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Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

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Major Antinutrients Found in Plant Protein Sources: Their Effect on Nutrition

K.E. Akande[1], U.D. Doma[1], H.O. Agu[2] and H.M. Adamu[3]
[1]Animal Production Programme, School of Agriculture,
Abubakar Tafawa Balewa University, Bauchi, Bauchi State, Nigeria
[2]Department of Food Science and Technology, Federal Polytechnic, Bauchi, Bauchi State, Nigeria
[3]Chemistry Programme, School of Science, Abubakar Tafawa Balewa University,
Bauchi, Bauchi State, Nigeria

Abstract: Compounds or substances which act to reduce nutrient intake, digestion, absorption and utilization and may produce other adverse effects are referred to as antinutrients or antinutritional factors. Seeds of legumes and other plant sources contain in their raw state wide varieties of antinutrients which are potentially toxic. The major antinutrients includes: toxic amino acids, saponins, cyanogenic glycosides, tannins, phytic acid, gossypol, oxalates, goitrogens, lectins (phytohaemagglutinins), protease inhibitors, chlorogenic acid and amylase inhibitors. These antinutrients pose a major constraint in the use of plant protein sources in livestock feeds without adequate and effective processing. The level or concentration of these anitnutrients in plant protein sources vary with the species of plant, cultivar and post-harvest treatments (processing methods). This paper reviews the nutritional effect of major antinutrients present in plant protein sources.

Key words: Antinutrients, plant protein, legumes

INTRODUCTION

Antinutrients or antinutritional factors may be defined as those substances generated in natural feedstuffs by the normal metabolism of species and by different mechanisms (for example inactivation of some nutrients, diminution of the digestive process or metabolic utilization of feed) which exerts effect contrary to optimum nutrition. Being an antinutritional factor is not an intrinsic characteristic of a compound but depends upon the digestive process of the ingesting animal. Trypsin inhibitors, which are antinutritional factors for monogastric animals, do not exert adverse effects in ruminants because they are degraded in the rumen (Cheeke and Shull, 1985). Many plant components have potential to precipitate adverse effects on the productivity of farm livestock. These compounds are present in the foliage and seeds of virtually every plant that is used in practical feeding (D'Mello, 2000).

Nutritional effect of major antinutrients in plant protein sources: The major antinutrients mostly found in plant protein sources are toxic amino acids, saponins, cyanogenic glycosides, tannins, phytic acid, gossypol, oxalates, goitrogens, lectins (phytohaemagglutinins), protease inhibitors, chlorogenic acid and amylase inhibitors.

Toxic amino acids: A wide range of toxic non-protein amino acids occur in the foliage and seeds of plants. These toxic non-protein amino acids appear to play a major role in determining the nutritional value of a number of tropical legumes (D'Mello, 1982). It has been proposed that these amino acids act antagonistically towards certain nutritionally important amino acids (Liener, 1980). Fowden (1971) suggested that the metabolic pathways culminating in the synthesis of certain non-protein amino acids might reflect subtle alteration in the genome responsible for directing the formation of crucial amino acids. Bell (1971) reported that while non-protein amino acids function primarily as storage metabolites, they may also provide an adaptive advantage to the plants, for example to render the plant less susceptible to attack by various animals and lower plants. Some of these toxic amino acids includes; djenkolic acids, mimosine and canavanine.

Mimosine, a toxic non-protein amino acid structurally similar to tyrosine, is contained in the legume *Leucaena leucocephala* (D'Mello and Acamovic 1989; D'Mello, 2000). Mimosine has been proven effective in defleecing sheep and goats (Jacquemet *et al.*,1990; Luo *et al.*, 2000). Mimosine a pyridoxal antagonist, which inhibits DNA replication and protein synthesis; thus, it may elicit defleecing by arresting cell division in the follicle bulb (Reis, 1979). In monogastric animals, mimosine causes poor growth, alopecia and reproductive problems. Levels of *Leucaena* meal above 5-10% of the diet for swine, poultry and rabbits generally result in poor animal performance.

The major symptoms of toxicity in ruminants are poor growth, loss of hair and wool, lameness, mouth and

Corresponding Author: K.E. Akande, Animal Production Programme, School of Agriculture, Abubakar Tafawa Balewa University, Bauchi, Bauchi State, Nigeria

oesophageal lesions, depressed serum thyroxine level and goitre. Some of these symptoms may be due to mimosine and others to 3, 4-dihydroxypyridine, a metabolite of mimosine in the rumen (Jones and Hegarty, 1984).

Djenkol beans (*Pithecolobium lubatum*) when ingested sometimes lead to kidney failure which is accompanied by the appearance of blood and white needle-like clusters in the urine. The clusters are sulphur-containing amino acids known as djenkolic acids which are present in the bean in the free state, to the extent of 1-4%. This toxic amino acid is structurally similar to cystine, but it is not degraded in the animal body. Due to its insolubility it crystallizes out in the kidney tubules and escapes through urine (Enwere, 1998).

The toxic, non-protein amino acid, canavanine, occurs widely in unbound form in various legume plants of the sub-family *Papillonoideae* (Bell *et al*., 1978) and abundantly in jack bean (*Canavalia ensiformis* (L). DC), constituting up to 63 g/kg dry weight of the seed (Ho and Shen, 1966). Canavanine, a structural analogue of arginine, was first isolated from jackbean by Kitagawa and Tomiyama (1929).

Canavanine is believed to exert its toxic influence by virtue of its structural similarity with the nutritionally indispensable amino acid, arginine. Canavanine may antagonize arginine and interfere with Ribonucleic Acid (RNA) metabolism (Rosenthal, 1982). Canavanine has been demonstrated to reduce feed intake of non-ruminants but this was observed only at the equivalent of about 300 g/kg dietary level of raw jackbean (Tschiersch, 1962).

Saponins: Saponins are a heterogeneous group of naturally occurring foam-producing triterpene or steroidal glycosides that occur in a wide range of plants, including pulses and oil seeds such as kidney bean, chickpea, soybean, groundnut, lupin and sunflower (Liener, 1980; Price *et al*., 1987; Jenkins and Atwal, 1994). It has been reported that saponins can affect animal performance and metabolism in a number of ways as follows: erythrocyte haemolysis, reduction of blood and liver cholesterol, depression of growth rate, bloat (ruminants), inhibition of smooth muscle activity, enzyme inhibition and reduction in nutrient absorption (Cheeke, 1971). Saponins have also been reported to alter cell wall permeability and therefore produce some toxic effects when ingested (Belmar *et al*., 1999). Saponins have been shown to bind to the cells of the small intestine thereby affecting the absorption of nutrients across the intestinal wall (Johnson *et al*., 1986).

The effect of saponins on chicks have been reported to reduce growth, feed efficiency and interfere with the absorption of dietary lipids, cholesterol, bile acids and vitamins A and E (Jenkins and Atwal, 1994).

Cyanogenic glycosides: Some legumes like linseed, lima bean, kidney bean and the red gram contain cyanogenic glycosides from which Hydrogen Cyanide (HCN) may be released by hydrolysis. Some cultivars of *Phaseolus lunatus* (lima bean) contain a cyanogenic glycoside called phaseolutanin from which HCN is liberated due to enzyme action, especially when tissues are broken down by grinding or chewing or under damp conditions (Purseglove, 1991). Hydrolysis occurs rapidly when the ground meal is cooked in water and most of the liberated HCN is lost by volatilization. HCN is very toxic at low concentration to animals. HCN can cause dysfunction of the central nervous system, respiratory failure and cardiac arrest (D'Mello, 2000).

Tannins: Tannins are water soluble phenolic compounds with a molecular weight greater than 500 daltons. They have the ability to precipitate proteins from aqueous solution. There are two different groups of tannins:- hydrolyzable tannins and condensed tannins. Condensed tannins are widely distributed in leguminous forages and seeds. Cattle and sheep are sensitive to condensed tannins, while goats are more resistant (Kumar, 1983; Kumar and Horigome, 1986; Kumar and Vaithiyanathan, 1990; D'Mello, 2000).

Tannins may form a less digestive complex with dietary proteins and may bind and inhibit the endogenous protein, such as digestive enzymes (Kumar and Singh, 1984). Tannin-protein complexes involve both hydrogen-bonding and hydrophobic interactions. The precipitation of the protein-tannin complex depends upon pH, ionic strength and molecular size of tannins. Both the protein precipitation and incorporation of tannin phenolics into the precipitate increase with increase in molecular size of tannins (Kumar and Horigome, 1986). However, when the molecular weight exceeds 5,000 daltons, the tannins become insoluble and lose their protein precipitating capacity and degree of polymerization becomes imperative to assess the role of tannins in ruminant nutrition (Kumar, 1983; Lowry, 1990). Tannins have been found to interfere with digestion by displaying anti-trypsin and anti-amylase activity. Helsper *et al*. (1993) reported that condensed tannins were responsible for the testa-bound trypsin inhibitor activity of faba beans. Tannins also have the ability to complex with vitamin B_{12} (Liener, 1980). Other adverse nutritional effects of tannins have been reported to include intestinal damage, interference with iron absorption and the possibility of tannins producing a carcinogenic effect (Butler, 1989).

Phytic acid: Phytic acid occurs naturally throughout the plant kingdom and is present in considerable quantities within many of the major legumes and oilseeds. This includes soybean, rapeseed and cotton seed. Matyka *et al*. (1993) reported that about 62-73% and 46-73% of the total phosphorus within cereal grains and legume seeds being in form of organically bound phytin phosphorus, respectively. As phytic acid accumulates in storage sites in seeds, other minerals apparently chelates to it forming the complex salt phytate (Erdman, 1979). Studies by Martinez (1977) revealed that in oilseeds, which contain little or no endosperm, the phytates are distributed throughout the kernel found within subcellular inclusions called aleurone grains or protein bodies.

Whole soybeans have been reported to contain 1-2% phytic acids (Weingartner, 1987; Osho, 1993). The major part of the phosphorus contained within phytic acid are largely unavailable to animals due to the absence of the enzyme phytase within the digestive tract of monogastric animals. Nwokolo and Bragg (1977) reported that in the chicken there is a significant inverse relationship between phytic acid and the availability of calcium, magnesium, phosphorus and zinc in feedstuffs such as rapeseed, palm kernel seed, cotton seed and soybean meals. Phytic acid acts as a strong chelator, forming protein and mineral-phytic acid complexes; the net result being reduced protein and mineral bioavailability (Erdman, 1979; Spinelli *et al.*, 1983; Khare, 2000). Phytic acid is reported to chelate metal ions such as calcium, magnesium, zinc, copper, iron and molybdenum to form insoluble complexes that are not readily absorbed from gastrointestinal tract. Phytic acid also inhibits the action of gastrointestinal tyrosinase, trypsin, pepsin, lipase and α-amylase (Liener, 1980; Hendricks and Bailey, 1989; Khare, 2000). Erdman (1979) stated that the greatest effect of phytic acid on human nutrition is its reduction of zinc bioavailability.

Gossypol: Gossypol is a naturally occurring polyphenolic compound present in the pigment glands of cotton seed (*Gossypium spp*). The average gossypol content varying from 0.4-2.4% within glanded cotton seeds to less than 0.01% free gossypol within some low-gossypol cotton seed meals (Liener, 1980; Robinson and Brent, 1989; Castaldo, 1995). Reduced lysine availability has been reported with cotton seed protein due to the ability of gossypol to bind with the reactive epsilon amino group of lysine during heat processing (Wilson *et al.*, 1981; Robinson, 1991; Church, 1991). The general symptoms of gossypol toxicity are depressed appetite, loss of weight, laboured breathing and cardiac irregularity. Death is usually associated with reduced oxygen-carrying capacity of the blood, haemolytic effects on erythrocytes and circulatory failure. Dietary gossypol also causes olive-green discolouration of yolks in eggs (Church, 1991; Olomu, 1995; McDonald *et al.*, 1995).

Oxalates: Oxalates affects calcium and magnesium metabolism and react with proteins to form complexes which have an inhibitory effect in peptic digestion. Ruminants, however unlike monogastric animals can ingest considerable amounts of high-oxalate plants without adverse effects, due principally to microbial decomposition in the rumen (Oke, 1969).

The hulls of sesame seeds contain oxalates and it is essential that meals should be completely decorticated in order to avoid toxicities (McDonald *et al.*, 1995). Chemical analysis carried by Alabi *et al.* (2005) on locust bean seeds revealed that the testa of locust bean seeds had the highest concentration of oxalate (4.96 mg/100 g) followed by the pulp (3.40 mg/100 g) and the cotyledon (1.15 mg/100 g). Olomu (1995) reported that pigeon pea contains about 0.38% oxalic acid. Oxalic acid binds calcium and forms calcium oxalate which is insoluble. Calcium oxalate adversely affects the absorption and utilization of calcium in the animal body (Olomu, 1995).

Goitrogens: Goitrogenic substances, which cause enlargement of the thyroid gland, have been found in legumes such as soybean and groundnut. They have been reported to inhibit the synthesis and secretion of the thyroid hormones. Since thyroid hormones play an important part in the control of body metabolism their deficiency results in reduced growth and reproductive performance (Olomu, 1995). Goitrogenic effect have been effectively counteracted by iodine supplementation rather heat treatment (Liener, 1975).

Lectins (phytohaemagglutinins): Phytohaemagglutinins or lectins are glycoproteins widely distributed in legumes and some certain oil seeds (including soybean) which posses an affinity for specific sugar molecules and are characterized by their ability to combine with carbohydrate membrane receptors (Pusztai, 1989). Lectins have the capability to directly bind to the intestinal muscosa (Almeida *et al.*, 1991; Santiago *et al.*, 1993), interacting with the enterocytes and interfering with the absorption and transportation of nutrients (particularly carbohydrates) during digestion (Santiago *et al.*, 1993) and causing epithelial lesions within the intestine (Oliveira *et al.*, 1989).

Although lectins are usually reported as being heat-labile, their stability varies between plant species, many lectins being resistant to inactivation by dry heat and requiring the presence of moisture for more complete destruction (Ayyagari *et al.*, 1989; Poel *et al.*, 1990; Almeida *et al.*, 1991).

Protease inhibitors: Protease inhibitors are widely distributed within the plant kingdom, including the seeds of most cultivated legumes. Protease inhibitors have the ability to inhibit the activity of proteolytic enzymes within the gastrointestinal tract of animals (Liener and Kakade, 1980).

Trypsin inhibitor and chymotrypsin inhibitor are protease inhibitors occurring in raw legume seeds. Protease inhibitors are the most commonly encountered class of

antinutritional factors of plant origin. These inhibitors have been reported to be partly responsible for the growth-retarding property of raw legumes. The retardation has been attributed to inhibition of protein digestion but there is evidence that pancreatic hyper-activity, resulting in increased production of trypsin and chymotrypsin with consequent loss of cystine and methionine is also involved (McDonald *et al.*, 1995).
Trypsin inhibitors have been implicated in reducing protein digestibility and in pancreatic hypertrophy (Liener, 1976). Trypsin inhibitors are polypeptides that form well characterized stable complexes with trypsin on a one-to-one molar ratio, obstructing the enzymatic action (Carlini and Udedibie, 1997). Protease inhibitors are inactivated by heat especially moist heat, because of even distribution of heat (Bressani and Sosa, 1990; Liener, 1995).

Chlorogenic acid: Sunflower meal contains high levels of chlorogenic acid, a tannin like compound that inhibits activity of digestive enzymes including trypsin, chymotrypsin, amylase and lipase (Cheeke and Shull, 1985). Because chlorogenic acid is uncondensed and non-hydrolyzable, its content of 1% or more of a total of 3-3.5% phenolic compounds in sunflower meal is not reported in tannin assays. Chlorogenic acid is also a precursor of ortho-quinones that occur through the action of the plant enzyme polyphenol oxidase. These compounds then react with the polymerize lysine during processing or in the gut. Although the toxic effects of chlorogenic acid can be counteracted by dietary supplementation with methyl donors such as choline and methionine. Chlorogenic acid is reported to be readily removed from sunflower seeds using aqueous extraction methods (Dominguez *et al.*, 1993).

Amylase inhibitors: Amylase inhibitors are also known as starch blockers because they contain substances that prevent dietary starches from being absorbed by the body. Starches are complex carbohydrates that cannot be absorbed unless they are first broken down by the digestive enzyme amylase and other secondary enzymes (Marshall and Lauda, 1975; Choudhury *et al.*, 1996). Pigeon pea have been reported to contain amylase inhibitors. These inhibitors have been found to be active over a pH range of 4.5-9.5 and are heat labile. Amylase inhibitors inhibit bovine pancreatic amylase but fail to inhibit bacterial, fungal and endogenous amylase. Pigeon pea amylase inhibitors are synthesized during late seed development and also degraded during late germination (Giri and Kachole, 1998).

Conclusion: The presence of antinutrients in plant protein sources for livestock feeding is a major constraint that reduces their full utilization. To be able to justify the overall nutritional potential or value of any plant protein source, proper assessment of the type, nature and concentration of the antinutrients present in the protein source and also the bioavailability of nutrients to the ingesting animal is necessary. Employing appropriate and effective processing techniques or combination of techniques could help reduce or eliminate the adverse effects of these antinutritive constituents in plant protein sources and thereby improve their nutritive value. Supplementation of some minerals, animo acids and vitamins could help reduce or neutralize the negative effect of antinutritional factors in plant protein sources for livestock nutrition. The concentration or level of the antinutritive constituents in these protein sources vary with the species of plant, cultivar and post-harvest treatments (processing methods). Since antinutrients vary among plant cultivars, therefore the use of genetically improved low-antinutritive cultivars or varieties could be a possible option for livestock feeding.

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