# Various Anti-Nutrients in Fish Feed Ingredients (a Review)

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**ABSTRACT**

*When plants are used as feed components for fish and animals, the presence of anti-nutrients is a concern. The utilization of nutrients from plant food or plant products that are used and play a significant part in their usage as feed can be reduced by antinutrients, which are chemicals or compounds that are naturally produced by plants. When ingested by animals or people, antinutrients in feed ingredients have an impact on the gastrointestinal, neurological, and cerebral systems. Cyanic acid, anti-trypsin, aflatoxin, mimosin, gossypol, and tannin are a few examples of known anti-nutrients found in plants. Plants can be used as feed without resulting in losses because of the antinutrient content they possess by undergoing physical, chemical, and biological treatment.*

**Keyword:** *anti-nutrients, feed, fish, plant*

## 1. INTRODUCTION

Antinutrients are substances that can inhibit the growth, development, health, behaviour, or spread of populations of other organisms. Antinutrients are substances or compounds that are naturally produced by plants and can reduce the utilization of nutrient intake from plant food or plant products that are used and play an important role in their use as feed [1]. Generally, they are secondary metabolites of plants, and are derived from both glucose and amino acid metabolism pathways (Figure 1).

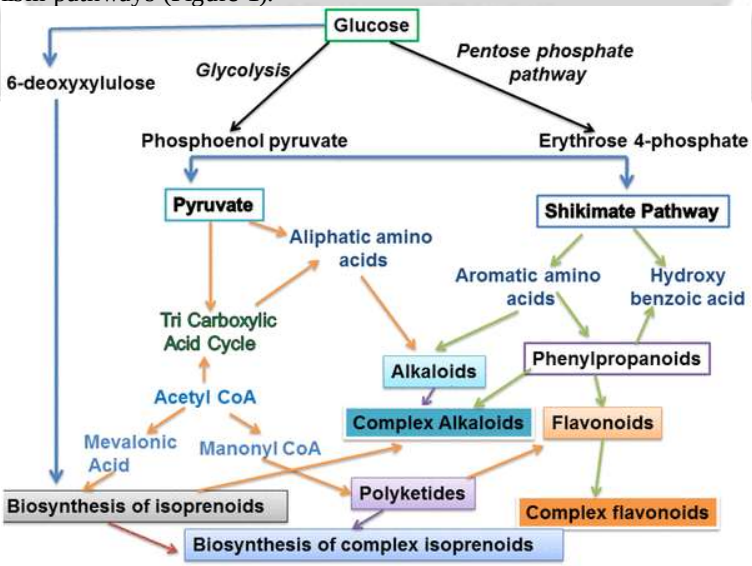


Figure 1. Plant secondary metabolism cycle [2]

The presence of anti-nutrients in plants is a concern when these plants are used as feed ingredients for livestock and fish. According to [3], antinutrients in feed ingredients when consumed by animals or humans will affect the gastro intestinal, nervous system and cerebral system. However, at the application level of plant-based feed ingredients for livestock and fish, many processes can be carried out to reduce or eliminate anti-nutrient content in plants, physically, chemically and biologically. This paper describes several types of antinutrients that are widely found in plants that are often used in fish feed ingredients, so that they become information for their use when formulating feeds.

## 2. TYPES OF ANTI NUTRIENT

**Cyanic Acid**

Cyanic acid is an anti-nutritional product resulting from the hydrolysis of cyanogenic glucoside compounds such as linamarin, loteustralin and durian. Cyanogenetic glycosides are compounds found in feed ingredients and are potentially very toxic because they can decompose and release hydrogen cyanide [4]. More than 100 types of plants are capable of producing cyanide, one of which is cassava. Cyanic acid is produced by plants as a protection mechanism against disturbance and will only be released when the plant is injured.

Cyanide acid is a poison that is classified as strong and works very quickly [5]. The lethal dose of cyanide acid from plants also cannot be determined precisely because the amount of cyanogenic glycosides from each plant consumed may vary [6]. The impact of cyanide acid on fish at low doses will not be lethal, nevertheless it will interfere with the growth process and cause diarrhea. Meanwhile, at high doses, metabolized cyanide acid in the body will interfere with the cell's respiratory process due to an obstacle to the alternating reaction of $Fe^{3+}$ in cells. If cyanide acid reacts with haemoglobin in the blood, it will form cyano-Hb which causes the blood to be unable to carry oxygen.

Cyanide acid in feed ingredients can be reduced by physical treatment, such as drying, soaking, boiling, grinding, and cooking processes. Meanwhile, biological treatment using the fermentation method can also be carried out to reduce cyanide acid.

***Anti-Trypsin***

Anti-trypsin or trypsin inhibitor is a compound that inhibits the action of trypsin which is naturally found in soybeans, lima bean (kara), wheat, sweet potatoes, potatoes, winged bean, peas, legume tubers, alfalfa, sorghum, fava beans, rice and ovomucoid. All of them are low molecular weight proteins, except for the anti-trypsin contained in ovomucoid which consists of 75 percent amino acids and 25 percent carbohydrates. In general, anti-trypsin is a compound consisting of amino acids.

The mechanism of action of anti-trypsin in the body of livestock begins with the interaction between trypsin (T) with inhibitor substrate (I) which contains lysine and arginine and forms a peptide bond in the form of a tetrahedral (TI)t. normal substrate (TI)t. Then by breaking the peptide bond of the original enzyme (TI)a, a second tetrahedral intermediate compound (TI)t is formed, and then a second inhibitory intermediate (I) is produced.

Anti-trypsin will stimulate the formation and at the same time the release of substances such as pancreozymin which is like a hormone from the intestinal wall. This substance will stimulate the release of enzymes from the pancreas. It is well known that the secretion of enzymes from the pancreas is regulated by a feedback mechanism due to the presence of trypsin from chymotrypsin in the intestine. It is clear that the reduced amount of trypsin and como-trypsin in the intestine will stimulate the release of pancreatic enzymes by binding to active trypsin and chymotrypsin in the small intestine. Thus, in the presence of antitrypsin, the pancreas will secrete excessive enzymes. Because it is a protein in itself, then livestock fed antitrypsin-containing feed not only cannot use the protein in the feed, but also loses body protein through enzymes that are secreted in excess. As a result, fish that consume feed containing antitrypsin will experience several symptoms, such as difficulty in consuming feed, pancreatic hypertrophy with an increase in the number of pancreatic tissue cells, protein digestion disorders, impaired fat absorption, reduced sulfuric amino acids and stunted growth.

Fish feed containing antitrypsin tends to form an expansion of the pancreas. Species whose pancreas weight exceeds 0.3 percent of body weight will tend to increase the expansion of the pancreas, where reduction of the size of the pancreas becomes impossible. The expansion of the pancreas will increase the secretion of trypsin which is abundant from the expansion of the pancreas causing a deficiency of sulfuric amino acids. The last effect that occurs is stunted growth. Almost all anti-trypsin in plants can be damaged by heat. More than 95 percent of its activity was destroyed by heat treatment within 15 minutes at $100^{o}$C. Feed milling using an extruder is very effective in destroying anti-trypsin. Important factors in controlling the destruction of anti-trypsin are temperature, heating time,

particle size and water content. Excessive heating will damage other food substances such as amino acids and vitamins.

**Aflatoxin**

Aflatoxins are a group related to the bisfuranocoumarin structural family that are produced mainly by toxic strains of *Aspergillus flavus* and *Aspergillus parasiticus*. Only half of these strains are known to produce toxins. Although other fungi such as *Penicullum* spp, *Rhizopus* spp, *Mucor* spp and *Streptomyces* spp can produce aflatoxins, their relevance to livestock production is unknown.

Aflatoxins are produced by aspergillus strains that are widely distributed in water and soil. When the environmental conditions are favourable, there is a substrate (in the form of feed or seeds) a source of nutrients, the fungus will be able to grow and develop properly. The final form of aflatoxin will depend on environmental conditions (temperature, humidity and aeration), substrate and type of fungus. In soybeans, only a small amount of aflatoxin $B_1$ can be produced by both types of aspergillus. *Aspergillus flavus* is a fungal colony that can attack seeds. *Aspergillus flavus* can colonize a variety of important animal feed sources, including maize, grains, beans, kapok seeds, cassava, copra, and various other grains. In general, the environmental factors needed for the growth of the aflatoxin-producing fungi are approximately 14 percent humidity and 25 percent temperature and certain aeration ($O_2$). If these requirements are achieved, mushroom investment will occur quickly.

Aflatoxicosis is the term used for diseases caused by eating food contaminated with aflatoxins [7]. Poisoning due to aflatoxins that occurs in fish can be categorized into two levels, namely acute and chronic poisoning levels. Basically, the target organ for aflatoxin toxins in all fish is the liver. The fatal cumulative effect on fish is impaired liver function. After a number of AFB1 toxins are formed, hepatocytes will undergo rapid changes involving lipids, resulting in necrosis (cell death). Damaged liver function will be followed by the emergence of other effects such as impaired blood clotting mechanism, jaundice and decreased serum production of essential proteins synthesized in the liver. Weakening of the blood clotting system and increased capillary permeability affect the extent of haemorrhage, including accumulation of blood in the gastrointestinal tract. In addition to liver damage, higher doses than some species can cause necrosis of the renal tubules. Although the thymus gland is the target organ in acute aflatoxin cases, according to the immune system, it is more associated with chronic aflatoxicosis.

**Mimosin**

Mimosin is a heterocyclic amino acid compound, which is an amino acid that has a circular carbon chain with a different group. Mimosin is often called leuseunina with the molecular formula $C_8H_{10}O_4N_2$. Assessing from its structure, it is a derivative of protein, it is characterized by the presence of the element N in its structure. Because the thing that distinguishes between proteins with carbohydrates and fats structurally is the presence of the element N.

Mimosin is structurally similar to tyrosine, but differs in function. That is an anti-nutrient substance that is in one of the feed ingredients, where the substance when consumed by livestock can cause a decrease in the performance of the livestock. In non-ruminant animals, mimosin causes stunted growth and reproductive problems [8]. Even one of the other anti-nutritional substances can cause death if consumed in large quantities and continuously for a long period of time. While tyrosine is a hormone that functions as a goitre prevention. Mimosin has a structure formula in Figure 2.

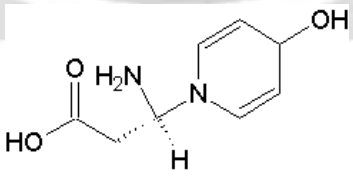


Figure 2. Mimosin structure formula[1]

Mimosin is found in many plants of the leguminous family, especially in *lamtoro* or *petai* (*Leucena leucoceaphala*). If found in the leaves and stems then the seeds contain as much as 1-4%. There are also wild plants in the form of shrubs, namely the *putri malu* (*Mimosa pudica*) and the leguminosae family known as bushes where the plant is known to contain a lot of protein and is suitable for use as fish feed. It is known that this plant has high palatability, fast growth, easy to grow and has a protein content of 25-30%, and is a plant that thrives in the tropics.

[1] https://upload.wikimedia.org/wikipedia/id/d/ed/Mimosin.gif

Basically mimosin is a factor that causes blood deficiency (anemia) in the fish body, hence that it can cause other disorders that can reduce fish appearance. While other effects that occur in fish is that it can cause stunted growth and reproductive disorders. According to its function, mimosin has the opposite function with the amino acids needed by the fish body. If the fish is under stress, it will result in the absorption of mimosin in the body faster than the absorption of amino acids. Thus, the fish will suffer from more poisoning.

Mimosin content can be reduced through several methods, one of which is through immersion in water [9]. Mimosin is not easily damaged in ordinary heating, the level of damage begins to occur if high heating is carried out, around 227-228$^{o}$C, this can be used as a prevention of poisoning by heating the feed ingredients containing these compounds first before being given to fish.

**Gossypol**

Gossypol is one of the many anti-nutritional substances found in fish feed with the chemical formula $C_{30}H_{30}O_8$. Gossypol has a functional group that is relative to compounds in the body, especially those that have amino groups and iron ions, hence that they interfere with the body's biochemical reactions. Gossypol is generally found in seeds such as cotton seeds, *kapok* seeds, or okra seeds, but it is also found in other parts of plants such as stems, leaves, stamens and cotton husks. The cotton plant is one of the producers of oilcake which is a producer of high protein and energy for fish food. Nevertheless, it is very unfortunate that the protein cannot be used freely by fish because it contains polyphenols, free or bound gossypol can poison the fish that consume it. Free gossypol is the most dangerous, cottonseed cake rich in gossypol contains ± 0.517% while gossypol bound for example with $FeSO_4$ compounds is not harmful.

It is known that the gossypol first accumulates in various body tissues before causing symptoms of poisoning. Gossypol toxins in feed will be able to inhibit and reduce egg quality (egg yolks turn bluish green and egg whites turn slightly pink), reduce appetite, body weight and Hb levels in the blood or reduce red blood cells in the body.

Gossypol can be released from glandular pigments by extracting cake with an azeotropic mixture of hexene, acetone and water (44: 53: 5) but this process is not used commercially. Another way of prevention is by various treatments in the fat extraction process. In removing the fat mechanically, the process will be easier/better if the cottonseed is first heated (with hot steam) while squeezing it. The heat will break the gland gossypol out with fat/oil and cause it to mix with seed protein. Proteins and gossypols form complex bonds mainly because gossypols are associated with the free amino acid lysine of the protein in question. The protein complex is less digestible by protease enzymes so that the gossypol cannot be absorbed, thus the nutritional value of the protein expected from the cottonseed will decrease. This processing not only reduces the efficacy of lysine but also valine, threonine, leucine and methionine.

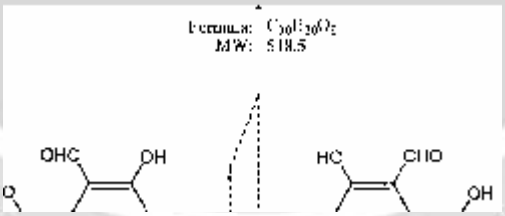


Figure 3. Gossypol structure [10]

**Tannin**

Tannins are polyphenolic compounds with high molecular weights and have the ability to bind proteins. Tannins consist of catechins, leukoanthocyanins and hydroxy acids, each of which can cause color when reacted with metal ions. Tannins have high reactive properties to the sulfhydryl groups and amino groups of proteins which cause the formation of bonds between tannins and proteins [11]. Tannins cannot crystallize in the form of colloidal compounds. Tannins are also known as tannic acid and gallotanic acid. Tannins range from colorless to yellow or brown. Tannins consist of two groups, namely coded tannins and hydrolizable tannins.

Feed ingredients that contain tannins include sorghum seeds, sunflower seeds, cotton seeds, peanuts, radish seeds, winged bean alfaalfa, pomegranate, lamtoro and many other plants that contain tannins. Tannins have the ability to precipitate proteins, because tannins contain a number of functional groups that have strong bonds with protein molecules and produce large and complex crosslinks, namely protein-tannins. Tannins can interfere with the

digestive process by binding to digestive enzymes and minerals [12]. There are three reaction mechanisms between tannin and protein so that a strong bond occurs between the two, namely:

1) Hydrogen bonds with OH groups on tannins and their receptor groups. For example, between NH and OH in protein.
2) Ionic bonds between the anionic groups in tannins with cation groups in proteins.
3) Covalent branch bonds between quinones and various reactive groups on proteins

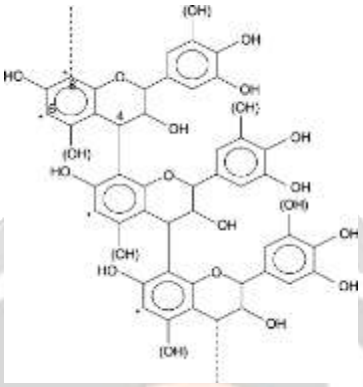

Figure 4. Tannin structure[2]

The aforementioned bonds cause tannins to immediately bind to feed protein in the digestive tract and cause the feed to be difficult to digest by digestive enzymes. The interaction of tannins with proteins in saliva (saliva) and glycoproteins in the mouth causes a feeling of shrinking (narrowing) in the mouth. In the body of fish, feeding that contains a small amount of tannins is not harmful. However, if the tannin content in the feed increases, it will begin to have an effect that can suppress fish growth, because tannin suppresses nitrogen retention and causes a decrease in the digestibility of amino acids that should be absorbed by the intestinal villi and utilized for growth and development of body tissues. Symptoms seen due to the presence of tannins are slow growth, decreased appetite due to the bitter taste of tannins and decreased egg-producing ability.

Prevention that can be done to eliminate the effect of tannins is by immersion in water, immersion in alkaline solution, mechanical means and supplementation of methyl donors. Soaking with water can be done with distilled water at a temperature of 30$^{o}$C for 24 hours, which can reduce tannin levels by 31%. Soaking with an alkaline solution can be done with a solution of 0.05 M NaOH and KOH at a temperature of 30$^{o}$C for 24 hours, which can reduce tannin levels by 75-85%. The reduction of tannins by mechanical means can be done by grinding by peeling the pericarp of sorghum. If feed containing tannin is already consumed by livestock, additional methyl donors can be given, such as methionine, choline, and arginine in pure form.

## 3. PLANTS THAT CONTAIN ANTI-NUTRIENTS

Almost all plants have anti-nutrients, however, Table 1 only contains anti-nutrients in plants that are often used as fish and livestock feed ingredients.

Table 1. Classification of anti-nutrients based on plant origin

| Plant Source | Anti Nutrients |
|---|---|
| Grains | |
| a. Rye | Trypsin inhibitor |
| b. Milo | Tannin |
| Tubers | |
| a. Potato | Solanum Alkaloids |
| b. Cassava Bulbs | Cyanogenic glucoside |
| Protein supplements | |

[2] https://ars.els-cdn.com/content/image/3-s2.0-B9780857095213500235-f23-01-9780857095213.gif

| | |
|---|---|
| a. Soybeans | Trypsin inhibitor |
| b. Cotton | Gossippol |
| Greeneries | |
| a. Alfalfa | Saponins |
| b. *Leucaena* spp | Mimosin |
| Grass | |
| a. Tropical grass | Oxalate |
| b. Sorghum forage | Cyanogeme |

## 4. CONCLUSIONS

Anti-nutrients are natural ingredients that occur naturally in plants. Utilization of plants as feed without causing losses due to the anti-nutrient content contained in them can be done by doing physical, chemical and biological treatment.

## 5. REFERENCES


[1]. Yanuartono, Y., Indarjulianto, S., Nururrozi, A., and Purnamaningsih, H. (2019). "Review: Hidrogen Sianida dan Implikasinya pada Ternak" *Jurnal Ilmu dan Teknologi Peternakan Tropis* 6(2), pp. 214.

[2]. Isah T. (2018) "Secondary metabolism of pharmaceuticals in the plant in vitro cultures: strategies, approaches, and limitations to achieving higher yield" *Plant Cell Tissue and Organ Culture* 132(13), pp 239-265

[3]. Handajani and Widodo (2010) "Pakan Ikan" UMM Press. Malang

[4]. Kurnia, N., and Marwoten, F. (2018) "Penentuan Kadar Sianida Daun Singkong Dengan Variasi Umur Daun dan Waktu Pemetikan" *Jurnal Ilmiah Pendidikan Kimia "Hydrogen,"* 1(2), pp. 117–121.

[5]. Yatno, Murni, R., Nelwida, and Yani, E. N. (2015). "Kandungan asam aianida, bahan kering dan bahan organik tepung biji karet hasil pengukusan" *Jurnal Ilmiah Ilmu-Ilmu Peternakan Universitas Jambi*, 18(2), pp. 58–65.

[6]. Yanuartono, Nururrozi, A., Indarjulianto, S., Purnamaningsih, H., and Raharjo, S. (2020). "Traditional methods of processing livestock feed to reduce antinutrient factor content: a brief review" *Jurnal Ilmu Ternak Universitas Padjadjaran*, 19(2), pp. 13

[7]. Broto, W. (2018). "Status Cemaran dan Upaya Pengendalian Aflatoksin Pada Komoditas Serealia dan Aneka Kacang" *Jurnal Litbang Pertanian*, 37, pp. 81–90

[8]. Argadyastro, D., Retnani, Y., and Diapari, D. (2015). "Pengolahan daun lamtoro secara fisik dengan bentuk mash, pellet dan wafer terhadap performa domba" *Buletin Makanan Ternak*, 102(1), pp. 19–26.

[9]. Padmiswari, A. A. I. M., Wiratmini, N. I., and Kasa, I. W. (2017). "Histologi Testis Tikus (*Rattus norvegicus*) Jantan yang Diberi Tepung Daun Lamtoro (*Leucaena leucocephala* Lamk. de Wit) Hasil Perendaman: *Metamorfosa: Journal of Biological Sciences*, 4(2), pp 178.

[10]. Kenar JA. (2006) "Reaction Chemistry of Gossypol and Its Derivative" *JAOCS*, 83(4), pp 269-302

[11]. Sakinah, N., Prangdimurti, E., and Palupi, N. S. (2019). "Kandungan Gizi Dan Mutu Protein Tepung Biji Kelor Terfermentasi" *Jurnal Teknologi dan Industri Pangan*, 30(2), pp. 152–160

[12]. Rolin, F., Setiawati, M., and Jusadi, D. (2017). "Evaluasi Pemberian Ekstrak Daun Kayu Manis *Cinnamomum burmannii* pada Pakan terhadap Kinerja Pertumbuhan Ikan Patin *Pangasianodon hypophthalmus* Sauvage, 1878." *Jurnal Iktiologi Indonesia*, 15(3), pp 201–208