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The reduction of mimosine and tannin contents in leaves of *Leucaena leucocephala*

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Abstract

Mimosine and tannin are chemicals present in lead tree leaves (*Leucaena leucocephala*). They are both toxic when ingested by herbivores, and their inactivation or reduction would enhance the use of the plant as livestock fodder. Antinutritional effects include interference with the digestive processes either by binding the enzyme or by binding to feed components like proteins or minerals (Liener, 1989). This study aims at reducing toxic substances in lead tree leaves. The latter were dried at 60 °C for 24 hrs, then soaked in water for 72 hrs, at room temperature and dried again at 60 °C for 48 hrs. Mimosine content was reduced from 4.4 to 0.2% or an overall 94% of reduction was achieved. In addition to that, tannin content was reduced from 37.6 to 0.3% or overall 99.33% reduction was achieved.

Keywords: Mimosine, Tannin, Lead tree leaves, *Leucaena leucocephala*, Antinutritional agents

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Introduction

Leucaena is a tropical tree with a wide assortment of uses. Increasingly, foresters and farmers in the tropics are exploring its potential, and the area planted to leucaena is expanding rapidly. Under optimum growing conditions, leucaena stands have yielded extraordinary amounts of wood-indeeds, among the highest annual totals ever recorded (Pantastico and Baldia, 1980;

Vogt et al., 1986). Leucaena also makes quality forage and is responsible for some of the highest weight gains of cattle in the tropics. Of all tropical legumes, Leucaena offers the most multiple uses. It can produce nutritious forage firewood, timber and rich organic fertilizer. (Pantastico and Baldia, 1980; Vogt et al., 1986; Makkar et al., 1995)
Leucaena foliage (leaflets plus stems) contains both nutrients and roughage and makes a ruminant feed roughly comparable to alfalfa forage. When dried, the leaflets readily fall off, providing a feed that is 25-30 per cent protein, even after correcting for mimosine nitrogen. In Malawi, Thailand and Philippines, leaflets are sun dried both for local forage and for export to Europe, Japan and Singapore. It has been found that dried leaves can be compressed into feed pellets without milling and without adding water, molasses, or other binders (Pantastico and Baldia, 1980; Vogt et al., 1986; Santiago et al., 1988). Leucaena's protein is of high nutritional quality. Its amino acids are well balanced, much as in alfalfa. The leaves are also a rich source of carotenoids and vitamins. Their provitamin A content, among the highest ever recorded in plant speciments, yellows the fat of leucaena-fed cattle, which consumers consider undesirable in some countries. This dose not happen with goats, and in chickens fed leucaena the yellowing of the skins and egg yolks is a desirable characteristic. (Thompson, 1975; Vogt et al., 1986; Santiago et al., 1988)

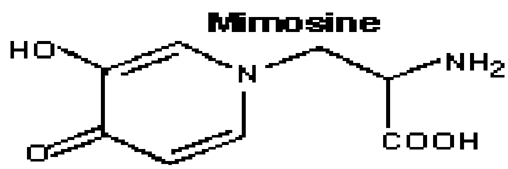

Figure 1: Chemical Structure of Mimosine (Source : www.bio.miami.edu/mimosa /Mimosine.gif (28 September 2003))

Mimosine is a free amino-acid very often present in certain legumes which include *Leucaena leucocephala* and *L. glauca*, both considered as excellent sources of protein for animal feed. Its presence limits the use of the leaves and seeds in feed for mono-gastric animals since it affects thyroid function, leading to poor growth.

Mimosine, a chemical present in *Leucaena leucocephala*, and its degradation product 3-hydroxy-4(1H)-pyridone (DHP) are both toxic when ingested by herbivores, and their inactivation or reduction would enhance the use of the plant as livestock fodder. Mimosine, although insoluble in distilled water, is soluble in ionic solutions. Leaves of *L. leucocephala* were leached in 0-100 per cent seawater and various acidic and basic solutions for 24 h at 25 °C. Rate of mimosine extraction depended on reagent concentration. The most effective was 0.05 N CH_3COONa, which extracted 95 percent. No loss of important nutrients was observed (Tacon, 1979). The method is simple, also removes DHP and is applicable for both small farmers and commercial use. In the leucaena types now available, mimosine has raised more concern than is justified. Fear of its effects has for years been a barrier to leucaena's wider use as forage. Today, much of this fear is being dispelled. Ruminant animals in Indonesia, India, Hawaii and several other countries have been showed to thrive without ill effect on diets of 100 percent leucaena plus a salt supplement (Vogt et al., 1986).

Mimosine, found in the leave of *L. leucocephala* and comprises about 3-5 percent of the dry weight of total protein (Vogt et al., 1986 ; Wee and Wang, 1987), acts as an inhibitor of pyridoxal containing transaminases, tyrosine decarboxylase, several metal-containing enzymes, and both cystathionine synthetase and cystathionase (Haslam,1989). Reported toxicity signs in fish and shrimp have included reduced growth and feed efficiency, and increased mortality

(Tilapia, *Oreochromus mossambicus*/ *niloticus*) (Ghatnekar et al., 1983; Vogt et al., 1986; Wee and Wang, 1987; Santiago et al., 1988). In the case of shrimp, marked histological alterations were evident within the mid gut gland-cells of post-larvae fed mimosine containing diets, including the progressive destruction of the mid-gland epithelial cell (Vogt et al., 1986; Haslam, 1989). Histopathological signs of mimosine toxicity were also reported by Pantastico and Baldia (1980) within the liver tissues of Indian major carp *L. rohita* fed diets containing *L. leucocephala* leaf meal, liver lesions included congestion of the blood vessels and fatty changes in the hepatocytes.

Mimosine, the toxic, non protein amine acid contributes as much as 14.8 percent to the total nitrogen content of *L. leucocephala* seeds. The mimosine content varies from 2.2 per cent to 10 per cent. Pantastico and Baldia (1980) reported, variations in Leucaena mimosine concentration range from 1.89 to 4.89 percent. The concentration is higher in the seeds than in other parts of the plant, second only to the immature tender leaves.

However, its use has been limited, because of the presence of the toxic non-protein amino acid, mimosine. A few studies have been conducted to evaluate the nutritive value of *Leucaena leucocephala* leaf meal as a protein source and pigment in fish feeds, but the data obtained are conflicting. Pantastico and Baldia (1980) reported improved growth responses of *Tilapia mossambica* and *T. nilotica* fed diets containing 100 percent Leucaena leaf meal. However, Jackson et al. (1982) obtained very poor growth of *Sarotheodon mossambicus* fed diets in which 25 percent of the fish meal was replaced by *Leucaena* leaf meal. This growth reduction was attributed to the toxic effects of mimosine. A trend of reduced growth performance and feed efficiency with increased dietary levels of *Leucaena* leaf meal was also reported by Wee and Wang (1987) for Nile tilapia. The reproductive performance and growth of *Oreochromus niloticus* broodstock were also affected by the presence of high levels of *Leucaena* leaf meal in diets. Santiago et al. (1988) recommended that *Leucaena* leaf meal should not be incorporated to more than 40 per cent of the diet of *Nile tilapia* broodstock.

The nutritive value of the *Leucaena* leaf meal can be improved if most of the mimosine is removed or degraded to a relatively less toxic compound. About 90 percent of mimosine can be extracted by soaking the leaves in freshwater for 36 hours. Wee and Wang (1987) found that soaked *Leucaena* leaf meal gave a significantly better growth of *Nile tilapia* than sundried or commercial leaf meal. However, they indicated that the nutritive quality of soaked leaf meal appears to be limited by other nutritional factors, such as the lack of certain amino acids.
Tannins are defined as water-soluble polyphenols with molecular weights ranging from 500 to 3000. They differ from other natural phenolic compounds in their ability to precipitate proteins (e.g. gelatin) from solution. (Spencer et al., 1988)

Tannins are water-soluble polypeptide glucosides present in many plants, their sourness and astringency give feed an unpleasant taste and odour. They affect protein utilization, because they bind to lysine and so make it unavailable to mono gastric animals and fish. Tannins are usually determined by Lowenthal, s permanganate oxidation process (Peason, 1976).
Tannins are generally found in a large array of higher plant species of both herbaceous and woody types. Tannin accumulation is reported in large amounts (often more than 10% of the dry weight) in particular organs or tissues; for example, bark, wood, leaves, fruits or roots (Haslam, 1989).Tannins can be classified into two distinct groups, according to their structures of hydrolyzable tannins and condensed tannins

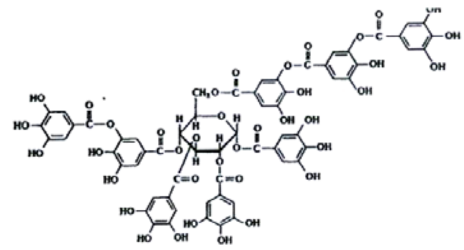

Figure 2: Chemical structures of hydrolysable tannins (Source: Haslam, 1989)

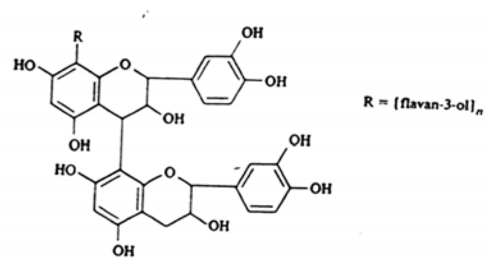

Figure 3: Chemical structures of condensed tannins (Source: Butter, 1992)

Tannins are secondary compounds of various chemical structures widely occurring in plant kingdom and generally divided into hydrolysable and condensed tannins. Their antinutritional effects include interference with the digestive processes either by binding the enzyme or by binding to feed components like proteins or minerals (Liener, 1989). Tannins also reduce the absorption of vitamin B12. Common carp has been shown to be to tolerate a 2 percent addition of quebracho tannin powder (a condensed tannin) without any effect on growth while a similar level of hydrolysable tannin (tannic acid) induce feed rejection after 28 days of feeding (Becker and Makker, 1999). Contrary to condensed tannins, the hydrolysable tannins are easily degraded in biological systems, forming smaller compounds that can enter the blood stream and over a period of time cause toxicity to the organs (liver and kidney). How far the purified commercially available tannins simulate those naturally occurring in plant products needs to be investigated. Condensed tannins present in copra at a level of approximately 2.4 percent could have been the cause of growth depression in tilapia and rohu (*Labeo rohita*) fingerlings even at such low levels of inclusion as 25 percent or 20 percent (Jackson et al., 1982; Mukhopadhyay and Ray, 1999). Other tannin containing feeds, like rapeseed and pea seed meal, have reportedly been tolerated by different fish species at moderate to high levels of inclusion. Broad bean (*Vicia faba*) meal, with a high condensed tannin content, had lower protein digestibility than soybean in *in vitro* experiments (Grabner and Hofer, 1985). The differences in digestibility were more pronounced after digestion under the simulated conditions of the carp gut than those of the rainbow trout gut. These differences probably indicate the differences in tolerances of different fish species and differences in the structure of the tannins or their interactions with other components in the diet. Tannins are also known to interact with other antinutrients. For example, interaction between tannins and lectins removed the inhibitory action of tannins on amylase (Fish and Thompson, 1991), and interactions between tannins and cyanogenic glycosides reduced the deleterious effect of the latter (Goldstein and Spencer, 1985).

Tannins, like gossypol, are a diverse group of polyphenol compounds but in contrast to gossypol on hydrolysis they yield sugar residues, phenol carboxylic acids, and condensed tannins (Santiago et al., 1988). Widely distributed in nature, food crops and legumes which have been reported to contain significant quantities of tannins include sorghum (contain up to 5 percent condensed tannins), faba bean, lima bean, sunflower seed meal (containing 1.2-2.7 percent chlorogenic acid), and rapeseed. Although no information is available concerning their toxicity within feeds for fish or shrimp, in higher animals tannins have been found to interfere with digestion by displaying anti-trypsin and anti-amylase activity Santiago et al. (1988) report that condensed tannins were responsible for the testa-bound TI activity in faba bean) and by binding to digestive enzyme or by binding directly with the dietary protein. Tannins are also have the ability to complex with vitamin B12 (Santiago et al., 1988). Although the toxic effects of chlorogenic acid can be counthionine, chlorogenic acid is reported to be readily removed from sunflower seeds using aqueous extraction methods for the effect of different processing methods on the tannin content of fababeans.

Recommended methods for the removal of condensed tannins include de-hulling the seeds to remove the tannin rich outer layer, autoclaving or treatment with alkali (Griffiths, 1991) Mukhopadhyay and Ray (1999) observed a reduction in the tannin content of sesame seed meal from 20 to 10 g/kg after fermentation with lactic acid bacteria. The treatment of tannin-containing feeds with oxidizing agents and supplementation with a tannin-complexing agent, polyethylene glycol, could mitigate their negative effects on animals (Makkar et al., 1995; Markkar and Becker, 1996). Limited literature on the effects of purified tannins on fish suggest that fish are sensitive to tannins and caution should be exercised in incorporating seeds and agro industrial byproducts containing high amounts of tannins

Methodology

Effective of soaking Leucaena leucocephala in water and drying on reduction of mimosine and tannin contents

Leaves of lead tree (*Leucaena leucocephala*) were harvested and dried at 60 ºC for 24 h. They were soaked in water for 24, 48 and 72 h at room temperature, respectively. Then, they were dried at 60 ºC for 48 h and milled. The treatments were as follows

M_1 = Fresh leaves of *Leucaena leucocephala*
M_2 = M_1 + drying at 60 ºC for 24 hrs (as control)
M_3 = M_1 + autoclaving at 121 ºC for 20 min + drying at 60 ºC for 24 hrs
M_4 = M_2 + autoclaving at 121 ºC for 20 min + drying at 60 ºC for 24 hrs
M_5 = M_2 + drying at 80 ºC for 24 hrs
M_6 = M_2 + drying at 80 ºC for 48 hrs
M_7 = M_2 + drying at 80 ºC for 72 hrs
M_8 = M_2 + soaking in water 24 hrs + drying at 60 ºC for 48 hrs
M_9 = M_2 + soaking in water 48 hrs + drying at 60 ºC for 48 hrs
M_{10}= M_2 + soaking in water 72 hrs + drying at 60 ºC for 48 hrs

Finally, mealed leaf of *Leucaena leucocephala* was used to analyse for mimosine and tannin contents. (Matsumoto and Sherman, 1951 and Peason, 1976)

Results and Discussion

Effective of soaking Leucaena leucocephala in water and drying on reduction of mimosine and tannin contents

Leaves of Leucaena leucocephala were harvested and dried at 60 ºC for 24 h. They were soaked in water at room temperature for 24, 48 and 72 h, respectively. Then, they were dried again at 60 ºC for 48 h and milled. Mimosine and tannin contents in Leucaena leucocephala were analyzed. The results were shown in table 1 and 2, respectively.

Table 1: Mimosine contents in jeaves of *Leucaena leucocephala*.

Treatments	Mimosine (percent)	Reduction of Mimosine Contents (percent)
M_1	4.346	0.000
M_2	2.925	32.697
M_3	2.847	34.491
M_4	0.914	78.969
M_5	1.644	62.172
M_6	1.434	67.004
M_7	1.251	71.215
M_8	0.816	81.224
M_9	0.458	89.462
M_{10}	0.227	94.777

From table 1, mimosine was reduced by 94.777 percent using simple method by firstly drying at 60 °C for 24 hrs, soaking in water for 72 hrs and finally drying once more at 60 °C for 48 hrs. Similar results were reported elsewhere (Ghatnekar et al., 1983; Vogt et al., 1986; Wee and Wang, 1987; Haslam, 1989).

Table 2: Tannin contents in leaves of *Leucaena leucocephala*.

Treatments	Tannin (percent)	Reduction of Tannin Contents (percent)
M_1	37.582	0.000
M_2	7.155	80.962
M_3	7.049	81.244
M_4	1.664	95.572
M_5	5.574	85.168
M_6	2.246	94.024
M_7	2.163	94.246
M_8	0.832	97.786
M_9	0.582	98.451
M_{10}	0.249	99.337

From table 2, the tannin contents could be reduced from 37.582 to 0.249 percent by using the easy method as previous described.

To reduce toxic substances in lead tree leaves of *Leucaena leucocephala*, they were dried at 60 °C for 24 hrs, then soaked in water for 72 hrs, at room temperature and dried again at 60 °C for 48 hrs. Mimosine content was reduced from 4.346 to 0.227 percent or 94.777 percent of reduction was achieved. In addition, tannin content was reduced from 37.582 to 0.249 percent or 99.337 percent of reduction was achieved.

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