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RESEARCH PAPER

Anti-Nutritional Factors in Food and Plant Crops

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ABSTRACT

Some of plants mainly contains toxic compound such as saponins, tannins, phytic acid, gossypol, lectins, protease inhibitors, amylase inhibitor, goitrogens etc. which limits their consumption and also reduces nutrient bioavailability when consumed. These factors are responsible for micronutrient malnutrition and mineral deficiencies. On the other hand they act as self defense system to the food. These are categorized into different categories and causes various symptoms upon consumption such as acute toxicity include respiratory distress, impaired body weight gain, anorexia, weakness, apathy and in some cases even death after several days. But there are various traditional methods and improved technologies are available, which can be used to reduce the levels of these anti-nutrient factors. Various processing methodologies such as fermentation, germination, debranning, autoclaving, soaking, drying, irradiation etc. are used to reduce the anti-nutrient contents in foods. The review is focused on some common recently important anti-nutritional factors (ANF) which were of major concern.

Keywords: Anti-nutrients, Saponins, Trypsin Inhibitors, Cooking, Fermentation, Irradiation

The anti-nutritional factors (ANF) are the toxic compounds synthesized naturally in plant, microbes and animals cause serious problems in human and animal nutrition. These are compounds which reduces the nutrient utilization or food intake when consumed as foods (Soetan and Oyewole, 2009). The presence of these endogenous compounds results in limiting use of food or feed stuff.

The ANFs can be divided into two major categories. One category is of proteins (such as lectins and protease inhibitors) which are sensitive to normal processing temperatures and second is of other substances which are stable or resistant to these temperatures and include among many others polyphenolic compounds (mainly condensed tannins), non-protein amino acids and galactomannan gums. According to Aletor (1993) there are several ANFs that are very significant in plants used for human foods and animal feeds (Fig. 1). They are enzyme inhibitors (trypsin and chymotrypsin inhibitors, plasmin inhibitors, elastase inhibitors), haemaglutinnins (concanavalin A, ricin), plant enzymes (urease, lipoxygenase), cyanogenic glycosides (phaseolunatin, dhurrin, linamarin, lutaustralin), goitrogens (pro-goitrins, glucosinolates), oestrogens (flavones, genistein), saponins (soya sapogenin), gossypol from Gossypium species e.g., cotton, tannins (condensed and hydrolysable tannins), amino acid analogues (BOAA, DAP, mimosine, N-methyl-1- alanine), alkaloids (solanine, chaconine), anti-metals (phytates, oxalates), anti-vitamins (anti-vitamins A, D, E and B12) and favism factors.

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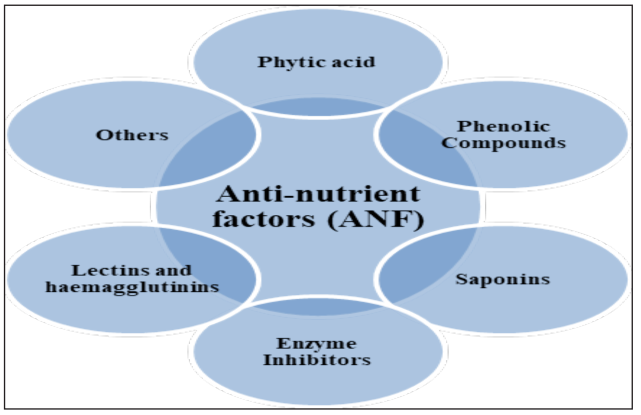


Fig. 1: Classification of anti-nutrients present in food (Samtiya *et al.* 2020)

Although these factors vary in their individual effect, a large proportion of them can be destroyed or inactivated simply by heat treatment processes during cooking. Unfortunately, detailed toxicological studies have not been performed on the majority of these anti-nutritional factors. Broadly speaking, their presence in untreated foodstuffs normally results in anorexia, reduced growth and poor food conversion efficiency when used at high dietary concentrations. Most of legumes are under-used because of the presence of anti-nutritional compounds, such as enzyme (trypsin, chymotrypsin, alpha-amylase) inhibitors, phytic acid, flatulence factors, saponins and toxic factors (Lyimo *et al.* 1992). Root crops, in common with most plants, contain small amounts of potential toxins and antinutritional factors such as trypsin inhibitors. Apart from cassava that has cyanogenic glucosides, cultivated varieties of most edible tubers and roots do not contain any serious toxins. Thus, wild species must be properly processed before their consumption. These wild species, however, are useful reserves in times of famine or food scarcity. Local people are aware of their potential risks in their use and have developed indigenous techniques to detoxify such crops before consumption.

SAPONINS

Saponins are glycosides present in plants and due to hydrophobic nature of aglycone and hydrophilic nature of sugar chains results in distinctive foaming characteristic (Price *et al*. 1987). The word is derived from Latin word *sapo* from soapwort plant (*Saponaria*). They are either a choline steroid or triterpenoid attached via C3 and an ether bond to a sugar side chain. Most of the saponins occur as insoluble complexes with 3-b-hydroxysteroids which interact with bile acid and cholesterol, similarly, saponin with either of iron, zinc, and calcium form insoluble complexes (Oakenfull *et al*. 1989; Milgate and Roberts, 1995; Rafińska *et al*. 2017). Soybean, chickpea, faba bean, pea, lentil and peanuts are the different sources of saponins. Erythrocytes lyse in saponin solution, therefore these compounds are toxic when injected intravenously (Khalil and El-Adawy, 1994). Saponin content may be variable, even among the same species of edible beans, because of variations in cultivars, varieties, locations, irrigation condition, type of soil, climatic conditions, and year during which they are grown (Price *et al*. 1987; Khokhar & Chauhan, 1986; Fenwick & Oakenfull, 1983; Gholami *et al*. 2014; Bianca *et al*. 2020). High concentrations impart a bitter taste and astringency in dietary plants causes its limited use as food (Liener, 1994). The anti-nutritional effects of saponins have been mainly studied using alfalfa saponins. Saponins cause hypocholesterolaemia by binding cholesterol, making it unavailable for absorption. They also cause haemolysis of red blood cells and are toxic to rats (Johnson *et al*. 1986). Saponins have wide applications in beverages, confectionery and pharmaceutical products (Sparg *et al*. 2004; Petit *et al*. 1995; Uematsu *et al*. 2000).

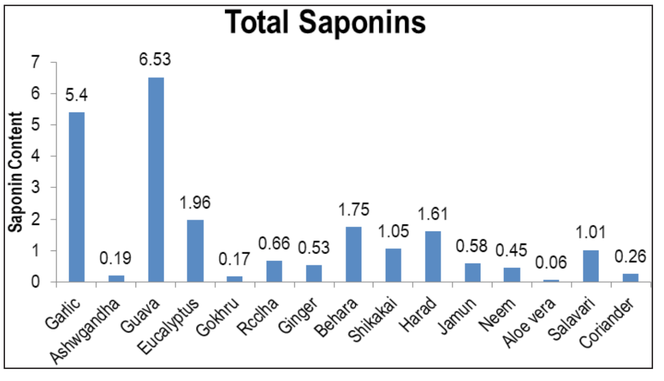
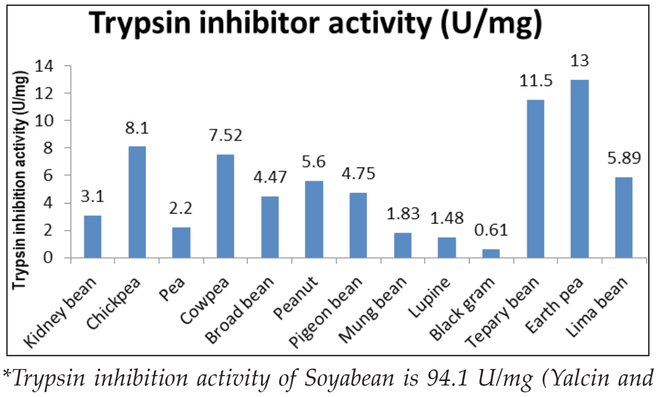


Fig. 2: Presence of total saponins content in commonly used spices (Sirohi *et al.* 2009)

Soaking, blanching, cooking and other treatments reduce the saponins content. Cooking of legumes reduces the amount of saponins by 7–53% (Jood *et al*. 1986; Sharma and Sehgal, 1992). Cooking of chickpea and black gram and of faba beans lowered the saponin content by 7–17% and 35%, respectively (Kataria *et al*. 1988).

TRYPSIN INHIBITOR

Legumes are low cost source of protein in the diet (Borade *et al.*, 1984). But another side they also contains some anti-nutritional factors like trypsin which is injurious to human health (Soetan and Oyewole, 2009). Trypsin inhibitior strappingly prevent the activity of key pancreatic enzymes trypsin and chymotrypsin thus reducing digestion and absorption of proteins by the formation of different complexes (Gemede and Ratta, 2014). These are classified in 2 families as per their molecular size: Kunitz (KTIs), having molecular weights around 20 kDa and Bowman-Birk (BBTIs) around 8 kDa (Avilés-Gaxiola *et al*. 2018). The tripsin inhibitor content of legumes increased during germination. The intake of trypsin inhibitor with the diet causes a rise of fecal nitrogen loss (Combs *et al*. 1967).



**Trypsin inhibition activity of Soyabean is 94.1 U/mg (Yalcin and Basman, 2015)*

Fig. 3: Trypsin inhibitor activity in different legumes (Avilés-Gaxiola *et al.* 2018)

Trypsin inhibitor may have different modes of action, such as the antifibrinolytic or antithrombinogenic or antiproteolytic or a promoter of conversion of methionine to cysteine manifesting ultimately in growth retardation. Trypsin (protese inhibitor) causes pancreatic enlargement and growth depression (Aletor and Fetuga, 1987). Cooking and germination seem to be good procedures to improve the quality of lentil flour from the nutritional point of view, despite the fact that a large variation on the effects of processing, related to the different legume varieties, has been observed (Vidal *et al*. 1994).

Table 1: Different treatments to reduce the Trypsin inhibitor

Treatments	Effect	References
Thermal treatments	Pressure cooking reduces trypsin inhibitors by 50% while microwaving 25%	Habiba, 2002
	Chickpeas needed minimum 90 minutes of boiling or 35 minutes of autoclaving or 15 minutes of microwaving	Alajaji and El-Adawy, 2006
	Lentils, require only 1 min roasting at 80 °C to reduce up to 95.6% trypsin inhibitors and also total tannins and phytic acid.	Embaby, 2010a; Ma and other 2011
	4 h soaking followed by cooking at 95 °C for 1 h the trypsin inhibition of pea, lentil, faba bean, chickpea, and common bean reduced 81.25%, 100%, 100%, 88.37%, and 93.7 %, respectively	Pedrosa and others 2015; Shi and others 2017
Extrusion	Extrusion cooking after pre-treatment at different high temperatures to the legumes field pea, chickpea and faba bean flour resulted in reductions of up to 53.7%, 91.8%, and 58.9%, respectively	Adamidou and others 2011
	Soybeans and lentil, extrusion at 150 °C found to be enough to inactivate trypsin inhibitors activity up to 95%	Žilić and others 2012; Rathod and Annapure 2016

Ultrasound	Ultrasound waves 30 kHz for 20 min in soybeans decreases 55% of Kunitz Trypsin inhibitor	Huang and others 2008
High hydrostatic pressure	Protein digestibility was improved by 4.3% and 8.7% in split peas and white beans	Linsberger-Martin and others 2013
Infrared radiation (IR)	IR exposure completely inactivates trypsin inhibitor and lipoxygenase enzymes in soybean	Bellido *et al*. 2006
Soaking	Simplest way of inactivation of trypsin inhibitor activities	El-Adawy *et al*. 2000; Mumba *et al*. 2004
Germination	Germination reduces trypsin inhibitor	Sangronis and Machado 2007
Fermentation	Solid-State Fermentation in *A. oryzae* over a period of 5 days reduces 89.2% trypsin activity	Gao *et al*. 2013

MIMOSINE

Mimosine [ß-N-(3-hydroxy-4-pyridone)-a-amino-propionic acid], a non-protein amino acid structurally resembles to tyrosine and found in the genera *Leucaena* and *Mimosa*. The mimosine contents in different parts varies differently as in seeds 4 to 5%, in roots 1 to 1.5% and in shoot 1 to 12% similarly old stems have the smallest and growing tips contains largest amounts (Jones and Lowry, 1984). The mechanism of action of mimosine in producing its effect, however, 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 B_6-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, goitre etc. (Jones and Hegarty, 1984). Moist heat at 70 to 100°C reduce 50% mimosine content (Akbar and Gupta, 1985) and dry heat treatment at 100°C reduced 17 to 19% mimosine content whereas autoclaving reduced 19 to 23% (Mali *et al*. 1990).

CYANOGENS (CNglcs)

Cyanogenic glucosides are phytoanticipins of more than 60 different types and known to be present in more than 2500 plant species (Conn, 1980; Bak *et al*. 2006). Generally, CNglcs acts as defense mechanism to plants which resulted in release of toxic hydrogen cyanide (HCN), an aldehyde or ketone and glucose compounds (Nahrstedt, 1996; Vetter, 2000). The lethal dose of cyanide for vertebrates lies in the range of 35–150 µmol/kg (Zagrobelny *et al*. 2008). Generally, cyanogenic glycoside is considered as not toxic on its own (Bolarinwa *et al*. 2016).

A cyanogenic food of particular economic importance is cassava, which is also known by the names manioc, yuca and tapioca. Cassava roots have been reported to contain cyanide content of 10–500 mg/kg of dry matter (Siritunga and Sayre, 2003). Amygdalin is the cyanogenic glycoside responsible for the toxicity found in the range of 8.84–48.33 mg/g in Cassava (Bolarinwa *et al*. 2016) and also in many species of Rosaceae, such as bitter almonds, peaches and apricots (Bolarinwa *et al*. 2014). Swe*et al*monds are low in amygdalin as a result of breeding processes. 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). Cyanide content of bamboo shoot ranged from 1000 to 8000 mg/kg hydrogen cyanide (Ferreira *et al*. 1995). 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. 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.

Table 2: Different treatments to reduce the cyanogens

Treatments	Effect	References
Soaking	Soaking of cassava root for72 h reduces 90% of cyanide content	Kemdirimi *et al*. 1995
	Soaking of apricot kernels at 20°C significantly reduces amygdalin	Tuncel *et al*. 1995
Fermentation	Fermentation of cassava pulp or dough for 4–5 days has been reported to decrease its total cyanide by 52–63%	Kemdirimi *et al*., 1995
	Fermentation of cocoyam flour reduces cyanide by 98.6%	Igbadul *et al*. 2014
	Fermentation results in reduction of 84.6% in the cyanide content in sorghum leaves	Prasad and Dhanya, 2011
Storage	Cyanide content decrease by 50–64% in the cassava product when stored for 4 weeks at room temperature	Onabolu *et al*. 2002

OXALATES

Oxalates were found in various families including aroid / arum family *e.g., Colocasia* (taro) and *Xanthosoma* (Calodium) (Noonan and savage, 1999). Oxalic acid result of oxidative breakdown of carbohydrate and protein, accumulated in plants especially during dry conditions (Bressani *et al*. 1993). Reports of some tropical leafy vegetables revealed that dry vegetables had higher oxalate concentrations than the fresh vegetables (Aletor and Adeogun, 1995). Oxalate content in taro (*Colocasia esculenta*) contain 278-574 mg /100 g fresh weight, sweet potato (*Ipomoea batatas*) 470 mg/100 g fresh weight and yam (*Dioscorea alata*) tubers 486- 781 mg/100 g dry were reported (Holloway *et al.* 1989; Mosha *et al.* 1995). Oxalate was widely distributed in plants in a readily water soluble form as potassium, sodium and ammonium oxalate and as insoluble calcium oxalates (Fassett, 1973; Connor, 1977; Smith, 1982).

The content of total oxalate, soluble oxalate, calcium oxalate and free calcium for various aroids as summarized in Table 3. Holloway *et al.* (1989) found that the stems of giant swamp taro, elephant foot yam, skin of giant taro and taro leaves contained about 400 mg/l00 g fresh weight of calcium oxalate, about 10 times the amount present in taro *Xanthosoma* and *Colocasia*.

Table 3: Content of calcium oxalates and calcium (mg/100 g fresh weight) in roots and stems of tropical root crops (standard deviation in parentheses)

Root crop and source	Total oxalate	Soluble Oxalate	Calcium Oxalate	Total Ca
1. **taro**, *Colocasia esculenta*				
(a) Fiji croms, mean of 5 Cvs.	65 (19)	35 (4)	43	23 (5)
(b) Fiji, suckers, mean of 2 Cvs.	60 (1)	—	—	14 (2)
2. **taro**, *X. sagittifolium* tonga corms, mean of 3 Cvs.	60 (30)	44 (22)	23	6 (1)
3. **giant taro**, *Alocasia*				
(a) Western Samoa, mean of 6 Cvs.	38 (18)	17 (9)	31	26 (5)
(b) Western Samoa mean of 6 Cvs.	30(8)	—	—	28 (9)
4. **giant swamp taro**, *Crytosperma*				
(a) Fed. States of Microneisa (Ponape), Mean of 2 Cvs.	319 (77)	45 (39)	399	135 (33)
(b) Kiribati, mean of 5 Cvs.	300 (218)	—	—	219 (156)

Source: *Holloway et al. (1989).*

Raw, unpeeled Colocasia (*Colocasia esculenta*) corm meal contained total oxalate 1234 mg/kg of dry matter. About a third of the oxalates were removed during peeling the outer skin of the corms, and when peeling was combined with boiling, oxalate contents were reduced to 272 mg/kg. The data suggested that oxalate was a major factor contributing to the antipalatability and antinutritive effect of raw *Colocasia* corm. Shajeela *et al.* (2011) observed that *D. bulbifera* var. vera content highest amount of total free phenolics (2.20g/100g on dry wt. basis) and total oxalate (0.78g/100g on dry wt. basis) in all species of yam. Bhandari and Kawabata (2004) studied on oxalate content in four species of yam and found that the *D. deltoidea* content highest amount of total oxalate, soluble oxalate and calcium oxalate followed by *D. triphylla, D. versicolor* and *D. Bulbifera*.

Singh *et al.*, (1999) studied on biochemical composition and nutririve value of promising collections of elephant foot yam of five genotype like Am-1, Am-2, Am-3, Am-4, Am-5, and found that Am-5 contained highest amount of oxalate (0.96 %) and Am-1 (0.58 %) was the lowest. Chattopadhyay *et al.* (2010) studied on quantitative and qualitative aspects of elephant foot yam corm and found that the genotype NDA-4 contained highest amount of soluble oxalate (18.50 mg/100g) while, Midnapur Finger was lowest in amount (6.65 mg/100g). Oscarsson and Savage (2007) studied on composition and availability of soluble and insoluble oxalates in raw and cooked taro (*Colocasia esculenta* var. Schott) leaves and found that young taro leaves contained 589 mg total oxalate/100g fresh wt. while older taro leaves contained 443 mg total oxalate/100g fresh wt. and soluble oxates were 74% of total oxalate content of the young and old leaves. They also studied on soluble and insoluble oxalate in young and old leaves with treatment of fresh, baked and baked with milk and found that the old leaves contained highest per cent of soluble and lowest per cent of insoluble oxalate, while, old leaves baked with milk contained lowest soluble and highest insoluble per cent of oxalate. Frank *et al.*, (2001) studied on oxalate, insoluble calcium and soluble oxalate in *Phaseolus vulgaris* leaves and found that the adult leaf contained more amount of oxalte than the growing leaf on dry weight basis.

Hang *et al.* (2012) studied on effect of simple washing processing methods on oxalate content of taro petioles and found that petioles and leaves of cv. Chia Voi contained lowest soluble and total oxalate in washed sample, and highest in raw sample. Cooking at 45 minute at 15 psi pressure reduced the total, soluble and insoluble content of all vegetables (silverbeet leaf, silverbeet stem, New Zeland spinach, Spinach, Rhubarb stalk, Beetroot, Broccoli, Carrot, Parsnip) Savage *et al.* (2000). Freezing followed by grilling reduces the availability of calcium as 76.7% of calcium was bound to oxalate to insoluble oxalate form (Brogren and Savage, 2003). Similarly, the oxalate content of spinach pasta was found to contained highest amount of total (91.8 mg/100g wet weight) and soluble oxalate (53.8 mg/100g wet weight (Liebman and Okombo, 2009).

GLYCOALKALOIDS

Potatoes are good source of glycol-alkaloids alpha-solanine and alpha-chaconine, concentrated mainly in the flowers and sprouts (200 to 500 mg/100 g). For food safety purposes, an upper limit for glycoalkaloid content of 20 mg per 100 g of potato is generally accepted. Concentrations of glycoalkaloids are 3 to 10 times greater in the peel than in the flesh. In bitter varieties, the alkaloid concentration can go upto 80 mg/100 g in the tuber as a whole and up to 150-220 mg/100 g in the peel. At these concentrations of solanine and other potato glycoalkaloids are toxic. But because of higher temperature of decomposing (243 °C) they remain unaffected at normal cooking. To avoid toxic levels of glycoalkaloids, potato cultivar selection is very important. However, improper postharvest handling conditions are the main cause of toxic levels in potatoes. To keep glycoalkaloid content low, store potatoes at lower temperatures, such as 7°C, keep potatoes away from light, market in opaque plastics films and paper bags, and rotate frequently on retail displays.

LECTINS

Lectins are proteins or glycoproteins with molecular weight of 10, 000–124, 000. Lectins, also referred to as phyto-haemagglutinins, are glycoprotein compounds which have been shown to agglutinate red blood cells *in vitro* (Gatel, 1994). It is also defined as 'proteins or glycoproteins of non-immune origin with one or more binding sites per subunit, which can reversibly bind to specific sugar segments through hydrogen bonds and Van Der Waals interactions' (Lis and Sharon, 1998). Lectin activity has been determined in more than 800 varieties of the legume family. Of the total protein 2- 10 per cent legume seeds are lectins. One of their most important characteristics is that they prevent absorption of digestive end products in the small intestine. In general, plant lectins are most abundant in the seeds but they are also found in different vegetative tissues such as in roots, leaves, barks, flowers, bulbs and rhizomes (Ratanapo *et al*. 1998; Van Damme *et al*. 2000). Soybean, common bean, jack bean, chickpea, faba bean, pea, lentil and peanut are good sources of lectins. Peas generally have higher lectin activities than faba beans, but both show considerably lower amounts in comparison to raw defatted soybeans. Effects of feeding lectin containing diets include changes in gut immune function, reduced production of endocrine cells and gut hormones, interference with the bacterial ecology in the gut lumen, and damage to mucosal cells (King *et al*., 1983). Lectins have the capability to directly bind to the intestinal mucosa interacting with the enterocytes and interfering with absorption and transportation of nutrients (particularly carbohydrates) during digestion and causing epithelial lesions within the intestine (Santiago *et al*. 1993, Oliveira *et al*. 1989). The main toxic components in *P. vulgaris* are lectins, sugar-binding proteins which bind and agglutinate red blood cells. The toxicity of lectins is characterized by growth inhibition in experimental animals and diarrhoea, nausea, bloating and vomiting in humans. But thorough cooking can destroy this to a safe limit. Armour *et al*. (1998) reported a complete inactivation of soy lectin and protease inhibitory activity by aqueous heat treatment of fully imbibed soy seeds at 100°C for 10 min.

GOSSYPOL

Gossypol is a phenolic compound first isolated in 1899 and its name is derived from the genus scientific name (*Gossypium*) combined with the "ol" from phenol (Soto-Blanco, 2008). Gossypol is produced by pigment glands in cotton stems, leaves, seeds, and flower buds but the highest concentration is seen in seeds (Rogers *et al*. 2002; Kenar, 2006; Alexander *et al*. 2008). General signs of acute toxicity include respiratory distress, impaired body weight gain, anorexia, weakness, apathy and in some cases even death after several days (Fombad and Bryant, 2004). The amount of free gossypol in the cottonseed can be quite variable. Many factors influence gossypol content such as: specie of cotton plant, climatic conditions, soil conditions, fertilizer, etc. Since gossypol affects the heart, gossypol toxicity has been manifested as two types of clinical syndromes particularly in young animals. Dietary gossypol can also bring about increased requirement, not only for lysine, but also for iron which it can chelate. At the physiological level, gossypol reduces oxygen availability in the blood. One syndrome of sudden death (resembling a heart attack) has frequently been reported in calves and lambs. Dietary free gossypol of up to 0.02-0.03% has been reported to cause death in growing pigs while poultry can tolerate fairly high dietary levels (Aletor and Onibi, 1990). These animals seem healthy, have good appetites and are

Table 4: Treatments to Reduce Gossypol Toxicity

Treatments	Effect	References
Heat treatment	Roasting & Extrusion both reduces the gossypol content in milk	Arieli,1998; Noftsger *et al*. 2000
Irradiation	Gamma irradiation & Electron beam irradiation reduces gossypol content in cottonseed meal	Quintana *et al*. 2000; Sijun *et al*. 2012; Shawrang *et al*. 2011; Ebrahimi-Mahmoudabad and Taghinejad-Roudbaneh, 2011
Fermentation	Fungus *Aspergillus niger, Aspergillus oryzae, Candida tropicalis, Saccharomyces cerevisiae, Geotrichum candidum* found to be very effective in reducing Gossypol Toxicity	Barros *et al*. 2002; Yildirim *et al*. 2003; Lim and Lee, 2011

often one of the best ones in the group, but are found dead. If cotton seed or cotton seed meal is bought in bulk, it would be worthwhile to have it tested before feeding it. Depending on the level of free gossypol, the cottonseed could be utilized in the best interest of the livestock.

SUMMARY

Antinutritional factors like saponin, trypsin inhibitors etc. are the defense mechanism of plants, yet these cause many ill effects when consumed. Antinutritional factor is a serious problem in wild species while most of cultivated species are devoid of it. Beside these, many processing practices are developed in these days to avoid the ill effect of antinutritional factors. Since, the antinutrional factors are defense mechanism of plants so these need to be exploited for the benefits of human use.

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