International Journal of Veterinary Sciences and Animal Husbandry

ISSN: 2456-2912
VET 2023; 8(1): 18-27
© 2023 VET
www.veterinarypaper.com
Received: 11-11-2022
Accepted: 15-12-2022

F Kemboi
Department of Animal Science, Egerton University, P.O Box 536-20116 Egerton, Kenya

JO Ondiek
Department of Animal Science, Egerton University, P.O Box 536-20116 Egerton, Kenya

AM King'ori
Department of Animal Science, Egerton University, P.O Box 536-20116 Egerton, Kenya

PA Onjoro
Department of Animal Science, Egerton University, P.O Box 536-20116 Egerton, Kenya

Corresponding Author:
F Kemboi
Department of Animal Science, Egerton University, P.O Box 536-20116 Egerton, Kenya

Effects of anti-nutritive factors on ruminants and methods to alleviate them: A review

F Kemboi, JO Ondiek, AM King'ori and PA Onjoro

DOI: https://doi.org/10.22271/veterinary.2023.v8.i1a.461

Abstract
Anti-nutrient substances are compounds originating in most animal feed materials that are toxic to animals and limit the accessibility of nutrients to the animal body. But, some Anti-nutrients substances might exercise useful special effects at lower concentrations in feeding material. Main anti-nutritional factors in eatable forage and fodder contain tannins, saponins, phytic acid, gossypol, lectins, amylase inhibitors, goitrogens and protease inhibitors. The balance between anti-nutritional factors' beneficial plus lethal effects depends on their time of exposure, concentration, biochemical structure, and interaction with other dietary constituents. Numerous anti-nutritional factors and noxious elements are present in various foliage utilized as animal feed. Anti-nutrient substances decrease the maximal use of nutrients, particularly minerals, proteins, and vitamins, therefore inhibiting optimum utilization of the nutrients in the feeds and reducing their nutritive quality. High concentrations of tannins (>5.0 g/100 g DM) can be lethal to animals, especially ruminants feeding on tannin-rich forages. Tannins mostly found in legumes binds with proteins, leading to the activation of numerous digestive enzymes and reduction protein digestibility. It is concluded that numerous anti-nutrient substances that affect protein digestibility and the poisonous effects of these substances in plants can be removed or treated to reduce the negative effects using a variety of processing methods such as boiling, fermentation, soaking, germination, and chemical treatment. From the various studies, processing methods can greatly improve the nutritive value of feeding material as feed for ruminants. This review focuses on various categories of anti-nutritional factors, effects of anti-nutritive factors on ruminants, and likely treating methods that could be employed to lessen these factors in animal feeds.

Keywords: Anti-nutrients, tannins, oxalate**,** cyanide, phytate, protein digestibility

Introduction

Anti-nutritional factors are substances that, when present in feed, either directly or through their metabolic products, reduce nutrient accessibility, thereby affecting animal performance (Yacout, 2016) [108]. Anti-nutritional factors are naturally occurring substances found in feeds that may be responsible for decreased nutrient utilization in animals after intake (Jeyakumar *et al.*, 2022) [36]. Plant toxins can be found in the foliage, seeds, bark, and nearly every plant part (D'Mello, 2004) [13]. Chemical constituents in feeds that, by themselves or through metabolic products produced in the system, inhibit feed use, reduce animal performance, or negatively impact animal health (Ramteke *et al.*, 2019) [73]. ANFs are chemical substances produced naturally in plants by the normal metabolism of plant species or by various mechanisms such as metabolic utilization of feed (Akande *et al.*, 2010) [3]. As a result, they are feed additives that inhibit animal growth and performance. The anti-nutritional factors (ANFs) exert an effect contrary to optimal nutrition (Akande *et al.*, 2010) [3]. Anti-nutrients are secondary metabolite substances evolved by plants for their protection (Habtamu & Negussie, 2014) [28] and, therefore, protect the plant from defoliation by insects and animals, especially browsers.

There are several classifications of plant toxins. D'Mello, (2004) [13] classified anti-nutrients into heat-labile groups comprising cyanogens, lectins, and proteinase inhibitors and heat-stable groups as condensed tannins, antigenic proteins, saponins, gossypol, and Phyto-oestrogens. According to Aletor (2005) [4], classification can be based on their chemical structure, specific action when ingested, or biosynthetic origin. The biological effects of these chemicals are complex and diverse (Habtamu & Negussie, 2014) [28]. These factors might not always be harmful, especially when consumed below the lethal dose. However, the balance of harmful and beneficial effects of anti-nutrients is determined by their concentration, chemical structure, interaction with other dietary components, time of exposure, and other factors (Habtamu & Negussie, 2014) [28]. The majority of these complexes' toxic effects from anti-nutritional factors can be removed using treatment methods such as fermentation, soaking, germination, genetic manipulation, autoclaving, and boiling (Jeyakumar *et al.*, 2022) [36].

Table 1: Anti-nutritional factors in forage crops

S. No	Anti-nutritional factors	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

Source: Ramteke *et al.* (2019) [73]

Types of anti-nutrients

Cyanogens

Cyanogens are glycosides that readily yield hydrogen cyanide (HCN) on activation (D'Mello, 2000) [14]. Cyanogens are products of secondary metabolism, naturally existing in plants. Cyanogens are sugar glycosides, and glucose is frequently joined with aglycone cyanide (Thakur *et al*., 2019) [95]. These compounds consist of a hydroxyl nitrile aglycone and a sugar moiety. They are derived from the proteinogenic amino acids isoleucine, leucine, valine, tyrosine, and phenylalanine, as well as the non-proteinogenic amino acid cyclopentenyl glycine (Habtamu & Negussie, 2014) [28]. For instance, among the feed ingredient, cassava is famous for with high levels of cyanide, a toxic respiratory substance lethal to animals (Amanabo *et al.*, 2011) [5]. The primary role and release of cyanogens in plants are reliant on stimulation by glucosidases to discharge poisonous volatile hydrogen cyanide in addition to ketones or aldehydes to keep away pathogen attack and herbivores (Zagrobelny *et al*., 2004) [110]. They impair central nervous system (CNS) function, resulting in cardiac arrest and respiratory failure (D'Mello, 2000) [14].

Saponins

Saponins are secondary compounds that are non-volatile and surface-active that are widely distributed in natural environments, primarily in the plant kingdom. Saponins are composed of non-polar aglycones linked by one or more monosaccharide units (Oleszek, 2002) [65]. They are structurally distinct particles composed of non-polar aglycones linked to one or more monosaccharide moieties. Their soap-like behavior in aqueous solutions is explained by the arrangement of polar and non-polar structural components in their molecules (Tadele, 2015) [91]. They are not only bitter, but they also induce throat inflammation, which reduces feed intake and animal performance (Shi *et al.*, 2004) [83]. Saponins have been shown in chicken to lower feed efficiency and growth, as well as to interfere with vitamin (A and E) and dietary fat absorption (Jenkins & Atwal, 1994) [34]. Saponins in ruminants can interfere with the eructation process in the reticulo-rumen, causing bloat (Ibrahim, 2019) [32]. Furthermore, they diminish nutritional bioavailability and enzyme action that influences protein breakdown by inhibiting certain digestive enzymes such as trypsin and chymotrypsin, which are nevertheless vital in the breakdown of dietary protein into peptides and amino acids (Simee, 2011) [85].

Tannins

Tannins are phenolic compounds present in all plants and climates across the world (Habtamu & Negussie, 2014) [28], particularly in leguminous forages, seeds (D'Mello, 2004) [13], and pods (Odero-Waitituh, 2015) [61]. Tannins are secondary compounds generated by plants and found in their leaves, bark, and fruits (Timotheo & Lauer, 2018) [99]. Tannins are classified into 1) hydrolyzable tannins found in garlic acid, 2) condensed tannins found in most legumes, and 3) pseudo-tannins found in brown-green algae (Habtamu & Negussie, 2014) [28], with brown, green algae having the lowest concentration of tannins. Hydrolyzable tannins are typically broken down in the ruminant gastrointestinal tract during digestion (Samtiya *et al.*, 2020) [79]. High tannin concentrations (>5.0 g/100 g DM) can be harmful to animals eating tannin-rich forages, causing intestinal mucosa irritation and peeling, liver and kidney abrasions, and even death (Reed, 1995) [75]. Tannins, which are mostly found in legumes, form compounds with proteins, inactivating numerous digestive enzymes and lowering protein digestibility. By generating alterable and irreversible tannin-protein complexes, tannins impair protein digestion, resulting in a reduction in critical amino acids (Raes *et al.*, 2014) [72]. They have an astringent and bitter taste, which confers their protection from browsing and perhaps growth regulation (Habtamu & Negussie, 2014) [28]. When browsed, they bind or precipitate proteins and other organic compounds like as amino acids and alkaloids. (Harold, 2004) [31], lowering the efficiency of animal feed. Proteins may be precipitated from aqueous solutions, with the quantity varied according to pH, ionic strength, and tannin molecular weight (Akande *et al.*, 2010) [3]. It can also be found in cereals like sorghum (Nyachoti *et al.*, 1997) [59]. More than 1.5 percent dry matter in the diet has a considerable impact on trypsin, alpha-amylase, and total proteinase activity, lowering the tract's absorptive capacity and resulting in poor animal performance (Singh & Mohanty, 2014) [88]. Oxalates and polyphenols are anti-nutritive chemicals that prevent food minerals from reaching the animal's body (Kaushik *et al.*, 2018) [38].

Protease and Amylase inhibitors

Proteinase inhibitors can be present in many legumes and seed plants (Akande *et al.*, 2010) [3]. They are a separate class of heat-labile anti-nutritional factors that react very specifically with various proteolytic enzymes in animals' gastrointestinal systems (D'Mello, 2004) [13]. Therefore, they reduce protein digestion, cause endogenous losses of amino acids, and lower animal performance. Protease inhibitors' anti-nutrient activity is linked to growth suppression, particularly in raw legumes (D'Mello, 2004; Habtamu & Negussie, 2014) [13, 28] and pancreatic hypertrophy (Habtamu

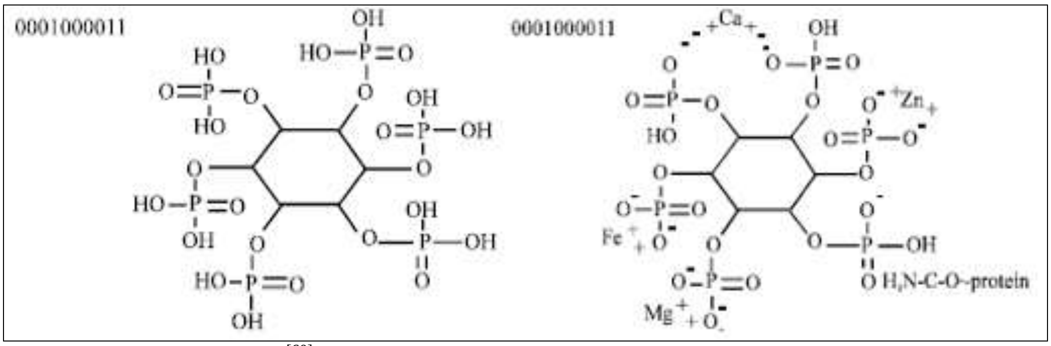
& Negussie, 2014) [28]. Amylase suppressors inhibit bovine pancreatic amylase but not endogenous amylase, as well as fungal and bacterial activity in the animal intestine (Thakur *et al.*, 2019) [95]. Amylase inhibitors or starch blockers such as tannins (Singh & Mohanty, 2014) [88] contain elements that prevent nutritional starches from absorption (Habtamu & Negussie, 2014) [2]. Amylase suppressors work in the pH range of 4.5 to 9.5 and are generally destroyed or changed by heat (Marshall & Lauda, 2007) [49]. However, because these inhibitors are unsteadiness in the gastrointestinal tract (GIT), they fail to reduce insulin responses and boost feed caloric production when used as a starch blocker (Giri & Kachole, 1998) [25].

Goitrogens

Goitrogens can interfere with the thyroid gland's function through altering the gland's regulatory systems, as well as its outer metabolism and thyroid production (Habtamu & Negussie, 2014) [28] and thereby causing thyroid hypertrophy (goiter). It is present in some members of the Brassica family, legumes such as groundnut and soybean (Akande *et al.*, 2010) [3]. They obstruct the generation and secretion of thyroid hormone, resulting in stunted growth and poor reproductive performance (Olomu, 1995) [66]. Sulphur glycosides in goitrogens stimulate the thyroid gland to develop by blocking the thyroid gland from absorbing iodine (Thakur *et al*., 2019) [95]. This poisonous effect from goitrogens can be reduced by adding iodine to the diet to reduce the adverse effects (Nagaraj, 2009) [56].

Phytates

Phytate is a type of phytic acid that can be found in plants, animals, and soil. They occur naturally as constituents of plant roots, tubers, and seeds (Brooks, 2001) [10], with considerable quantities in many oilseeds and legumes (Akande *et al.*, 2010) [3]. They accumulate in the seeds as a salt (phytic acid) of the monovalent and divalent cations K+, Mg2+, and Ca2+ throughout the ripening period. Phytic acid is a negatively charged molecule that forms complexes with positively charged metal ions including calcium, iron, magnesium, and zinc (figure 1), limiting their availability in the body through decreased absorption rates (Samtiya *et al.*, 2020) [79]. These phytates bind around two-thirds of the phosphorus in legumes and seeds. They bind to zinc and other minerals, decreasing absorption and threatening animal development (Brooks, 2001) [10]. Phytates impair calcium bioavailability, and the Ca: Phy molar ratio has been proposed as a measure of calcium bioavailability. The essential molar ratio of Ca: Phy is stated to be 6:1. (Oladimeji *et al.*, 2000) [64] The negatively charged phytate molecule is thought to bind key divalent cations such as Fe2+, Zn2+, Mg2+, and Ca2+ (Tadele, 2015) [91]. Phytate is regarded the principal phosphorus storage form (Habtamu & Negussie, 2014) [28], and efforts should be made to release the phosphorus by the use of enzyme phytase/Fitase as a feed supplement to minimize deficiencies. Phytate has also been identified to modulate insulin excretion in animals (Shamsuddin, 2002) [82]. Phytate, as a complexing agent, may remove heavy metal ions. Boiling has been shown to reduce phytate levels by up to 20% (Thakur *et al.*, 2019) [95] and is inexpensive.



Source: Oatway *et al.* (2001) [60]

Fig 1: Structure of phytate Structure of phytate, mineral, protein, starch complex

Oxalates

Oxalate is a dicarboxylic acid that accumulates in many plants as insoluble calcium oxalate, soluble oxalate, or a mix of the two. Animals can be poisoned by high oxalate concentrations in feed plants (Goyal, 2018) [27]. Oxalates impact calcium and magnesium metabolism, forming compounds having inhibitory effects on stomach digestion when they combine with proteins (Akande *et al.,* 2010) [3]. Oxalate binds to calcium, generating an insoluble calcium oxalate that impairs calcium uptake and use in animals (Olomu, 1995) [66]. Soluble oxalate commonly forms complexes with ammonium (NH4 +) ions, potassium (K+), and sodium (Na+). Insoluble oxalate forms compounds with calcium ($Ca^{2+),}$ iron (Fe^{2+}) ions, and magnesium (Savage *et al*., 2000) [81]. Due to a high level of oxalates in the system, the insoluble calcium-oxalate complex has a tendency to precipitate in the urinary tract of the kidneys, generating calcium oxalate crystals (Veer *et al.*, 2021) [104]. When acid is excreted in the urine, calcium oxalate crystals form in the urinary tract, causing kidney stones (Noonan & Savage, 1999) [58].

Lectins

Lectins (Hemagglutinins) are proteins or glycoproteins present in over 800 different varieties of legumes, accounting for around 2-10% of total protein in bean seeds (Thakur *et al.*, 2019) [95]. Lectins are carbohydrate-binding proteins present in cereal seeds, beans, and tubers that preferentially bind carbs and, more critically, glycoprotein carbohydrate moieties on the surface of most animal cells, causing nausea, vomiting, diarrhea, gut hyperplasia, and weight loss (Sauvion *et al.*, 2004) [80]. Lectins are protein antigens that bind to glycolipids or glycoproteins found on the surface of red and white blood cells (Sauvion *et al.*, 2004) [80]. Lectins may also have systemic effects such as increased protein metabolism and glycogen catabolism, accumulation of fat, and disruption of mineral breakdown (Fereidoon, 2014) [19].

Processing methods and their effect on anti-nutrient content in feeds

Successful attempts at using genomic technologies to reduce anti-nutrient content in feeds as the reduction of phytate in seeds have been successful. However, this success had disadvantages as phytate reduction in the seed interfered with germination as it is important in seed germination (Sing & Mohanty, 2011) [88]. This opens research to methods that do not interfere with plant physiology. Various methods have been used to reduce anti-nutrients in animal feeds and their effects. Among these methods are mechanical or physical procedures (e.g., wilting, processing, and ensiling), microbe inoculation, and chemical approaches (treatment with alkalis, organic solvents, and precipitants) (Tadele, 2015) [91].

Soaking and germination

At 60ºC for 6 hours, it effectively reduced anti-nutrient content in tiger nut tannins by 61%, polyphenols by 48%, phytate by 44%, oxalate by 58%, and alkaloids by 13%, as well as improving the nutritional value (Oladele *et al.,* 2009) [63]. Soaking nuts and other edible seeds provides a crucial wet environment for germination and the reduction of enzyme inhibitors and other anti-nutritive elements, which improves nutritional quality and digestibility (Kumari, 2018) [41]. Due to microbial enzyme activity for their sustenance, sprouting or germination reduces crude fibre content (Okoye & Ene, 2018) [62].

When grain seeds germinate, the enzyme phytase is activated, which reduces phytate and so lowers phytic acid levels in the seed grain (Samtiya *et al.,* 2020) [79]. Because minerals and water-soluble vitamins in legumes and grains are leached during soaking and fermentation, phytochemicals are reduced (Kruger *et al.,* 2014) [40]. The presence of the enzyme phenolic oxidase during the germination process might explain the drop in polyphenol levels (Tian *et al.,* 2019) [98]. Furthermore, germination in conjunction with dehulling enhances legume quality by boosting nutritional bioavailability and digestibility while decreasing anti-nutrients (Tadele, 2015) [91]. Seed germination and fermentation Improve the nutritional value of legumes and grains by changing their chemical composition and decreasing the quantities of antinutritional chemicals that are harmful to animals (Samia *et al.,* 2005) [78].

Heat toasting and sun drying

Boiling or cooking improves feed nutritional value by lowering anti nutritional content, particularly trypsin inhibitors and tannins (Patterson *et al.,* 2017) [68]. In the feed manufacturing sector, heat processing is a standard practice that increases feed safety and nutritional value (D'Mello, 2004; Rao *et al.,* 2013) [13, 74]. Because of the unique nature of proteinase inhibitors, heat processing is successful in denaturing them; yet, some residual activity may exist in commercially made products (Habtamu & Negussie, 2014)) [28]. Heat processing can also denatured Lectins and cyanogens (D'Mello, 2004) [13]. The anti-nutrients in tiger nut were reduced by toasting in an open-air pan at 100 °C for 30 minutes; tannins by 71%; polyphenols by 65%; phytate by 40%; oxalate by 77%, and alkaloid by 27% (Oladele *et al.,* 2009) [63]. Sun drying is a feasible heat treatment method in the tropics as it removes some aflatoxin (Rao *et al.,* 2013) [13] and micro-organisms when poultry waste is fed to ruminants. Numerous anti-nutritional factors, particularly in leguminous plants, can be significantly reduced by soaking autoclaving, and cooking (Torres *et al.,* 2016) [100].

Chemical treatment

Ammoniation is the chemical treatment of aflatoxin-contaminated feed using ammonium hydroxide or gaseous ammonia in industrial feed mills at high temperatures and pressures, or at room temperature and moderate pressure in small-scale operations (D'Mello, 2004; Egwim *et al*., 2013) [13, 16]. If the ammoniation processes are allowed to complete, the detoxification process is permanent, and aflatoxin contamination is almost eliminated. As long as the residual ammonia is evaporated, the feedstuff remains aflatoxin-free. Decontaminated meals are readily consumed by animals with no negative consequences (D'Mello, 2004) [13].

Polyethylene glycol treatment of tannin-rich forages is accomplished by spraying foliage with polyethylene glycol, but the economic feasibility of this procedure remains to be determined (D'Mello, 2004) [13].

Fermentation

Fermentation is the process through which microorganisms such as bacteria, yeast, and fungus transform carbohydrates into organic acids or alcohol. End products include biomass, enzymes, primary and secondary metabolites, and recombinant products. The conversion of sugars and other carbohydrates to usable end products, as well as the synthesis of critical nutrients such peptides (Jyoti *et al.,* 2016) [37] and the elimination of anti-nutrients and mycotoxins are the main advantages of fermentation (Aremu *et al.,* 2015; Okoye & Ene, 2018; Sukhanandi *et al.,* 2014) [62, 6, 90]. Grain fermentation increases the quantity of amino acids, particularly limiting amino acids like methionine, tryptophan, and lysine, which improve nutritional quality (Mohapatra *et al.,* 2019) [53]. Probiotic fermentation with *Lactobacillus spp* also enhanced protein and starch *in-vitro* digestibility while lowering anti-nutrients including phytic acid, polyphenols, and trypsin inhibitors (Sindhu & Khetarpaul, 2001) [86]. In cassava roots, fermentation has also been demonstrated to lower the potential toxicity of cyanogenic glycosides (Bakshi & Wadhwa, 2004) [8]. This improves the nutritional content of grains by lowering phytic acid levels and releasing soluble calcium, iron, and zinc (Gibson *et al.,* 2010) [24].

Effects of Tannins on Ruminant Animals

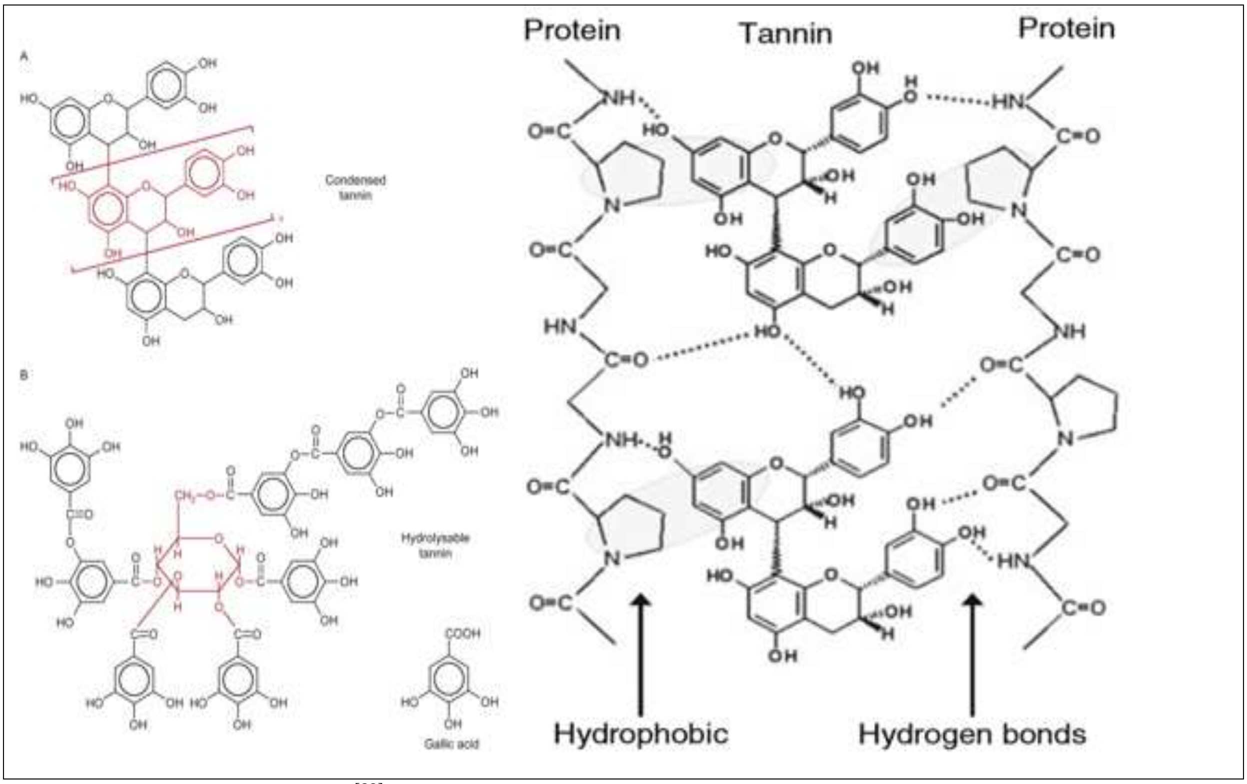
Tannins are polyphenols found in plants that differ from other polyphenols in their capacity to form complexes (Figure 2) and precipitate proteins (Hagerman, 2012) [29]. Tannins are phenolic chemicals present in all plants and in all regions across the world (Habtamu & Negussie, 2014) [28], particularly in leguminous forages, seeds, and their pods (D'Mello, 2004) [13]. (Odero-Waitituh, 2015) [61]. Tannins are classified into 1) hydrolyzable tannins (HT) found in garlic acid, 2) condensed tannins (CT) found in most legumes, and 3) pseudo-tannins found in brown-green algae (Habtamu & Negussie, 2014) [28], with brown-green algae having the lowest concentration of tannins. Hydrolyzable tannins are more soluble in water than condensed tannins, have a smaller molecular weight, and are more sensitive to enzymatic and non-enzymatic hydrolysis (Jerónimo *et al*., 2016) [35]. Only dicotyledonous plants have hydrolyzable tannins, while angiosperms and gymnosperms have condensed tannins, according to Silanikove *et al* (2001) [84]. Despite this, according to Waghorn, both hydrolyzable and condensed tannins can be present in the same plant.

The most prevalent tannin found in forage legumes, shrubs, and tree leaves is condensed tannins (Min *et al.,* 2003) [52]. Many of these plants are common and excellent for ruminant diet (Vasta *et al*., 2008) [103]. These forages are increasingly being employed as a replacement feeding resource to replace part of the cereal concentrate in animal diets, according to Vasta & Luciano (2011) [102], particularly in small ruminants.

This is done to lower livestock feeding expenses and increase the quality of their edible products as part of nutritional programs. Tannins are polyphenolic secondary plant chemicals or metabolites that have been demonstrated to inhibit microbial activity in fermentation, protein breakdown, methane synthesis, and the ability to reduce food-borne pathogens (Addisu, 2016) [2].

When browsed, tannins bind or precipitate proteins and other organic compounds, including amino acids and alkaloids, reducing animal feed utilization efficiency (Harold, 2004) [31]. They also have an astringent and bitter taste, which confers protection from browsing and possibly growth regulation (Habtamu & Negussie, 2014) [28]. Proteins may be precipitated from aqueous solutions, and the rate of precipitation is influenced by pH, ionic strength, and the tannins' molecular weight (Akande *et al*., 2010) [3]. Sorghum and other grains contain them as well (Nyachoti *et al.,* 1997) [59]. More over 1.5 percent dry matter in the diet reduces trypsin, alpha-amylase, and total proteinase activity, as well as the tract's absorptive capacity, resulting in poor animal performance (Singh & Mohanty, 2014) [88]. Leguminous seeds and forages contain a lot of tannins. Goats can handle condensed tannins better than cattle and sheep, because to their high proline levels in their saliva.

Condensed tannins are more digestible than hydrolyzable tannins, which can generate a range of harmful effects in the rumen owing to hydrolysis. Mules, deer, rats, and mice have been seen secreting proline-rich proteins in their saliva, which function as a first line of defense against ingested tannins (Niezen *et al*., 1995) [77]. Ruminal function, feed intake, wool development, and live weight increase are all influenced by condensed tannins (Kumar & Dutta 2018) [42]. By lowering protein degradability in the rumen and improving protein flow to the small intestine, condensed tannins may provide nutritional advantages for cattle, such as bloat control and enhanced by-pass protein availability.



***Source*:** Smolander *et al.* (2012) [89]

Fig 2: Structure of tannins Structure of tannin-protein complexes

Digestion Kinetics

Tannin-protein complexes render protein inaccessible to the animal, lowering the nutritional value of the forage (Taiz & Zeiger, 2002) [92]. The rate of gas generation is influenced by purified tannins from diverse plants, which reduce microbe adhesion to feed particles. According to McAllister *et al.* (1994) [5], condensed tannins cause considerable detachment of *Fibrobacter succinogenes*, lowering rumen fibrolytic activity. At least for condensed tannins, which are not destroyed by rumen bacteria, microbial adaptation to tannins with a delay in tannin breakdown appears to be responsible (Makkar *et al.*, 1995) [48]. The astringent taste of tannin-rich meals is due to their decreased consumption. Aside from the unpleasant or bitter taste, high-tannin diets have a slower rate of digestion (Makkar *et al.*, 1995) [48], which may explain for the decreased feed intake.

Post-Absorptive Effects

Because condensed tannins are not absorbed into the bloodstream (Terril *et al.*, 1994) [94], they are unlikely to affect organs including the liver, kidney, and spleen under normal physiological settings (Garg *et al.*, 1992) [22]. Condensed tannins, like hydrolyzable tannins, can be taken into the circulation once excessive amounts of tannins or other intestinal membrane irritants induce intestinal injury. Saponins have also been related to an increase in permeability in the intestine (Price *et al.*, 1987) [71]. Increased absorption of both condensed and hydrolyzable tannins, as well as nutrients like glucose and amino acids, may be a result of consuming saponins and tannin-containing diets. Tannins also have antihelminthic effects and assist ruminants avoid bloating. Legume tannins may enhance silage quality by limiting excessive protein breakdown during ensiling (Hagerman *et al.*, 1999) [30].

Microbial Protein Synthesis Effectiveness

In the presence of tannins, microbial protein synthesis is more efficient, as assessed by the amount of nitrogen incorporated into bacteria per unit of short-chain fatty acid biosynthesis (Makkar *et al.*, 1995) [48]. Despite the fact that tannins lower nutritional availability, they promote a change in nutrient partitioning, directing a higher percentage of available resources to microbial mass synthesis and less to short-chain fatty acid biosynthesis. Rumen fermentation efficiency can also be determined by the ratio of volatile fatty acids to microbial protein (VFA: MCP). Tannins from native browsing species can influence ruminant fermentation, particularly in the rumen, resulting in improved rumen by-pass of critical amino acids and increased animal output (Rivera-Méndez *et al.*, 2017) [76]. As a result, tannins can control rumen fermentation to enhance microbial protein production at low concentrations (Poungchompu *et al.*, 2009) [70]. Tannins may assist to coordinate the release of different nutrients by delaying feed digestion, which might explain the increase in microbial efficiency (Makkar *et al.*, 1995) [48]. Increased microbial protein synthesis efficiency and lower feed protein degradability in the rumen benefit ruminants. They enhance the availability of non-protein nitrogen to the small intestine, resulting in more milk, meat, and wool being produced. Furthermore, these effects result in ruminant protein sparing, as well as lower methane generation and nitrogen excretion into the environment. According to Frutos *et al.*, (2004) [20] tannins have long been regarded to be toxic to ruminants The type of tannin ingested, its chemical structure and molecular weight, the amount consumed, and the animal species involved all influence the outcome.

Antihelmintic Effects of Tannins

Helminthiasis is a severe impediment to ruminant productivity. Tannins have long been known to have anthelmintic characteristics, particularly condensed tannins, and employing CT-containing forages to reduce digestive system parasites has been investigated as a strategy to reduce the usage of synthetic anthelmintic medications in ruminant production (Wang *et al*., 2015) [107]. The bulk of parasite control initiatives, according to FAO (2002) [18], relied mainly on chemotherapeutic control. Several issues with this practice have been identified, including the growing problem of parasite resistance to various classes of drenches (Chartier *et al.*, 2001; Leathwick *et al.*, 2001) [12, 43], the risks of chemical residues in animal products and the environment in general, toxicity, and drug non-availability in inaccessible regions. Herd health is harmed by nematode infections, which can make farming less lucrative (Charlier *et al.*, 2014) [11]. Tannins in fodder have been found to minimize the negative effects of gastrointestinal parasites by eradicating larval and adult worms, according to Athanasiadou *et al.* (2001) [7]. Similarly, when sheep were fed varying amounts of condensed tannins, multiple researchers (Molan *et al.*, 2002; Paolini *et al.*, 2005) [54, 67] observed decreased fecal tally and worm load with no difference in parasite species. The resistance of Haemonchus contortus to anthelmintics is a worldwide problem, thus finding and developing new chemicals that can help with nematode control is critical (Emery *et al.*, 2016) [17]. As a result, forages containing CT can be utilized as a parasite management option. Condensed tannins offer antihelmintic capabilities against roundworms (*H. contortus*), allowing ruminants to better use nutrients (Paolini *et al.*, 2005) [67]. Condensed tannins bind tightly to free proteins in the gastrointestinal system, decreasing food availability and resulting in the death of larvae and worms (Athanasiadou *et al.*, 2001) [7]. Increased digestible protein availability boosts nematode resistance and resilience in the gastrointestinal tract (Donaldson *et al*., 1997; VanHoutert & Sykes, 1996) [15, 101]. Furthermore, tannins would bond with the high-glycoprotein cuticle of larvae, resulting in larval mortality (Thompson & Geary, 1995) [97]. Molan *et al.* (2002) [54] reported that CT derived from sainfoin prevented lungworm and gastrointestinal nematode egg development in a dose-dependent manner (mixed species of Ostertagia, Oesophagostomum, Cooperia, Trichostrongylus, and Strongyloides).

Frothy Bloat and Bloat Safety

The quick creation of fermentation gas and the release of soluble protein into the rumen fluid from highly digested, high-protein forage causes frothy bloat in ruminants (Wang *et al*., 2012) [106]. According to Li *et al.* (1996) [44], alfalfa (Lucerne) foamy bloat might be stopped with as low as 1.0 mg CT g-1 DM. According to Addisu, (2016) [2] the production of stable protein broth in the rumen of animals fed high-nutritive-value legumes like Lucerne or white clover causes bloat in small ruminants Animal feed with moderate tannins destabilizes protein foams and reduces bloat (Tanner *et al*., 1995) [93]. Gas created in the rumen during fermentation cannot be discharged regularly when grazing ruminants ingest significant amounts of leguminous plants because it is caught in continuous foam formed by the fast release of soluble proteins during chewing and ruminal breakdown (Addisu, 2016) [2]. When these animals graze on leguminous plants containing CT, however, this does not happen (McMahon *et al.*, 2000) [51].

Tannins' Impact on Ruminal Methanogenesis

Because 6-8 percent of feed gross energy is lost as methane, which contributes to the greenhouse impact in the environment, methane gas generated by anaerobic fermentation in the rumen constitutes a feed energy loss. As a result, controlling methane emissions is crucial to ruminant production's long-term survival (Knapp *et al.*, 2014) [39]. Tannins, particularly condensed tannins, are present in a variety of temperate and tropical plant species and have been proven to lower urine nitrogen (N) excretion in ruminants when taken during browsing (Aboagye, & Beauchemin, 2019) [1]. Tannins, which are typically present in leguminous plants, have anti-methanogenic characteristics, blocking methanogens and indirectly targeting protozoa in ruminants, according to *in-vitro* research (Bhatta *et al.*, 2009; Jayanegara *et al.*, 2015) [9, 33]

CT had an influence on the formation of enteric methane (CH4) from fresh or dried sainfoin, according to Chung *et al.* (2013) [11], and it also had an effect on methane emissions from beef calves fed at maintenance.

For example, condensed tannins (CT) have been demonstrated to lower methane generation by 13–16 percent (DMI basis), owing to a direct toxic action on methanogens (Several alternative plant forages, such as broccoli leaves and some native plants such as *Eremophila glabra* and *Acacia saligna*, have been demonstrated to minimize methane emissions in laboratory studies. Low to moderate tannin concentrations (i.e., 3.0 to 5.0 g/100 g DM) in tannin-rich native browse species might benefit browsing ruminants by preventing bloat, reducing CH4 production, improving N utilization, controlling internal parasites, acting as an anti-oxidant, and improving animal performance (Gladineet *et al.*, 2007; Mueller-Harvey *et al.*, 2019; Theodoridou, 2010) [26, 55, 96].

Detanninification of Feedstuffs
Consumption of mature tannin-rich leaves does not usually result in animal death; however, it does impair production. Various approaches to tannin detoxification or removal in various tannin-rich feed resources are;

Drying
Cassava and leucaena leaves were dried at different temperatures (60 °C for 48 hours, shade drying for 24, 48, and 72 hours, and sun drying for 24 and 48 hours) to minimize tannin content (Makkar & Singh, 1991) [46]. For feedstuffs with a greater moisture content, drying is more effective; for example, drying leaves from multifunctional trees (MPTS) decreases tannin levels.

Chemicals
Aqueous organic solvents (30% acetone, 50% methanol, 40% ethanol) are 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 *et al.*, 1993) [87]. Polyethylene glycol (PEG), ferrous sulfate, and a tannin-complexing agent are also used to inactivate and lower tannin levels. PEG can be sprayed on a diet after being combined in water at 0.5 g PEG/ml (Getachew *et al.*, 2001) [23]. PEG is a non-absorbable, inert chemical that forms a stable combination with tannins (Figure 2), blocking tannin-protein binding while simultaneously releasing protein from the tannin-protein complex (Yisehak *et al.*, 2013) [109].

Wood ash
A useful source of alkali for decreasing overall phenol level is wood ash. High-tannin sorghum and millet have traditionally been treated with wood ash solutions for human consumption. Wood ash, a very inexpensive alkali source, has the ability to detannify tannin-rich feedstuffs (Makkar & Singh, 1991) [46].

Storage
Proper storage, as well as the use of urea, can aid in the reduction of phenols and condensed tannins. Tannin inactivation is increased 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, i.e., chop and store for 5-10 days before feeding (Makkar& Singh, 1991) [46]. The release of ammonia, which is required for tannin inactivation, requires urea.

Solid-state fermentation
White-rot fungi (*Sporotrichum pulverulentum*, *Ceriporiopsis subvermispora*, and *Cyathus stercoreus*) have been used to evaluate another approach for tannin biodegradation (Gamble *et al.*, 1996; Makkar *et al.*, 1994) [21, 47]. Total phenols and condensed tannins were decreased by 58 and 66 percent, respectively, after 10 days of fermentation with *S. pulverulentum* (Makkar, 2003) [45]. *C. subvermispora* and *C. steroreus* white-rot fungi, cause considerable degradation of condensed tannins (56–65%) in *Sericea lespedeza* leaves in 3 weeks (Makkar, 2003) [45].

Conclusions
Tannins, saponins, phytate, oxalate, and protease inhibitors are the most common anti-nutritive factors found in plant materials. When present in high concentrations in animals, these chemical compounds reduce protein digestibility and mineral absorption while also triggering toxicity and health conditions. Anti-nutritive variables have a negative link with micronutrient bioavailability, according to previous studies, since larger levels of anti-nutrients lower nutrient availability. Thus, several processing methods such as fermentation, soaking and germination, heat toasting, sun-drying, and chemical treatment can remove most of the poisonous and anti-nutritional factors effects of these complexes in animal feed; however, more research is needed to realize elimination methods for heat-stable (condensed tannins, gossypol, saponins, and other anti-nutritive factors present in various animal feeds without changing the nutritional value of feed.

References

1. Aboagye IA, Beauchemin KA. Potential of molecular weight and structure of tannins to reduce methane emissions from ruminants: A review. Animals. 2019;9(11):1-18. DOI:10.3390/ani9110856
2. Addisu S. Effect of dietary tannin source feeds on ruminal fermentation and production of cattle; A review. Online Journal of Animal and Feed Research. 2016;6(2):45-56.
3. Akande EK, Doma UD, Agu HO, Adamu HM. Major anti-nutrients found in plant protein sources: Their effect on nutrition. Pakistani Journal of Nutrition. 2010;9(8):827-932.
4. Aletor VA. Anti-nutritional factors as nature's paradox in food and nutrition securities. Inaugural Lecture series 15, delivered at the Federal University of Technology, Akure (FUTA); c2005.
5. Amanabo M, Ogbadoyi EO, Johnson AO, Mathew IS, Akanya HO. Effect of heading on some micronutrients, anti-nutrients, and toxic substances in Amaranthus cruentus grown in Mnna, Niger State, Nigeria. American Journal of Food and Nutrition. 2011;1(4):147-154.
6. Aremu MO, Awala EY, Opaluwa OD, Odoh R, Bamidele TO. Effect of processing on the nutritional composition of African locust bean (*Parkia biglobosa*) and mesquite bean (*Prosopis africana*) Seeds. Communications in Applied Sciences. 2015;3(1):22-41.
7. Athanasiadou S, Kyriazakis I, Jackson F, Coop RL. Consequences of long-term feeding with condensed tannins on sheep parasitized with *Trichostrongylus colubriformis*. Journal of Parasitology. 2001;30(9):1025-1033.
8. Bakshi MPS, Wadhwa M. Evaluation of forest tree leaves of the semi-hilly arid region as livestock feed. Asian-Australasian Journal of Animal Science. 2004;17(6):777-783.
9. Bhatta R, Uyeno Y, Tajima K, Takenaka A, Yabumoto Y, Nonaka I, *et al*. Difference in the nature of tannins on *in vitro* ruminal methane and volatile fatty acid production and on methanogenic archaea and protozoal populations. Journal of Dairy Science. 2009;92(11):5512-5522.
10. Brooks SPJ. Proposed phytic acid standard including a method for its analysis. Journal of AOAC International. 2001;84(4):1125-1129.
11. Charlier J, Van Der Voort M, Kenyon F, Skuce P, Vercruysse J. Chasing helminths and their economic

impact on farmed ruminants. Trends in Parasitology. 2014;30(7):361-367.
12. Chartier C, Soubirac F, Pors L. Prevalence of anthelmintic resistance in gastrointestinal nematodes of dairy goats under extensive management conditions in southwestern France. Journal of Helminthology. 2001;75(4):325-330.
13. D'Mello JPF. Contaminants and toxins in animal feeds. FAO Animal Production and Health paper. c2004, 107-128.
14. D'mello JPF. Anti-nutritional factors and mycotoxins. Farm animal metabolism and nutrition. c2000, 383.
15. Donaldson J, Van Houtert MFJ, Sykes AR. The effect of protein supplies on the periparturient parasite status of the mature ewe. In: Proceedings-New Zealand Society of Animal Production. 1997;57:186-189.
16. Egwim E, Amanabo M, Yahaya A, Bello M. Nigerian indigenous fermented foods: processes and prospects. Mycotoxin and Food Safety in Developing Countries. 2013;7:153-180. http://dx.doi.org/10.5772/52877.
17. Emery DL, Hunt PW, Le Jambre LF. Haemonchus contortus: The then and now, and where to from here? International Journal for Parasitology. 2016;46(12):755-769.
18. FAO. WHO working group report on drafting guidelines for the evaluation of probiotics in food. Food and Agriculture organization. London, Ontario, Canada; c2002. p. 30.
19. Fereidoon S. Beneficial health effects and drawbacks of anti-nutrients and phytochemicals in foods. Applied Microbiology and Biotechnology. 2014;97:45-55.
20. Frutos P, Raso M, Hervás G, Mantecón AR, Pérez V, Giráldez FJ, *et al*. Is there any detrimental effect when a chestnut hydrolyzable tannin extract is included in the diet of finishing lambs? Journal of Applied Animal Research. 2004;53(2):127-136.
21. Gamble GR, Akin DE, Makkar HP, Becker K. Biological degradation of tannins in Sericea lespedeza by the white-rot fungi *Ceriporiopsis subvermispora* and *Cyathus stercoreus* analyzed by solid-state 13C nuclear magnetic resonance spectroscopy. Journal of Applied and Environmental Microbiology. 1996;62(10):3600-3604.
22. Garg SK, Makkar HPS, Nagal KB, Sharma SK, Wadhwa DR, Singh B, *et al*. Toxicological investigations into oak (*Quercus incana*) leaf poisoning in cattle. Journal of Veterinary and Human Toxicology. 1992;34(2):161-164.
23. Getachew G, Makkar HPS, Becker K. Method of polyethylene glycol application to tannin-containing browses to improve microbial fermentation and efficiency of microbial protein synthesis from tannin-containing browses. Journal of Animal Feed Science and Technology. 2001;92(1-2):51-57.
24. Gibson RS, Bailey KB, Gibbs M, Ferguson EL. A review of phytate, iron, zinc, and calcium concentrations in plant-based complementary foods used in low-income countries and implications for bioavailability. Food and Nutrition Bulletin. 2010;31(22):S134-S146.
25. Giri AP, Kachole MS. Amylase inhibitors of pigeon pea (*Cajanus Cajan*) seeds. Phytochemistry. 1998;47(2):197-202.
26. Gladine C, Rock E, Morand C, Bauchart D, Durand D. Bioavailability and anti-oxidant capacity of plant extracts rich in polyphenols, given as a single acute dose, in sheep made highly susceptible to lipoperoxidation. British Journal of Nutrition. 2007;98(4):691-701.
27. Goyal M. Oxalate Accumulation in Fodder Crops and Impact on Grazing Animals-A Review. Forage Research. 2018;44(3):152-158.
28. Habtamu GF, Negussie R. Anti-nutritional factors in plant foods: potential health benefits and adverse effects. Global Advances Research Journal of Food Science and Technology. 2014;3(4):103-117.
29. Hagerman A. Fifty Years of Polyphenol-Protein Complexes. Recent Advances in Polyphenol Research. 2012;3:71-97. 10.1002/9781118299753.ch3.
30. Hagerman AE, Riedl KM, Rice RE. Tannins as biological anti-oxidants. Plant Polyphenols. 1999;2:495-505.
31. Harold. On Food and Cooking: The science and lore of the kitchen. New York: Scribner; c2004, p. 714.
32. Ibrahim EM. Clinical, Haematological and Biochemical Studies on Induced Frothy Bloat in Goats in South Darfur State, Sudan (Doctoral dissertation, Sudan University of Science and Technology); c2019.
33. Jayanegara A, Goel G, Makkar HP, Becker K. Divergence between purified hydrolysable and condensed tannin effects on methane emission, rumen fermentation and microbial population *in vitro*. Animal Feed Science and Technology. 2015;209:60-68.
34. Jenkins KJ, Atwal AS. Effects of dietary saponins on faecal bile acids and neutral sterols and availability of vitamins A and E in the chick. Journal of Nutritional Biochemistry. 1994;5(3):134-137.
35. Jerónimo E, Pinheiro C, Lamy E, Dentinho MT, Sales-Baptista E, Lopes O, *et al*. Tannins in ruminant nutrition: Impact on animal performance and quality of edible products. CA Combs (Ed.), In: Tannins: Biochemistry, food sources and nutritional properties, Nova Science Publishers Inc., New York, USA; c2016. p. 121-168.
36. Jeyakumar E, Lawrence R. Microbial fermentation for reduction of anti-nutritional factors. In Current Developments in Biotechnology and Bioengineering; c2022. p. 239-260. Elsevier
37. Jyoti K, Baunthiyal M, Singh A. Characterization of silver nanoparticles synthesized using *Urtica dioica* Linn. leaves and their synergistic effects with antibiotics. Journal of Radiation Research and Applied Sciences. 2016;9(3):217-227.
38. Kaushik G, Singhal P, Chaturvedi S. Food processing for increasing consumption: The case of legumes. In Food processing for increased quality and consumption. Academic Press; c2018. p. 1-28.
39. Knapp JR, Laur GL, Vadas PA, Weiss WP, Tricarico JM. Invited review: Enteric methane in dairy cattle production: Quantifying the opportunities and impact of reducing emissions. Journal of Dairy Science. 2014;97(6):3231-3261.
40. Kruger J, Oelofse A, Taylor JR. Effects of aqueous soaking on the phytate and mineral contents and phytate: mineral ratios of wholegrain normal sorghum and maize and low phytate sorghum. International Journal of Food Sciences and Nutrition. 2014;65(5):539-546.
41. Kumari S. The effect of soaking almonds and hazelnuts on phytate and mineral concentrations (Doctoral dissertation, University of Otago); c2018.
42. Kumar S, Dutta N. Use of condensed tannin as anthelmintic and organic protectant of proteins for ruminants. Journal of Entomology and Zoology Studies. 2018;6(4):1478-1486.
43. Leathwick JR. New Zealand's potential forest pattern as predicted from current species-environment relationships. New Zealand Journal of Botany. 2001;39(3):447-464.

44. Li YG, Tanner GJ, Larkin PJ. The DMACA-HCI protocol and the threshold proanthocyanidin content for bloat safety in forage legumes. Journal of Science and Food Agriculture. 1996;70(1):89-101.
45. Makkar HPS. Effects and fate of tannins in ruminant animals, adaptation to tannins, and strategies to overcome detrimental effects of feeding tannin-rich feeds. Small Ruminant Research. 2003;49(3):241-256.
46. Makkar HPS, Dawra RK, Singh B. Tannin levels in leaves of some oak species at different stages of maturity. Journal of the Science of Food and Agriculture. 1991;54(4):513-519.
47. Makkar HP, Becker K. Isolation of tannins from leaves of some trees and shrubs and their properties. Journal of Agricultural and Food Chemistry. 1994;42(3):731-734.
48. Makkar HPS, Borowy N, Becker K, Degen R. Some problems in fiber determination in tannin-rich forages. Animal Feed Science Technology. 1995;55(1-2):67-76.
49. Marshall JJ, Lauda CM. Purification and properties of phaseolamin, an inhibitor of alpha-amylase, from the kidney bean, Phaseolus vulgaris. Journal of Biological Chemistry. 2007;250(20):8030-8037.
50. McAllister T, Bae H, Jones G, Cheng K. Microbial attachment and feed digestion in the rumen. Journal of Animal Science. 1994;72(11):3004-3018.
51. McMahon LR, McAllister TA, Berg BP, Majak W, Acharya SN, Popp JD, *et al*. Effects of forage condensed tannins on ruminal fermentation and bloat in grazing cattle. A review. Canadian Journal of Plant Science. 2000;80(3):469-485.
52. Min BR, Barry TN, Attwood GT, McNabb WC. The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: A review. Animal Feed Science and Technology. 2003;106(1-4):3-19.
53. Mohapatra D, Patel AS, Kar A, Deshpande SS, Tripathi MK. Effect of different processing conditions on proximate composition, anti-oxidants, anti-nutrients and amino acid profile of grain sorghum. Food Chemistry. 2019;271:129-135.
54. Molan AL, Warghorn GC, McNabb WC. Effect of condensed tannins on egg hatching and larval development of *Trichostrongylus colubriformis in-vitro*. The Veterinary Record. 2002;150:65-69.
55. Mueller-Harvey I, Bee G, Dohme-Meier F, Hoste H, Karonen M, Kölliker R, *et al*. Benefits of condensed tannins in forage legumes fed to ruminants: Importance of structure, concentration, and diet composition. Crop Science. 2019;59(3):861-885.
56. Nagaraj G. Oil Seeds- Properties, Processing, Products and Procedures. New India Publishing Agency. New Delhi; c2009.p. 601.
57. Niezen JH, Waghorn TS, Charleston WAG, Waghorn GC. Growth and gastrointestinal nematode parasitism in lambs grazing either Lucerne (Medicago sativa) or sulla (*Hedysarum coronarium*) contain condensed tannins. Journal of Agricultural Science. Cambridge. 1995;125(2):281-289.
58. Noonan SC, Savage GP. Oxalic acid and its effects on humans. Asia pacific Journal of Clinical Nutrition. 1999;8(1):64-74.
59. Nyachoti CM, Atkinson JL, Lesson S. Sorghum tannins: A review. World's Poultry Science Journal. 1997;53:5-21.
60. Oatway L, Vasanthan T, Helm JH. Phytic acid. Food Reviews International. 2001;17(4):419-431.
61. Odero-Waitituh JA. Evaluation of inclusion levels of milled mature pods of *Prosopis juliflora* (Mathenge) in broiler finisher diets. (MSc Thesis, Egerton University, Njoro, Kenya); c2015. p. 28-62.
62. Okoye JI, Ene GI. Effects of processing on the nutrient and anti-nutrient contents of tiger nut (*Cyperus esculentus*). Journal of Food Technology and Food Chemistry. 2018;1(1):1-7.
63. Oladele AK, Osundahunsi OF, Adebowale Y. Influence of processing techniques on the nutrients and anti-nutrients contents of tiger nut (*Cyperus esculentus* l.). World Journal of Dairy and Food Science. 2009;4(2):88-93.
64. Oladimeji MO, Akindahunsi AA, Okafor AF. Investigation of the bioavailability of zinc and calcium from some tropical tubers. Nahrung. 2000;44(2):136-137.
65. Oleszek WA. Chromatographic determination of plant saponins. Journal of Chromatography A. 2002;967(1):147-162.
66. Olomu JM. Monogastric animal nutrition: Principles and practice. A Jachem Publication, Benin City, Nigeria; c1995. p. 112-118.
67. Paolini Jr RJ, Miller DD, Comiskey B. U.S. Patent No. 6,866,760. Washington, DC: U.S. Patent and Trademark Office; c2005.
68. Patterson CA, Curran J, Der T. Effect of processing on anti-nutrient compounds in pulses. Cereal Chemistry. 2017;94(1):2-10.
69. Paulová L, Patáková P, Brányik T. Advanced fermentation processes. Engineering Aspects of Food Biotechnology; c2013. p. 89-110.
70. Poungchompu O, Wanapat M, Wachirapakorn C, Wanapat S, Cherdthong A. Manipulation of ruminal fermentation and methane production by dietary saponins and tannins from mangosteen peel and soapberry fruit. Archives of Animal Nutrition. 2009;63(5):389-400.
71. Price KR, Johnson IT, Fenwick GR, Malinow MR. The chemistry and biological significance of saponins in foods and feeding stuffs. Critical Reviews in Food Science and Nutrition. 1987;26(1):127-135.
72. Raes K, Knockaert D, Struijs K, Van Camp J. Role of processing on bio accessibility of minerals: Influence of localization of minerals and anti-nutritional factors in the plant. Trends in Food Science and Technology. 2014;37(1):32-41.
73. Ramteke R, Doneria R, Gendley MK. Anti-nutritional factors in feed and fodder used for livestock and poultry feeding. Acta Scientific Nutritional Health. 2019;3(5):39-48.
74. Rao SBN, Prasad KS, Rajendra D. Recent Advances in amelioration of anti- nutritional factors in livestock feedstuffs. Animal Nutrition and Reproductive Physiology (Recent Concepts); c2013. p. 655-678.
75. Reed JD. Nutritional toxicology of tannins and related polyphenols in forage legumes. Journal of Animal Science. 1995;73(5):1516-1528.
76. Rivera-Méndez C, Plascencia A, Torrentera N, Zinn RA. Effect of level and source of supplemental tannin on growth performance of steers during the late finishing phase. Journal of Applied Animal Research. 2017;45(1):199-203.
77. Robertson HA, Niezen JH, Waghorn GC, Charleston WAG, Jinlong M. The effect of herbages on live weight gain, wool growth, and fecal egg count of parasitized ewe lambs. Proceedings of the New Zealand Society of Animal Production. 1995;55:199-201.

78. Samia M, AbdelRahaman B, Elfadil E. Effect of malt pretreatment followed by fermentation on anti-nutritional factors and HCl extractability of minerals of pearl millet cultivars. Journal of Food Technology. 2005;3(4):529-534.
79. Samtiya M, Aluko RE, Dhewa T. Plant food anti-nutritional factors and their reduction strategies: An overview. Food Production, Processing and Nutrition. 2020;2(1):1-14.
80. Sauvion N, Nardon C, Febvay G, Gatehouse AM, Rahbé Y. Binding of the insecticidal lectin Concanavalin A in pea aphid, *Acyrthosiphon pisum* (Harris) and induced effects on the structure of midgut epithelial cells. Journal of Insect Physiology. 2004;50(12):1137-1150.
81. Savage GP, Vanhanen L, Mason SM, Ross AB. Effect of cooking on the soluble and insoluble oxalate content of some New Zealand foods. Journal of Food Composition and Analysis. 2000;13(3):201-206.
82. Shamsuddin AM. Anti-cancer function of phytic acid. International Journal of Food Science & Technology. 2002;37(7):769-782.
83. Shi J, Arunasalam K, Yeung D, Kakuda Y, Mittal G, Jiang Y, *et al*. Saponins from edible legumes: Chemistry, processing, and health benefits. Journal of Medicinal Food. 2004;7(1):67-78.
84. Silanikove N, Perevolotsky A, Provenza FD. Use of tannin-binding chemicals to assay for tannins and their adverse post-ingestive effects in ruminants. Animal Feed Science and Technology. 2001;91(1-2):69-81.
85. Simee W. Isolation and determination of anti-nutritional compounds from root to shells of peanut (*Arachis hypogaea*). Journal of Dispersion Science and Technology. 2011;28:341-347.
86. Sindhu SC, Khetarpaul N. Probiotic fermentation of indigenous food mixture: effect on anti-nutrients and digestibilities of starch and protein. Journal of Food Composition and Analysis. 2001;14(6):601-609.
87. Singh U. Protein quality of pigeon pea as influenced by seed polyphenols and cooking process. Plant Food and Human Nutrition. 1993;43:171-179.
88. Singh V, Mohanty CS. Effort towards reducing the anti-nutrients in legumes through the application of genomic technologies. Biotechnology. 2014;5:01-23.
89. Smolander A, Kanerva S, Adamczyk B, Kitunen V. Nitrogen transformations in boreal forest soils-does composition of plant secondary compounds give any explanations? Plant and Soil. 2012;350(1):1-26.
90. Sukhanandi S, Bhatt S, Shah A. Effects of fermentation on nutritional quality of *Prosopis juliflora* pods as alternative fish feed. Research Journal of Animal, Veterinary and Fishery Sciences. 2014;2(12):1-7.
91. Tadele Y. Important anti-nutritional substances and inherent toxicants of feeds. Food Science and Quality Management. 2015;36:40-47.
92. Taiz L, Zeiger E. Plant Physiology. 3rd Edition, Sinauer Associates, Inc. Publishers, Sunderland, MA, USA; c2002.
93. Tanner GJ, Moate PJ, Davis LH, Laby RH, Li YG, Larkin PJ, *et al*. Proanthocyanidins (Condensed tannins) destabilize plant protein foams in a dose-dependent manner. Australian Journal of Agricultural Research. 1995;46:1101-1109.
94. Terrill TH, Waghorn GC, Woolley DJ, McNabb WC, Barry TN. Assay and digestion of condensed tannins in the gastrointestinal tract of sheep. British Journal of Nutrition. 1994;72(3):467-477.
95. Thakur A, Sharma V, Thakur A. An overview of anti-nutritional factors in food. International Journal of Chemistry Studies. 2019;7(1):2472-2479.
96. Theodoridou K. The effects of condensed tannins in sainfoin (*Onobrychis viciifolia*) on its digestion and nutritive value (Doctoral dissertation, Université Blaise Pascal-Clermont-Ferrand II); c2010.
97. Thompson D, Geary T. The structure and function of helminth surfaces. In: Biochemistry and Molecular Biology of Parasites, (Marr, J., M. Muller, (Eds.), Academic Press; c1995. p. 203-232.
98. Tian S, Sun Y, Chen Z, Yang Y, Wang Y. Functional properties of polyphenols in grains and effects of physicochemical processing on polyphenols. Journal of Food Quality. 2019;4:144-150.
99. Timotheo CA, Lauer CM. Toxicity of vegetable tannin extract from Acacia mearnsii in Saccharomyces cerevisiae. International Journal of Environmental Science and Technology. 2018;15(3):659-664.
100. Torres J, Rutherfurd SM, Muñoz LS, Peters M, Montoya CA. The impact of heating and soaking on the *in vitro* enzymatic hydrolysis of protein varies in different species of tropical legumes. Food Chemistry. 2016;194:377-382.
101. Van Houtert MFJ, Sykes AR. Implications of nutrition for the ability of ruminants to withstand gastrointestinal nematode infections. International Journal of Parasitology. 1996;26(11):1151-1167.
102. Vasta V, Luciano G. The effects of dietary consumption of secondary plant compounds on small ruminants' products quality. Small Ruminant Research. 2011;101(1-3):150-159.
103. Vasta V, Nudda A, Cannas A, Lanza M, Priolo A. Alternative feed resources and their effects on the quality of meat and milk from small ruminants. Animal Feed Science and Technology. 2008;147(1-3):223-246.
104. Veer SJ, Pawar VS, Kambale RE. Anti-nutritional factors in foods. The Pharma Innovation Journal. 2021;10:01-04.
105. Waghorn G. Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat Production-Progress and challenges. Animal Feed Science and Technology. 2008;147(1-3):116-139.
106. Wang Y, Majak W, Mcallister T. Frothy bloat in ruminants: Cause, occurrence, and mitigation strategies. Animal Feed Science and Technology. 2012;172(1-2):103-114.
107. Wang Y, McAllister TA, Acharya S. Condensed tannins in sainfoin: composition, concentration, and effects on the nutritive and feeding value of sainfoin forage. Crop Science. 2015;55(1):13-22.
108. Yacout MHM. Anti-nutritional factors and its roles in animal nutrition. Journal of Dairy, Veterinary and Animal Research. 2016;4(1):237-239.
109. Yisehak K, Janssens GP. Evaluation of nutritive value of leaves of tropical tanniferous trees and shrubs. Livestock Res Rural Develop. 2013, 25(2). Article #28. Retrieved September 13, 2014, from http://www.lrrd.org/lrrd25/2/yise25028.htm.
110. Zagrobelny M, Bak S, Rasmussen AV, Jørgensen B, Naumann CM, Møller BL, *et al*. Cyanogenic glucosides and plant– insect interactions. Phytochemistry. 2004;65(3):293-306.
111. Chung K, Wallace J, Kim SY, Kalyanasundaram S, Andalman AS, Davidson TJ, *et al*. Structural and molecular interrogation of intact biological systems. Nature. 2013;497(7449):332-7.