Credit seminar on **ANTI-NUTRITIONAL FACTORS IN FISH FEED INGREDIENTS**

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Introduction

The use of plant-derived materials such as legume seeds, different types of oilseed cake, leaf meals, leaf protein concentrates, and root tuber meals as fish feed ingredients is limited by the presence of a wide variety of anti-nutritional substances. Most of the potential, alternative, plant-derived nutrient sources are known to contain a wide variety of antinutritional substances (Francis et al., 2001).

Of the total global production of fishmeal, 2 Mtn was used in fish farming, with farming of shrimp accounting for 20.3%, of salmon 18.8%, carp 18.3%, marine fish 13.9%, trout 10.9%, eel 10.7%, milkfish 1.6% and catfish farming 1.3% of that tonnage (Sargent and Tacon, 1999). Antinutrients are chemicals which have been evolved by plants for their own defense, among other biological functions and reduce the maximum utilization of nutrients especially proteins, vitamins, and minerals, thus preventing optimal exploitation of the nutrients present in a food and decreasing the nutritive value. Some of these plant chemicals have been shown to be deleterious to health or evidently advantageous to human and animal health if consumed at appropriate amounts.

Antinutrients in plant foods are responsible for deleterious effects related to the absorption of nutrients and micronutrients. However, some antinutrients may exert beneficial health effects at low concentrations. For example, phytic acid, lectins, tannins, saponins, amylase inhibitors and protease inhibitors have been shown to reduce the availability of nutrients and cause growth inhibition. However, when used at low levels, phytate, lectins, tannins, amylase inhibitors and saponins have also been shown to reduce the blood glucose and insulin responses to starchy foods and/or the plasma cholesterol and triglycerides.
Antinutrients have been defined as substances which by themselves, or through their metabolic products arising in living systems, interfere with food utilisation and affect the health and production of animals. They could be broadly divided into four groups: 1. factors affecting protein utilisation and digestion, such as protease inhibitors, tannins, lectins; 2. factors affecting mineral utilisation, which include phytates, gossypol pigments, oxalates, glucosinolates; 3. antivitamins; 4. Miscellaneous substances such as mycotoxins, mimosine, cyanogens, nitrate, alkaloids, photosensitizing agents, phyto oestrogens and saponins (Francis et al., 2001).

**Major Antinutritional Factors in Fish Feed Ingredients**

1) Protease/trypsin inhibitors
2) Haemagglutinins (lectins)
3) Glucosinolates
4) Cyanogens
5) Saponins
6) Tannins
7) Estrogenic factors
8) Lathyrogens
9) Gossypol
10) Toxic amino acids
11) Phytates
12) Phytoestrogens
13) Alkaloids
14) Oligosaccharides and non-starch polysaccharides
15) Cyclopropenoic fatty acids
16) Antigenic compounds
17) Erucic acid
18) Antivitamin factors
19) Antienzymes
20) Mycotoxins

Anti-nutritional factors can be classified regarding their chemical description, their biological effects, or their ability to withstand heat treatments:

1. Chemical description (Tacon, 1985)
   a. Proteins
      ✓ Protease inhibitors
      ✓ Haemagglutinins (lectins)
   b. Glycosides
      ✓ Glucosinolates
      ✓ Cyanogens
      ✓ Saponins
      ✓ Estrogenic factors
   c. Phenol
      ✓ Gossypol
      ✓ Tannins
   d. Miscellaneous
      ✓ Anti-minerals
      ✓ Anti- vitamins
      ✓ Anti-enzymes
      ✓ Toxic amino acids
      ✓ Mycotoxins

2. Biological effects (Francis et al., 2001)
e. Factors affecting protein utilisation and digestion

- Protease inhibitors
- Tannins
- Haemagglutinins (lectins)

f. Factors affecting mineral utilisation

- Phytic acid
- Gossypol
- Glucosinolates

g. Antivitamins

h. Miscellaneous

- Mimosine
- Cyanogens
- Estrogenic factors

3. Heat resistance (Francis et al., 2001)

i. Heat labile factors

- Protease inhibitors,
- Phytic acid,
- Haemagglutinins,
- Glucosinolates,
- Anti-vitamins.

j. Heat stable factors

- Saponins,
- Non-starch polysaccharides,
- Anti-genic proteins,
✓ Estrogens,
✓ Phenolic compounds.

1) Protease/trypsin inhibitors

Protease inhibitors are widespread antinutrient substances in many plant-derived nutritional ingredients that could be used in fish feed, particularly the legumes. Their potency depends on their origin and the target enzyme. Protease inhibitors are protein-based molecules (Tacon, 1997). In soybean, there are two main groups of trypsin inhibitors: the heat-labile Kunitz inhibitors blocking mainly trypsin, and the Bowman-Birk inhibitors inhibiting either trypsin or chymotrypsin (Tacon, 1997; Francis et al., 2001). The protein digestibility is directly reduced.

Commercial soybean products mostly show trypsin inhibitors (TI) in the range of 2–6 mg/g, averaging 4 mg/g (Synder and Kwon, 1987). TI have a wide distribution in the plant kingdom and is present in most legume seeds and cereals. The common culture fish species differ in their ability to tolerate dietary TI.

TI levels of 1.6 mg/g or higher in the diet retarded Nile tilapia growth, but the fish tolerated and grew well at dietary levels of 0.6 mg TI/g diet (Wee and Shu, 1989). Makkar and Becker (1999) found that carp fed diets containing meal of J. curcas seeds of non-toxic provenance, with 24.8 mg TI/g and heat-treated meal with 1.3 to 8.3 mg TI/g, showed no differences in growth performance implying that the fish were able to tolerate the high levels of TI.

After a certain period a compensation process stimulates trypsin secretion and it seems that with trypsin inhibitors levels below 5 mg/g, most cultured fish are able to compensate (Francis et al., 2001). However, in many fish species, it leads to pancreas hypertrophy (Guillaume et al., 1999).
**Occurrences**

Protease inhibitors are widespread within the plant kingdom (Tacon, 1997). While their concentration is negligible in cereals, they can be highly concentrated in some legumes such as soybean (Guillaume et al., 1999) with trypsin inhibitors ranging between 2 to 6 mg/g (Francis et al., 2001).

**Treatment**

Most protease inhibitors are readily destroyed by heat. Moist heat treatment (autoclaving at 121° C for 15-30 min) or aqueous heat treatment (100° C for 10 min) is recommended (Norton, 1991; Armour et al., 1999).

2) **Glucosinolates**

Glucosinolates are thioglucosides commonly found in plants belonging to the family Cruciferae. They are always accompanied by thioglucosidase enzymes in plants but the two are kept separated in different cell compartments. When the contents of these two components come together by cellular damage, breakdown products like isothiocyanates and nitriles, capable of causing potentially harmful effects to animals, are released (Duncan, 1991).

They are not directly toxic but when hydrolysed by the enzyme myrosinase, they release more or less potent antithyroid agents (Tacon, 1997). Goitrin is the main antithyroid inhibiting the thyroid gland to bind with iodine. It cannot be compensated with supplementary iodine (Guillaume et al., 1999) and the increased thyroid activity is characterized by the presence of hyperplasia and follicular hypertrophy (goitre) (Francis et al., 2001).

**Occurrences**

Glucosinolates are commonly found in plants belonging to the family Cruciferae such as rapeseed and mustard oil seed (Francis et al., 2001).

**Treatment**
The enzyme myrosinase is readily destroyed by heat but the glucosinolates are more heat resistant. Microwave irradiation (2450 MHz for 2.5 min of pre-conditioned meal [moisture 13 g/kg, 24 h at 4°C], micronization (90 s at 195°C), dry extrusion, wet extrusion (150°C with 2% ammonia), soaking in copper sulfate solution (1 kg meal in 2 l water with 6.25 g CuSO4.5H2O, and drying at 60°C), soaking in water (6 h-12 h and drying at 60°C) and fermentation (60-96 h at 30°C under aerobic condition) have been reported to reduce the glucosinolates content of rapeseed meal by 7.0-25.4%, 37%, 19.0-42.8%, 67%, 90%, 36-90%, and 100%, respectively (Tripathi & Mishra, 2007). 95.6% of the glucosinolates has been removed from mustard seed by solvent extraction with hexane (10% anhydrous ammonia in methanol at 95%) (Shahidi & Gabon, 1990).

Heat treatment is effective in reducing the glucosinolate content of feed materials (in rapeseed meal from 40 to 26 μmol/g after wet pressure-cooking; Burel et al., 2000). Extracting with water was found to be a cost-effective method of removing glucosinolates from full-fat and fat-free Moringa oleifera kernels (Makkar and Becker, 1997). Besides, plant geneticists have selected rapeseed varieties with very low glucosinolate contents (Guillaume et al., 1999).

3) **Haemagglutinins (lectins)**

Plant lectins or phytohaemagglutinins are found in many legume seeds and are able to bind reversibly to carbohydrate moieties of complex glyco-conjugates present on membranes. Even though they are proteins, they are at least partially resistant to proteolytic degradation in the intestine. Their common biological effects include disruption of the small intestinal metabolism and morphological damage to the villi (Grant, 1991).

Phytohaemagglutinins or lectins are glycoproteins that can combine with carbohydrate membrane receptors (Tacon, 1997). They are partially resistant to proteolytic degradation in the intestine (Francis et al., 2001). They interact with the enterocytes disrupting the absorption
of nutrients and cause epithelial lesions within the intestine (Tacon, 1997). The consequent hypertrophic mucus production in the intestine also alters the nutrient absorption and the digestibility is further impaired (Francis et al., 2001). Irritation caused by lectins to the intestinal membrane resulting in over secretion of mucus may impair the enzymatic and absorptive capacity of the intestinal wall. Their deleterious effect may be more potent when present along with other antinutrients.

**Occurrences**

Lectins are widely distributed in legumes and some oilseeds (Tacon, 1997).

**Treatment**

Lectins can be removed by aqueous heat treatment (100 °C for 10-20 min). Autoclaving during 15 minutes is less efficient (Grant, 1991).

Aregheore et al. (1998) reduced the lectin content in *Jatropha* seed meal from 102 to 1.17 haemagglutination units by moist heating at 100°C for 10 min.

5) **Cyanogens**

Cyanogens or cyanogenetic J-glycosides (Tacon, 1997) are not directly toxic, but when hydrolysed with an associated extracellular enzyme, they liberate hydrogen cyanide (HCN) and probably other carbonyl compounds that suppress natural respiration (Francis et al., 2001). Furthermore, thiocyanate, a detoxication product of cyanide, acts as an antithyroid agent (Francis et al., 2001).

**Occurrences**

Cyanogens are found in many cereals, root tubers, legumes and oilseeds.

**Treatment**

Cyanogens are generally heat-stable and sparingly soluble in water, but the associated enzymes are readily destroyed by heat (Tacon, 1997). Drying of cassava at 60° C has been reported to remove up to 90% of the HCN (Charavanapavan, 1944). Soaking three days at 30°
C and sun drying for another two days (Ng & Wee, 1989), or incubating at 30 °C for 18 hours followed by steam heat to evaporate HCN (Yamashita et al., 2007) are efficient treatments. HCN content in linseed meal has been reduced by 34.4-53.1% with aqueous treatment (soaked in water at 25° C for 18h) (Hossain & Jauncey, 1990).

6) Cyclopropenoic fatty acids

Cyclopropenoic fatty acids (CFA) are toxic fatty acids and include sterculic and malvalic acid. They can cause abnormalities in the reproductive processes, disrupt the lipid metabolism, and impair the growth. Moreover, when associated with aflatoxins, CFA are suspected to be carcinogenic to fish (Francis et al., 2001).

Occurrences

CFA are present in cottonseed oil and meal.

Treatment

CFA are usually removed from the oilseeds during oil extraction but residual levels may persist (Tacon, 1997).

7) Erucic acid

Erucic acid (cis-13-docosenoic acid) is a 22-carbon monounsaturated fatty acid. Contradictory results have been published on its toxicity to fish. The reported signs are reduced growth, increased mortality, and histopathology of skin, gills, kidney and heart (Tacon, 1997).

Occurrences

Erucic acid is found in rapeseed and mustard oils and is therefore removed from the seed by oil extraction.

8) Estrogenic factors
These non-steroidal estrogenic substances are mostly isoflavones that occur in the form of glycosides (Francis et al., 2001). In soybean, for example, genistein is the most prominent isoflavone (Tacon, 1997). They can bind to oestrogen receptors or get converted into compounds that have estrogenic effects (Francis et al., 2001). Studies with Siberian sturgeon have shown that phytoestrogens could induce vitellogenesis (Francis et al., 2001).

**Occurences**

Phytoestrogens are present in many cereals, legumes and oilseeds such as barley, rice, wheat, corn, chick pea, lucerne, groundnut, soybean, cottonseed and linseed.

**Treatment**

Genistein has been reported to be heat-stable, but phytoestrogens are not a serious threat to fish (Tacon, 1997). They should however be taken into account while formulating the feed (Francis et al., 2001).

9) **Gossypol**

Gossypols are polyphenols, contained in the pigment glands of plants of the genus *Gossypium* and in certain other members of the order Malvales. Feeding diets containing gossypols cause negative effects such as growth depression and intestinal and other internal organ abnormalities (Berardi and Goldblatt, 1980). The formation of indigestible gossypol–protein complexes may produce deficiencies of some amino acids, such as methionine, which are essential for the normal fat metabolism (Herman, 1970).

The growth rate of fingerling carp was depressed to half of that of the control on a 1% dietary inclusion of gossypol–acetate (Roehm et al., 1967). A 2% gossypol level resulted in feed rejection in the same study. Significant amounts of gossypol became bound to liver, kidney and spleen tissue. This bound gossypol remained in the liver (which is the main organ responsible for metabolising these compounds) even after fish were fed a gossypol-free control diet for 10 weeks.
It causes problems to the reproductive system by affecting the reproductive tissues or pituitary and gonadal hormone secretion (Francis et al., 2001). Gossypol can also bind with the reactive amino group of lysine during heat processing (Tacon, 1997). Impaired protein digestibility, reduced growth, and toxicity signs in organs (kidney, liver, and spleen) have been reported in fish (Guillaume et al., 1999). Gossypol is also a strong carcinogen when fed to fish in combination with aflatoxin. It is recommended to keep levels of free-gossypol below 0.01% within the diet (Tacon, 1997).

**Occurrences**

Gossypol is present in the pigment glands of plants of the genus *Gossypium* (e.g., cottonseed) (Francis et al., 2001).

**Treatment**

Free gossypol pigments are bound to proteins (with the reactive epsilon amino group of lysine) when processing the cottonseed with heat. The level of free-gossypol can thus be reduced by 50-99% although the final protein bioavailability is consequently reduced (Tacon, 1997). Recently, cottonseed varieties free of pigment glands have been selected (Guillaume et al., 1999).

**10) Phytic acid**

Phytic acid is one of the most powerful anti-nutritional factors in plant feedstuffs. The antinutritional activity of phytic acid can be eliminated by the addition of enzymes such as phytase. Phytic acid or phytate in cereals, legume grains and oil seeds is bound with phosphorus, calcium and magnesium, trace elements such as iron and zinc, and protein and amino acids. Most fishes do not have endogenous enzymes to break down phytate and release nutrients; therefore, they pass through the gut undigested. This is why greater proportions of valuable nutrients from plant sources are not utilized by aquatic animals and are wasted as excreta.
Phytase enzyme releases phosphorus and bound minerals and amino acids from phytate, paving the way for maximum utilization of nutrients.

The advantages of phytase are:

✓ Phosphorus bound in phytate becomes more available as a nutrient. Therefore, inclusion levels of phosphorus can be reduced.

✓ Performance of aquaculture operations is under scrutiny because nutrients are discharged into surrounding ecosystems. Addition of phytase reduces the release of nutrients into the environment by making more bound phosphorus available to fish for growth.

✓ Phytase added to diets improves protein and amino acid digestibility in fishes.

✓ Phytase can improve the metabolic energy of feeds by breaking down the phytate-lipid complex.

✓ Plant protein sources can be substituted for animal protein sources (e.g., fishmeal), reducing feed costs.

21) Lathyrogens

Lathyrogens include J-amino propionitrile and the neurotoxic amino acid J-N-oxalyl-L-I,Jdiaminopropionic acid (Tacon, 1997). They are responsible of the disease called lathyrism (skeletal lesions, retarded sexual development and paralysis) (Tacon, 1997).

Occurrences

They are found in certain plants of the genus *Lathyrus* such as the grass pea.

Treatment

Lathyrogens are readily removed by cooking in water (100° C) and draining off the excess water (Tacon, 1997). Soaking dehusked seeds overnight followed by steaming for 30 minutes, or roasting of the seed at 140° C for 15-20 minutes have also been reported (Gupta, 1987).
22) Mycotoxins

Mycotoxins are secondary metabolites of fungi. The most important mycotoxicosis in fish is caused by aflatoxins, which is produced by *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxins appear fluorescent under U.V. light and are named aflatoxins B or aflatoxins G when the colour is blue or green, respectively. Aflatoxin B1 is the most potent one (Guillaume *et al.*, 1999). Their chemical characteristics and biological activities are very wide and able to cause different pathology and pathohistological changes in fish. Aflatoxins are also very potent carcinogenic (Guillaume *et al.*, 1999).

Aflatoxicosis signs include pale gills, impaired blood clotting, anaemia, poor growth rates or lack of weight gain, liver tumours, and increased mortality. Moreover, aflatoxins can depress the immune system by destroying some essential nutrients in the diet (e.g., vitamins A & C, and thiamine) (Royes & Yanong, 2002).

**Occurrences**

Moulds are common contaminants of oilseeds and associated by-products (particularly groundnut), but they can also be found in cereals and complete feeds. Ingredients become contaminated with fungi in the field, during processing, storage, transport and usage. Moisture levels in the feed (above 14%), temperature (above 27°C), humidity (above 62%), aeration and presence of other microorganisms are important factors influencing the presence of mycotoxins (Royes & Yanong, 2002; Jakic-Dimic *et al.*, 2005).

**Treatment**

Prevention is the recommended measure to avoid mycotoxins. Feeds should be stored in a cool and dry area. Regular testing is possible by inspecting visually the feed (with or without black light), or by the mean of commercial detection kits. When moulds are detected in small quantities, a commercial inactivator can be purchased. However, heavily contaminated feeds and ingredients should be discarded (Royes & Yanong, 2002).
23) Saponins

Saponins are naturally occurring foam-producing triterpene or steroidal glycosides (Tacon, 1997). Acting as a detergent, they can damage the intestinal mucosa disrupting the nutrients transport. They are capable of binding with proteins, forming sparingly digestible complexes and reducing consequently the protein digestibility (Francis et al., 2001). Their bitterness is known to affect the ingredients palatability (Guillaume et al., 1999). Saponins are commonly used as a fish-poison. When added to the water, they damage the respiratory epithelium of the gills (Francis et al., 2001). Levels below 1 g/kg are unlikely to affect growth performance of common aquaculture fish (Francis et al., 2001).

Occurrences

They occur in many plant species and are particularly concentrated in the leaves (Guillaume et al., 1999).

Treatment

Saponins are highly soluble in water and can be removed by aqueous extraction (Francis et al., 2001).

24) Anti-enzymes

Anti-enzymes factors are a group of compounds having an anti-enzymatic activity such as the amylase inhibitor, the invertase inhibitor, and the arginase inhibitor (derivative of chlorogenic acid) (Tacon, 1997).

Occurrences

They are found in many plant-derived ingredients (cereal, root tubers, legumes and oilseeds).

25) Anti-vitamin factors
Anti-vitamin factors are a group of compounds having an anti-vitamin activity. They include (Tacon, 1997):
- Anti-vitamin A destroys carotene
- Anti-vitamin D interferes with calcium and phosphorus absorption
- Anti-vitamin E causes liver necrosis and muscular dystrophy
- Anti-thiamine factor (thiaminase) causes deficiencies in vitamin B1
- Anti-pyridoxine
- Anti-vitamin B12

**Occurrences**

Anti-vitamin factors are present in many plants used in fish feeds.

**Treatment**

They are heat-labile and are readily destroyed by heat treatment. Some of them can also be removed by water extraction (Tacon, 1997 – Francis et al., 2001).

**26) Antigenic compounds**

Antigenic compounds in some legume seeds and cereals elicit antigenic effects in animals. These compounds are capable of inducing intestinal mucosal lesions, abnormalities in the villi, specific and non-specific immune responses, and abnormal movement of digesta through the gut. Soybean protein contains glycinin and beta conglycinin that act as allergens in several animals.

**27) Alkaloids**

Alkaloids are secondary metabolites found in plants. True alkaloids are basic, contain nitrogen in the heterocyclic ring and are derived from amino acid precursors. Alkaloid-containing grain legumes, such as lupins (Lupinus albinus), are otherwise ideally suited as a feedstuff in aquafeeds because of high digestible protein content (30-50 percent). Aqueous extraction removes alkaloids from some materials.
28) Miscellaneous anti-nutrients

Cyanogens are compounds found at high concentrations in a number of pulses, root crops (cassava), and some oilseeds (linseed). Cyanogens, when hydrolyzed, produce toxic products (e.g., hydrogen cyanide and probably other carbonyl compounds) that suppress respiration and cause cardiac arrest.

Mimosine is an unusual amino acid present in Leucaena leucocephala, comprising about 3-5 percent of total protein dry weight. Leucaena leaf meal is poorly digested by tilapia. Therefore, it is questionable whether sufficient mimosine enters the body to cause physiological effects.

Cyclopropenoic fatty acids, such as sterculic acid and malvalic acid present in cottonseed oil and meal, are known to cause abnormalities in the reproductive processes and alterations in the lipid metabolism in mammals. In rainbow trout, they interfere with long chain fatty acid metabolism and with stearic and palmitic dehydrogenation (Roehm et al. 1970). Cyclopropenoic fatty acids and other toxins, such as aflatoxins, are suspected carcinogens in fish.

Canavanine is a thermo resistant free amino acid present in legume species and is an antagonist of arginine. Jack bean (*Canavalia ensiformis*) meal treated for removal of canavanine is as effective as fishmeal as a feed ingredient for tilapia (Martinez-Palacios et al. 1988).

Major anti-nutritional factors commonly present in plant-derived feedstuffs used in aqua feed (Francis et al. 2001)
Elimination of antinutritional substances by technological treatments

<table>
<thead>
<tr>
<th>Anti-nutritional factors</th>
<th>Plant-derived nutrient source</th>
<th>Means of alleviation</th>
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<td>Interaction with protein nutrition</td>
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<tr>
<td>Protease inhibitors</td>
<td>Soybean, Jatropha kernel meal, rapeseed meal, lupin seed meal, pea seed meal, sunflower oil cake, alfalfa leaf meal, sesame meal</td>
<td>Heat, autoclaving</td>
</tr>
<tr>
<td>Heamagglutinins (lectins)</td>
<td>Soybean, Jatropha kernel meal, pea seed meal</td>
<td>Heat, autoclaving</td>
</tr>
<tr>
<td>Saponins</td>
<td>Peas, alfalfa, Jatropha kernel meal, lupin seed meal, pea seed meal, sunflower oil cake, alfalfa leaf meal</td>
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<tr>
<td>Polyphenols (Tannins)</td>
<td>Tannins, sorghum, Jatropha kernel meal, pea seed meal, mustard oil cake</td>
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<tr>
<td>Chlorogenic compounds</td>
<td>Supplementary methionine or choline</td>
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<tr>
<td><strong>Interaction with mineral availability</strong></td>
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<tr>
<td>Phytic acid</td>
<td>Soybean, Jatropha kernel meal, pea seed meal, cottonseed meal, sesame meal</td>
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<tr>
<td>Oxalic acid</td>
<td>Leaf protein</td>
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<tr>
<td>Glucosinolates</td>
<td>Rapeseed, mustard oil cake, plants with low content</td>
<td></td>
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<tr>
<td>Gossypol</td>
<td>Cottonseed meal</td>
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<tr>
<td><strong>Interaction with vitamin availability</strong></td>
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<tr>
<td>Vit A (lipoxygenase)</td>
<td>Soybean</td>
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<td>Vit D</td>
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<td>Vit E (oxidase)</td>
<td>Anti-nicotinic acid</td>
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<td></td>
<td>(niacinogen) corn</td>
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<td></td>
<td>Arginase inhibitor</td>
<td>Sunflower oil cake</td>
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<td></td>
<td>cyclopropenoic acid</td>
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<td></td>
<td>antivitamins</td>
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A number of treatments of food materials are able to eliminate some bioactive substances partially including soaking, dry and moist heat treatment, filtration, germination, and enzymatic treatments.

Chemical and physical characteristics determine the choice of appropriate treatment used to eliminate an undesirable compound from food.

**Heat treatment**

- Heat processing is widely accepted as an effective means of inactivating the thermo-labile anti-nutritional factors in food material.
- This improves protein quality by inactivating anti-physiological factors, particularly trypsin inhibitor and haemagglutinins and by unfolding the protein structure.
- Heat treatment process includes boiling, autoclaving, pressure cooking, extrusion cooking, toasting

**Cooking**

<table>
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<tr>
<th>Alkaloids</th>
<th>Alfalfa leaf meal, cottonseed meal, pea seed meal, soybean meal</th>
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<tr>
<td>Phytoestrogens</td>
<td>Lupin seed meal</td>
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<tr>
<td>Allergens</td>
<td>Soybean meal, lupin seed meal</td>
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<td></td>
<td>Soybean meal</td>
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</table>
Cooking or boiling generally inactivates heat sensitive anti-nutritive factors such as trypsin and chymotrypsin inhibitors and volatile compounds.

Cooking for 60 minutes at 100°C was sufficient to inactivates over 90% of the trypsin inhibitor activity in food materials.

**Autoclaving**

Autoclaving cooking under pressure includes the food materials are autoclaved for 30 minutes at 125 °C and 15 lb pressure, thermo labile inhibitory substances such as cyanogenic glycosides, saponins, terpenoids and alkaloids could be eliminated from the food materials.

**Pressure cooking**

- The food material is cooked under pressure for 30 minutes to remove trypsin inhibitors in food.

**Microwave treatment**

- Microwave treatment is the heats food by passing microwave radiation through it.
- Microwave ovens use frequencies 2.45 (GHz) and a wavelength is 12.2 centimetres for 10 minutes to eliminate the trypsin inhibitor and haemogglutinating activity in food.

**Extrusion cooking**

- The cooking process takes place within the extruder where the product produces its own friction and heat due to pressure generated (10-20 bar).
- The process can induce both protein denaturation and starch gelatinization, complete inactivation of haemagglutinins in food materials.

**Soaking**

Soaking could be one of the process to remove soluble antinutritional factors, seeds were soaked in water at 22°C for 18 hours to decrease in trypsin inhibitor activity in the food.
Germination

Germination (Sprouting) has been documented to be an effective treatment to remove some anti-nutritional factors in legumes by mobilizing secondary metabolic compounds which are thought to function as reserve nutrients.

Germination can lower the phytate content in legume seeds depending upon the type of bean and germinating condition.
## Conclusion

**Effects of antinutritional factors in fishes**

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<th>Antinutritional substances</th>
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<td>Gut wall damage, immune response, metabolic toxicity</td>
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<tr>
<td>Amylase inhibitors</td>
<td>Interference with starch digestion</td>
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<tr>
<td>Antigenic proteins</td>
<td>Gut wall damage, immune response</td>
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<td>Polyphenols</td>
<td>Decrease of protein digestion</td>
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<td>Formation of complexes with proteins and carbohydrates, digestibility reduced</td>
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<tr>
<td>Vicine, convicine</td>
<td>Haemolytic anaemia, interference with fertility and hatchability of eggs</td>
</tr>
<tr>
<td>Saponins</td>
<td>Haemolysis, affect intestinal permeability</td>
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<tr>
<td>Flatulence factors</td>
<td>Gastro-intestinal discomfort</td>
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<tr>
<td><strong>Glucosides</strong></td>
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<tr>
<td>Glucosinolates</td>
<td>Impaired iodine utilization, affect intestinal permeability</td>
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<tr>
<td>Alkaloids</td>
<td>Liver damage, bitter taste</td>
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<tr>
<td>Quinolizidine (lupin alkaloids)</td>
<td>Neural disturbances, affect liver function, bitter taste</td>
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</tbody>
</table>
The common processing techniques, like dry and especially wet heating, extracting with water, and addition of feed supplements have been widely and successfully used to reduce the concentration of antinutrients in plant feeds. Caution needs to be exercised when resorting to treatment methods because they sometimes have unintended adverse effects on the nutritional quality of the feed material, e.g., heat treatment reportedly alters the chemical nature and decreases the nutritional quality of proteins and carbohydrates.

The different tolerance limits of individual fish species to the presence of antinutrients also need to be considered before deciding on treatment procedures to reduce their levels. Tilapia species, for example, seem to be more tolerant than carp to the increased presence of antinutrients in general. Feeding experiments using purified individual antinutrients are needed to determine the threshold limits that will not affect the productivity of common culture fish.

Another important factor to be considered is the interactions between various antinutritional factors in a particular substance as these interactions in some instances lead to a decrease in the toxic effect of the interacting antinutrients. For example, saponin–tannin,
tannin–lectin and tannin–cyanogen interactions have all been shown to result in a reduction in the individual toxicity of the antinutrients.

**References**


