quality of the forage available. This can be managed and alleviated by different methods and help the farmers to increase the quality of the forage available, which in turn causes increased milk productivity and production.

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