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Review Article

Livestock Feed Anti-Nutritional Components: A Review

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Abstract:

Even though antinutritional factors, particularly those present in plants and plant-derived products, have both beneficial and negative effects on livestock, the majority of these compounds are in charge of the negative effects linked to nutrient and micronutrient absorption, which can interfere with the function of specific organs. Common and well-known antinutritional factors found in livestock feed include saponins, tannins, protease inhibitors, cyanogenic glycosides, lectins, mimosine, amylase inhibitors, etc. If an animal consumes more antinutritional factors than is recommended, undesirable effects may occur. The breakdown products of these compounds may also have some detrimental consequences. Feeding feed that is low or free of antinutritional factors will help to minimize or diminish the harmful effects of these compounds in livestock feed. Alternatively, numerous strategies to prevent or reduce excessive levels of these compounds can be used to reduce the levels of antinutritional factors. The best methods for reducing or eliminating anti-nutritional factors from animal feedstuffs include sprouting/germination, soaking, cooking/boiling, milling, fermentation, autoclaving, storing, applying wood ash, employing efficient chemical treatments, etc. Creating awareness among livestock keepers, producers of animal feed, and all other stakeholders engaged in the manufacture of animal feed regarding the impact of antinutritional factors also among the strategies.

Key words: antinutritional factors; fermentation; saponins; soaking; tannins

Introduction

The term "antinutritional factors" (ANFs) refers to organic or synthetic substances found in livestock feeds that can interfere with feed utilization and negatively impact an animal health and productivity (Makkar, 1993; Akande et al., 2010). They can also decrease animal intake, digestion, absorption, and nutrient utilization, which can have unfavorable effects (Jacob, 2015a; Yacout, 2016). Furthermore, ANFs are important in determining the use of plants in both humans and animal (Emire et al., 2013). These substances, which are sometimes referred to as antinutrients, antinutritive factors, secondary compounds, or plant secondary metabolites, are physiologically active, can be used directly or indirectly as metabolic products (Epafras, 2019; Gemede and Ratta, 2014).

The majority of ANFs compounds are present in plants that provide energy and protein and are detrimental to the general health and productivity of farm animals (Epafras, 2019). Nearly every plant used for practical feeding contains several chemicals. Variations in raw ANFs that are potentially dangerous to the entire plant state can be found in various plant parts, legume seeds, and other plant sources (D'mello, 2000). These plants produce and utilize these substances as organic insecticides to defend themselves against bacteria, molds, birds, and other insects that may pose a threat (e.g., unpleasant taste, unattractive color, toxic properties, foul odors, and immunosuppressive qualities) (Epafras, 2019). These ANFs have a variety of actions (some decrease the digestibility of proteins, bind to nutrients in food, or cause damage to the intestinal wall). Additionally, the ability of a compound to act as an ANFs depends on the animal ingesting it. For example, trypsin inhibitors, which are ANFs in animals with monogastric stomachs, do not negatively affect ruminants because they are broken down in the rumen (Cheeke & Shull, 1985).

Epafras (2019) stated that a number of recent studies have examined the potential physiological consequences of these chemicals in a variety of biological systems because of their positive and negative biological reactions. Negative effects include lowering feed intake, lowering feed conversion, binding to proteins and other vital nutrients required by animals in the feed, and in some cases, causing death. Positive effects include reducing parasite burden, protein degradation in the rumen, methane emission, and bloating in animals. As ANFs can be detrimental to animal production and health, and interfere with feed utilization, farmers must have a better understanding of these compounds and how to manage them to reduce their poisonous effects.

S/N	Type of ANFs	Feedstuff crops
1	Nitrate	Pearl millet, Oats and Sudan grass
2	Oxalates	Setaria grass, Paddy straw, Guinea grass, Bajra and Hybrid Napier, Kikuyu and Buffel grasses
3	Saponins	Lucerne
4	Tannins	Shrubs/ Fodder tree
5	Cyanogens	Sudan grass, Johnson grass and Sorghum
6	Glucosinolates (Goitrogens)	Turnips, Rapeseed and Mustard green
7	Mimosine	Leucaena and Subabul

Table 1: Some common ANFs in forage crops

2. Classification of Antinutritional factors

Antinutritional factors in plants and plant by products can be classified based on their chemical structure, biosynthetic origin, and resistance to heat. Although this classification does not encompass all known groups of antinutritional factors, it presents a list of those frequently found in human food and animal feedstuffs (Emire et al., 2013).

2.1. Based on their chemical compositions.

Tacon (1985) classified ANFs as proteins (protease inhibitors and lectins/hemagglutinins), glycosides (glucosinolates, cyanogens, saponins, and estrogenic factors), phenols (gossypol and tannins), and miscellaneous (anti-minerals, anti-vitamins, anti-enzymes, toxic amino acids, and mycotoxins) depending on their chemical composition.

2.2. Based on their biological action

Another scholar Francis et al. (2001) classified as factors affecting protein utilization and digestion (protease inhibitors, tannins and lectins/haemagglutinins), factors affecting mineral utilization (phytic acid, gossypol, and glucosinolates), antivitamins and miscellaneous (mimosine, cyanogen, and estrogenic factors) based on their biological action.

2.3. Heat resistance

The same author (Francis et al. (2001) classified these compounds as heat-labile factors (protease inhibitors, phytic acid, hemagglutinins, glucosinolates, and anti-vitamins) and heat-stable factors (saponins, non-starch polysaccharides, antigenic proteins, estrogens, and some phenolic compounds) based on their resistance to heat treatment.

3. Some common Antinutritional factors in livestock feed

According to Marcio et al. (2022), the formulation of animal diets is based on the price, available raw materials, and animal nutritional requirements. Any unnecessary loss owing to indigestibility is simply a loss of money for the farmer (loss of nutrients and animal performance). This means that we must better understand and control the ingestible fraction in animal feeds. Antinutritional factors are often tested through laboratory analyses of animal feedstuff and forage used to feed livestock. However, the list can be endless, depending on the interests of the individual, as feed safety is a concern in many countries, followed by some common antinutritional factors in animal feedstuffs and forage (Epafra, 2019).

3.1. Saponins

Saponins are glycosides found in many plants that exhibit soap characteristics owing to their wetter properties (Westendarp, 2005). The word 'saponin' is derived from the Lati letters 'sapo' means soap, and saponin-containing plants have traditionally been utilized for washing (Das et al., 2012). They are mainly produced by plants (Alfalfa, Soyabean Lucerne, Berseem, Yucca, Mahua, Guar, etc.), lower marine animals (sea cucumbers, starfish, etc.), and rhizobacteria (Yoshiki et al. 1998). Saponins are common in a large number of plants and plant products, and play an important role in human and animal nutrition (Das et al., 2012). Saponins function hemolytically and may cause symptoms of toxicity at high concentrations. Among the therapeutically relevant effects, expectorative, anti-inflammatory, and immunostimulatory effects had the highest rankings. Additionally, saponins exhibit antimicrobial properties, particularly against fungi, bacteria, and protozoa. In animal nutrition, saponins suppress intestinal and ruminal ammonia production (Westendarp, 2005). According to Das et al. (2012), saponins affect animals both positively and negatively. It has a biological role in membrane-permeabilizing, immunostimulant, and hypocholesterolemic properties, and has been found to have significant effects on growth and feed intake in animals. On the other hand, these compounds have been observed to kill protozoans, impair protein digestion, take up vitamins and minerals in the gut, and act as hypoglycemic agents.

Other scholars (Patra and Saxena, 2009; Gunun et al., 2019; Ramdani et al., 2023) have stated that saponins can modulate ruminal fermentation and improve animal production. Similarly, as reported by Kholif (2023) [20], saponins have been shown to act as membrane-permeabilizing, immunostimulant, hypocholesterolemic, and defaunating agents in the rumen for the manipulation of ruminal fermentation. Moreover, it has been reported that saponins impair protein digestion in the gut to interact with cholesterol in the cell membrane, causing cell rupture and selective ruminal protozoa elimination, thus improving N-use efficiency and resulting in a probable increase in ruminant animal performance. The US Food and Drug Administration has stated that saponins are Generally Recognized as Safe for human consumption (Patra and Saxena, 2009).

3.2. Tannins

Tannins are widespread throughout the plant kingdom, occurring as hydrolyzable and condensed tannins at different levels in several animal feeding sources. However, the use of tannin-rich feed in animal diets requires great care because of its possible detrimental effects on animal performance and induction of metabolic disorders (Jeronimo et al. (2016). Tannins may have important adverse and beneficial effects on animal performance and production quality, due to their chemical structure as well as their content in the diet; both aspects are intrinsically linked to animal physiology (Waghorn, 2008; Piluzza et al., 2014).

Some adverse effects may be listed as a reduction in feed intake, fiber digestibility, and consequently animal performance (Makkar et al., 2007), but others have shown how tannins may enhance protein utilization, control internal parasites, and also act on production (growth performance, milk, meat, wool) (Min et al., 2003; Maggiolino et al., 2019) and animal welfare (Salzano et al., 2021; Damiano et al., 2022).According to ASPCA (2018), tannins can be added to the diets of monogastric animals (chicken, hen, rabbit, and pig) and ruminants (meat cows, dairy cows, goats, and sheep), to which they have many benefits, including positive effects on the immune system, antibacterial activity, improvement of digestive system health, astringency, increased nutrient assimilation, and antiparasitic effects. Moreover, it has been shown that they may play an important role in improving antioxidant and immunity status of animals (Ciliberti et al., 2019 and Maggiolino et al., 2019).

3.3. Protease inhibitors

According to Jacob (2015b), protease inhibitors are small protein molecules that interfere with the action of proteolytic enzymes involved in breaking down proteins into amino acid components. Naturally occurring protease inhibitors (antiproteases) are proteins or peptides that control proteolysis within an organism and inactivate the proteases of competing or predatory species (Fear, 2007). They are widely distributed throughout nature and are found in plants, animals, and microorganisms (Burns 1987). Different plants, including most legumes, cereals, certain fruits (apples, bananas, pineapples, and raisins), and vegetables (cabbage, cucumbers, potatoes, spinach, and tomatoes) are considered the main sources of protease inhibitors (Richardson, 1977; Doell et al., 1981).

The typical animal response to the intake of protease inhibitors is to increase enzyme secretion, which results in an increased size of the pancreas (that secretes digestive enzymes into the duodenum). There are two types of protease inhibitors: Kunitz inhibitors, which only inhibit trypsin, and Bowman-Birk inhibitors, which inhibit trypsin and chymotrypsin (Jacob, 15b). Similarly, Kalac and Mika (1997) also noted that protease inhibitors have physiological effects by inhibiting growth intensity, restricting protein compatibility and digestibility in poultry, inducing pancreatic hypertrophy and hyperplasia, and increasing trypsin and chymotrypsin synthesis (requirement of methionine and cysteine increases), causing endogenous waste of nitrogen and sulfur, stimulating production matters that support pancreatic enzyme secretion, and adult animals are less sensitive to protease inhibitors.

3.4. Alkaloids

Alkaloids are naturally occurring toxic amines produced by plants, mainly as defence mechanisms to protect themselves against herbivores Kim et al., 2007). Based on Lima (2016), alkaloids are named because they are thought to be utilized for plant defense, and to prevent herbivory, they typically have a positive charge at an alkaline pH. Unlike tannins, many alkaloids are palatable to livestock and seem to be preferred in some instances. Within grain legumes, lupins contain considerable amounts of alkaloids, whereas faba beans, peas, and oilseeds do not. The main toxic effects of alkaloids result in disturbances to the central nervous system, digestive processes, reproduction, and the immune system, and they are toxic to livestock as well as humans. However, Van Leeuwen and Additives (2016) reported that plant alkaloids are beneficial for intestinal integrity and improve feed conversion rates in pigs. This is mainly due to the regulation of the inflammatory processes in the intestinal mucosa.

3.5. Non protein amino acids (mimosine)

Mimosine, a non-protein amino acid structurally similar to tyrosine, occurs in a few species of Mimosa and in all species of the closely related genus Leucaena (Emire et al., 2013). In non-ruminant animals, mimosine causes poor growth, alopecia, cataract, and reproductive problems. Leucaena meal levels 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 the catalytic action of B6-containing enzymes, such as trans-aminases, or may complex with metals, such as zinc (Hegarty, 1978; Emire et al., 2013). Rushkin (1984) reported that memosine can cause weight loss and poor health in non-ruminants when Leucaena is fed above 7.5% (dry mass) of the diet. Tang and Ling, (1975) also reported that, mimosine had adverse effects on the biosynthesis of collagen in embryonic cartilage from chick embryos, due to inhibition of the synthesis of hydroxyproline. Similarly, other scholars (Dewreede and Wayman (1970); Tang and Ling (1975) noted that the reduction in collagen content or the more fragile character of collagen in various organs might induce symptoms such as capillary hemorrhage, proteinuria, and uterine perforations in animals.

3.6. Lectins (phytohaemagglutinins)

Lectins are carbohydrate-binding proteins widely distributed in living organisms. In the plant kingdom, lectins are often called phytohemagglutinins (PHA) (Lannoo and Van Damme, 2014) and are present in many foods, especially beans and other dietary pulses, which can have toxic effects when consumed without adequate cooking, occasionally leading to acute gastroenteritis (Kumar et al., 2013). They are found in all plants, but raw legumes (beans, lentils, peas, soybeans, and peanuts) and whole grains such as wheat contain the highest amounts of lectins. Another author, Crowe (2024) also highlighted that, in plants, lectins form part of the defense system against predators and are found in a wide variety of plant foods, including legumes (such as beans and lentils), whole grains, fruits, and vegetables (such as tomatoes and eggplants).

Although the amount of lectins in foodstuffs can vary considerably, it can dramatically affect the entire digestive tract, bacterial population, body metabolism, and health (Pusztai and Bardocz, 1999). According to Freed (1999) and Vasconcelos and Oliveira (2004), animal and cell studies have shown that active lectins can interfere with the absorption of minerals, particularly calcium, iron, phosphorus, and zinc.

3.7. Trypsin inhibitors

Inhibitors of enzymes such as trypsin are present in many food products, including legumes, cereals, potatoes, and tomatoes (Friedman and Brandon, 2001). A report by Patterson (2023) indicated that trypsin inhibitors are natural anti-herbivore enzymatic compounds produced by soybeans and other legumes that block the production of the digestive enzyme trypsin. These compounds are well-described and understood to be problematic when fed to livestock above a certain threshold. For example, excessive exposure to trypsin inhibitors, in addition to reducing nutrient absorption through diarrhea generation, also reduces total health, thereby making animals more susceptible to enteric disease infections and exacerbating mycotoxicosis. Therefore, the knock-on effects associated with trypsin inhibitor exposure should not be ignored as all negative outcomes lead to reduced performance and profitability.

Gilani et al. (2005) noted that the presence of high levels of dietary trypsin inhibitors from soybeans, kidney beans, or other grain legumes can cause substantial reductions in protein and amino acid digestibility (up to 50%) in rats and pigs. Excessive quantities of trypsin inhibitors in feed can cause pancreatic hypertrophy, leading to poor growth and decreased performance (Pacheco et al. 2014; García-Rebollar et al. 2016; Rada et al. 2017). Pancreatic hypertrophy is a compensatory modulation by the body that offsets the effects of ingested trypsin inhibitors (Liener 1981; Waldroup et al. 1985). However, this mechanism is limited and ineffective in overcoming the continuous daily intake of high amounts of trypsin inhibitors, which ultimately reduces the digestibility of dietary proteins. Rada et al. (2017) observed the negative effect of increasing trypsin inhibitor activity on body weight gain and feed conversion ratio in broiler diets at 38 days of age.

3.8. Phytic acid

Plant feedstuffs contain phytic acid, which is a storage form of phosphorus. However, phytic acid is poorly hydrolyzed by pigs and poultry, and has the capacity to complex dietary nutrients, thereby reducing nutrient digestibility (Woyengo and Nyachoti, 2013). It is the principal storage form of phosphorus in many plant tissues, especially in bran and seeds, and hinders the digestibility of phosphorus (P), calcium, amino acids, proteins, minerals, starches, lipids, and energy (Romano and Kumar, 2018; Maricio et al., 2022). For non-ruminant animals, phytic acid is largely indigestible and therefore can reduce nutrient utilization, leading to reduced growth, bone mineralization, and excessive inorganic phosphorus discharge to the environment. Some secondary effects on the host animal include suppression of appetite, digestive enzymes, or irritating the gut (Romano and Kumar, 2018). On average, for each 1% phytate in the diet, feed digestibility decreases by between 0.49 to 0.89% in organic matter digestibility or apparent metabolizable energy (Marcio et al., 2022). Furthermore, it may compromise the utilization of other dietary nutrients including proteins, starch, and lipids (Humer et al., 2015).

3.9. Pyrimidine glycosides

Vicine and convicine are generally present in Vicia faba and belong to the group of pyrimidine glycosides, which are composed of one molecule of glucose linked to one pyrimidine nucleoside (Champ, 2002). In contrast, other grain legumes and oilseeds contained only negligible amounts compared with faba beans. Vicine and convicine act by reducing glutathione and glucose-6-phosphate dehydrogenase activity, which may result in hemolytic anemia due to biochemical abnormalities in the blood cells.

3.10. Oxalate

Oxalate, a salt of oxalic acid, has been found in several plants. Oxalate crystals may cause problems, either by mechanical action or because they retain calcium in a non-available form (Feedipedia, 2023). According to Savage et al. (2000), oxalate primarily accumulates as soluble oxalate, insoluble calcium oxalate, or a combination of these two forms. Insoluble oxalate forms with calcium ($Ca2+$), magnesium ($Mg2+$), and iron ($Fe2+$) ions, whereas soluble oxalate usually forms with sodium ($Na+$), potassium ($K+$), and ammonium ($NH4+$) ions. Soluble oxalate is one of several nutrients in forage plants. It exerts its effects by binding calcium, magnesium, and other trace minerals such as iron, making them unavailable for assimilation (Talapatra et al., 1948; Watts, 1959; Gorb and Maksakow, 1962). This leads to disturbances in calcium and phosphorus metabolism and causes excessive mobilization of bone mineral

Ruminants tend to be more tolerant to oxalate than are non-ruminants. A dose of 0.12 g oxalic acid/kg live weight/d resulted in a mild degree of hypocalcemia in sheep; however, prolonged grazing on tropical grasses could result in severe hypocalcemia in sheep and cattle (Goyal, 2018). Yorke (2011) reported that oxalate ingestion produces several syndromes, depending on the type of oxalate found in the plant. Some of these syndromes can result in sudden death or chronic renal damage due to the consumption of plants containing high concentrations of soluble oxalates, equine nutritional hyperparathyroidism (big head) due to insoluble calcium oxalate in the plant, and buccal mucosal irritation due to insoluble calcium oxalate crystals in the leaves. The demineralized bones become fibrotic, misshapen and thus causing lameness and 'bighead' in animals (McKenzie et al., 1981).

3.11. Amylase inhibitors

Wisessing and Choowongkomon (2012) showed that amylase inhibitors are found in the seeds of plants, such as cereal grains (wheat, maize, rice, and barley) and legumes (kidney beans, cowpea, and adzuki beans). Amylase inhibitors inhibit amylases of insects in general and inhibit the growth of insects, and thus serve as defense proteins in both cereal grains and bean seeds

3.12. Cyanogenic glycoside

Cyanogenic glycosides are found in several common feed ingredients, such as sorghum, cassava, linseed, and several forage legume species, including clovers and grasses (Monbaliu et al., 2012). Plants produce these substances in the form of a self-defense system against invaders such as herbivores, insects, and microorganisms (bacteria and fungi). Offensive glycosides are grouped into two broad categories (those that exist preformed and ready to cause damage), and those that exist in precursor forms that require activation by an enzyme that coexists in the plant tissue and is released only during tissue damage (Papanikou, 2019).

Cyanogenic glycosides include compounds such as amygdalin (present in bitter almonds and apricot kernels), dhurrin (present in sorghum), linamarin and lotaustralin (both present in cassava), linustatin and neolinustatin (both present in linseed), prunasin (present in Prunus species), and its diastereoisomer, sambunigrin (present in the elderberry plant Sambucus nigra, but not in ripe berries (Rietjens and Eisenbrand, 2023). Plant poisoning (acute or chronic) from cyanogenic glycosides frequently occurs in animals and humans. The selection of low-cyanide-content cultivars will reduce the risk of poisoning. However, prevention of poisoning requires proper preparation of forage (curing or ensiling) for animals and proper preparation of food (shredding, rinsing, and boiling) for people (Panter, 2018). Ruminants are highly susceptible to cyanide poisoning because the rumen environment is mildly acidic, usually has ample water content, and the microflora can rapidly convert cyanogenic glycosides to free cyanide gas (Arnold et al., 2014).

Cyanogenic glycosides	Plant species	
	Common name	Latin name
Amygdalin	almonds	*Prunus amygdalus*
Dhurrin	sorghum	*Sorghum album, Sorghum bicolor*
Linamarin	cassava	*Manihot esculenta, Manihot carthaginensis*
	lima beans	*Phaseolus lunatus*
Lotaustralin	cassava	*Manihot carthaginensis*
	lima beans	*Phaseolus lunatus*
Prunasin	stone fruits	*Prunus species e.g. P. avium, P. padus, P. persica, P. macrophylla*
Taxiphyllin	bamboo shoots	*Bambusa vulgaris*

Source: Conn, 1979a, b as adopted by Speijers, G. (1993)

Table 2: The occurrence of cyanogenic glycosides in major edible plants

3.13. Aflatoxin

Aflatoxins are a group of secondary metabolites produced by several Aspergillus species, with increased toxicity and carcinogenic potential (Cassel et al., 2001; Pleadin et al., 2015). These byproducts are produced as fungi grow in the feed grains, processed feed, and food products. The consumption of low concentrations of aflatoxins by animals can lead to death within 72 h. In general, at non-fatal levels, the health and productivity of animals fed contaminated feed is seriously impaired. Once aflatoxin is produced, it is stabilized. Heat, cold, and light did not affect this. It is also colorless, odorless, and tasteless, and because of the low concentrations and uneven distribution in grain bins, aflatoxins are difficult to detect (Cassel et al. (2001). Simple stomached (monogastric) farm animals and humans are more susceptible to aflatoxins than ruminants, because bacteria from the rumen section of the stomach can metabolize mycotoxins [79-81] (Cassel et al., 2001; Rheeder et al., 2002; Pleadin et al., 2015). Farm animals fed aflatoxin-contaminated feed can cause various severe toxic effects, leading to increased susceptibility to infectious diseases, increased mortality, weight loss, poor performance, and reduced reproductive capability. Following ingestion of contaminated foodstuffs, aflatoxins are metabolized and biotransformed differently in animals (Popescu et al., 2022).

3.14. Gossypol

Gossypol is a toxic compound found in cotton plants. It is concentrated in cottonseed, but can also be found in other parts of the cotton plant, such as hulls, leaves, and stems. It also exists in two forms (free and bound). The free form is toxic, whereas gossypol, which binds to proteins, is in a bound or non-toxic form (Morgan, 1989 and 2017). It primarily affects the heart and liver, but also the reproductive tract, abomasum, and kidneys. According to Garland (2021), although all animals are susceptible to gossypol toxicity, simple stomachs (monogastric), preruminants, immature ruminants, and poultry are affected most frequently. Affected animals may show cardiac failure and sudden death, hepatotoxicosis, liver necrosis secondary to congestive heart failure, hematologic effects, including anemia, increased red blood cell fragility, reproductive effects (including decreased libido and spermatogenesis in males, irregular cycling, disrupted pregnancy, and embryonic death in females, and green coloration of egg yolks and decreased hatchability in chickens), and thumping in swine.

3.15. Nitrate

Nitrate is the form of atmospheric nitrogen taken up by plant roots from the soil and transported to the leaves. Under stress conditions, excess nitrate accumulates in plants. Drought or hot, dry winds cause water stress, leading to nitrate accumulation. Damage caused by hail or frost impairs photosynthesis, resulting in excess nitrate accumulation and toxicity in livestock (Ramteke et al.,2019). Most of the nitrate accumulates in the stem, followed by the leaves, and very little accumulates in the grains (Singh et al., 2000). The rate and quantity of fodder consumption, type of forage, energy level, and adequacy of diet are factors that affect the severity of nitrate poisoning (Ramteke et al., 2019).

S/N	Nitrate content (ppm)	Effect on Animals
1	0-1000	This level is considered safe to feed under all conditions.
2	1000-1500	This level should be safe to feed to non-pregnant animals under all conditions. It may be best to limit its use to pregnant animals to 50% of the total ration on a dry basis.
3	1500-2000	Feeds are fed safely if limited to 50 % of total dry matter ration
4	2000-3500	Feeds should be limited to 35-40 % of total dry matter in the ration. Feeds containing over 2000 ppm nitrate nitrogen should not be used for pregnant animals
5	3500-4000	Feeds should be limited to 25 % of total dry matter in ration. Do not use for pregnant animals.
6	> 4000	Feeds containing over 4000 ppm are potentially toxic. Do not feed

Sources: Andrae and Pinkerton (2008) as adopted by Ramteke et al. (2019)

Table 3: Level of Nitrate in forage crops (DM Basis) and potential effects on animals

4. Methods counteract and reduce antinutritional factors in livestock feed

A better understanding and management of anti-nutritional factors in animal feed is necessary to allow farmers to apply more appropriate techniques to reduce deleterious antinutrient effects while enhancing their benefits, thus enabling the use of vast reservoirs of animal feedstuffs (Epafras, 2019). Various techniques have been used to reduce or eliminate the adverse effects of ANFs in animal feed and their effects. These methods include mechanical or physical procedures (e.g., wilting, processing, and ensiling), microbe inoculation, and chemical approaches (treatment with alkalis, organic solvents, and precipitants) (Tadele, 2015). Fermentation, germination, soaking, and processing techniques like autoclaving and milling (Deeksha et al., 2023).

4.1. Sprouting/germination: Sprouting/germination is considered to be a highly suitable method for reducing the anti-nutrient components of plant-based feeds (Nkhata et al. 2018). Owing to microbial enzyme activity for their sustenance, sprouting or germination reduces crude fiber content (Okoye & Ene, 2018). Germination has been shown to be an effective method for reducing ANF content by mobilizing secondary metabolic compounds. For example, germination at 25°C for 24, 48, and 72 h significantly decreases the levels of condensed tannins in faba beans by 56, 58%, and 60 %, respectively (Alonso et al., 2000).

4.2. Soaking: Whole seeds were soaked in distilled water for 24 h at room temperature at a bean: water ratio of 1:10(w/v). After soaking, the water was drained, and the seeds were dried at 550C for 6 h in a hot air oven (Doss et al., 2011). It provides essential moist conditions in grains and other edible seeds that are required for germination and for the reduction of enzyme inhibitors and other antinutritive elements, which improves nutritional quality and digestibility (Kumari, 2018). It is also commonly required for fermentation, which can be used to reduce the levels of various anti-nutrients in foods (Gupta et al. 2015). According to Oladele et al. (2009), at 60 °C for 6 h, it effectively reduced the anti-nutrient content of tiger nut tannins by 61%, polyphenols by 48%, phytate by 44%, oxalate by 58%, and alkaloids by 13%, as well as improved the nutritional value.

4.3. Cooking: Another set of seeds was cooked in distilled water (100 0C) at a bean: water ratio of 1:10 (w/v) for 20 min. The cooked seeds were rinsed with distilled water and dried at 550C for 6 h in a hot-air oven (Doss et al., 2011).

4.4. Milling: Milling is the most commonly used method for removing bran layer from grains. This technique removes anti-nutrients (e.g., phytic acid, lectins, tannins), which are present in the bran of grains. However, this technique has the main disadvantage of removing the important minerals (Gupta et al. 2015).

4.5. Boiling: Most foods become useful and healthy with heat treatment applications in the daily diet. Legumes and cereals are usually cooked either by simple boiling or using a pressure cooker for consumption (Ertop and Bektas, 2018). Simple boiling has been reported to improve the nutritional quality of food grains by reducing antinutrient levels (Rehman and Shah, 2005).

4.6. Fermentation: Fermentation is an important process that decreases the levels of antinutrients in food grains and increases mineral extractability, in vitro protein digestibility, and the nutritional value of grains (Ertop and Bektas, 2018). Fermentation decreases the antinutrient levels in food grains and increases mineral extractability (Badau et al., 2005). It reduces the amounts of phytic acid, tannins, and polyphenols during fermentation and increases the mineral bioavailability and digestibility of food (Gupta et al., 2015).

4.7. Autoclaving: Separated batches of seeds were autoclaved at 15 psi (1210C) in distilled water at a bean: water ratio of 1:10 (w/v) for 30 min. After treatment, the seeds were rinsed with distilled water and dried at 550C for 6 h in a hot-air oven (Doss et al. 2011). When this application is used on cereals and other plant-based foods, it activates phytase and increases acidity (Ertop and Bektaş 2018). Most previous studies concluded that autoclaving is the best method to reduce the levels of several anti-nutritional compounds when compared to other processing methods (Shimelis and Rakshit, 2007; Vadivel et al. 2008; Doss et al. 2011).

4.8. Storage: Proper storage as well as the use of urea can aid in the reduction of phenols and condensed tannins. Tannin inactivation increases when urea is used followed by storage. Chopping and keeping leaves rather than feeding them on the same day improves the degree of tannin deactivation; that is, chopping and store for 5-10 days before feeding (Makkar and Singh, 1991). The release of ammonia, which is required for tannin inactivation, requires the use of urea.

4.9. Wood ash: Wood ash is a useful alkali source for decreasing overall phenol levels. High-tannin sorghum and millet have traditionally been treated with wood ash solutions for human consumption. Wood ash, a very inexpensive alkali source, can detannify tannin-rich feedstuffs (Makkar et al., 1991).

4.10. Drying: Cassava and Leucaena leaves were dried at different temperatures (60°C for 48 h, shade drying for 24, 48, and 72 h, and sun drying for 24 and 48 h) to minimize the tannin content (Makkar et al., 1991). For feedstuffs with greater moisture content, drying is more effective; for example, drying leaves from multifunctional trees (MPTS) decreases tannin levels.

4.11. Chemical treatment: Various chemical treatments have been employed to improve the nutritional value of legumes, such as polyethylene glycol, for which tannins have a higher affinity than proteins, is by far the most used reagent to neutralize these secondary compounds (Muller-Havey, 2001). Aqueous organic solvents (30% acetone, 50% methanol, and 40% ethanol) were used to extract up to 70% of the tannins from the leaves. The use of an alkali, such as 0.05M Sodium hydroxide, is efficient in the treatment of phenolics because they are oxidized by oxygen at higher pH levels. Tannin levels were lowered by 95% using oxidizing agents like 0.02M potassium permanganate (Singh, 1993). Polyethylene glycol, ferrous sulfate, and tannin-complexing agents have also been used to inactivate tannins and lower tannin levels. Polyethylene glycol can be sprayed on a diet after being combined in water at 0.5 g PEG/ml (Getachew et al., 2001). Polyethylene glycol is a non-absorbable, inert chemical that forms a stable combination with tannins, blocking tannin-protein binding while simultaneously releasing proteins from the tannin-protein complex (Yisehak et al., 2013).

5. Summary

Plant and plant product-originated livestock feed may be limited by ANFs content, even if they are important for their nutritive value. Additionally, the ability of a compound to act as an ANF depends on the animal ingesting it. Being an ANFs by itself is not harmful, as its ability to be poisonous/toxic depends on the livestock ingesting it. However, most of them are responsible for adverse effects that are related to the absorption of nutrients and micronutrients, which may interfere with the function of certain organs and are present in feeds of plant and plant product origin. Saponins, tannins, protease inhibitors, cyanogenic glycosides, lectins, mimosine, and amylase inhibitors are among the most common ANFs in livestock feed and induce undesirable effects in animals if their consumption exceeds an upper limit. Certain harmful effects may also be caused by the breakdown products of these compounds. The concentration or level of these compounds can vary with plant cultivars, plant parts (leaf, seed, hull, stem, etc.), and postharvest treatments (processing methods). The negative effects of ANFs in livestock feed should be minimized or reduced by feeding with low or free ANFs feed sources, reducing ANFs content using different techniques (avoiding/reducing high levels of these factors), and by creating awareness about the effect of ANFs on livestock keepers, feed producers, and all actors involved in livestock feed starting from production until feeding.

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