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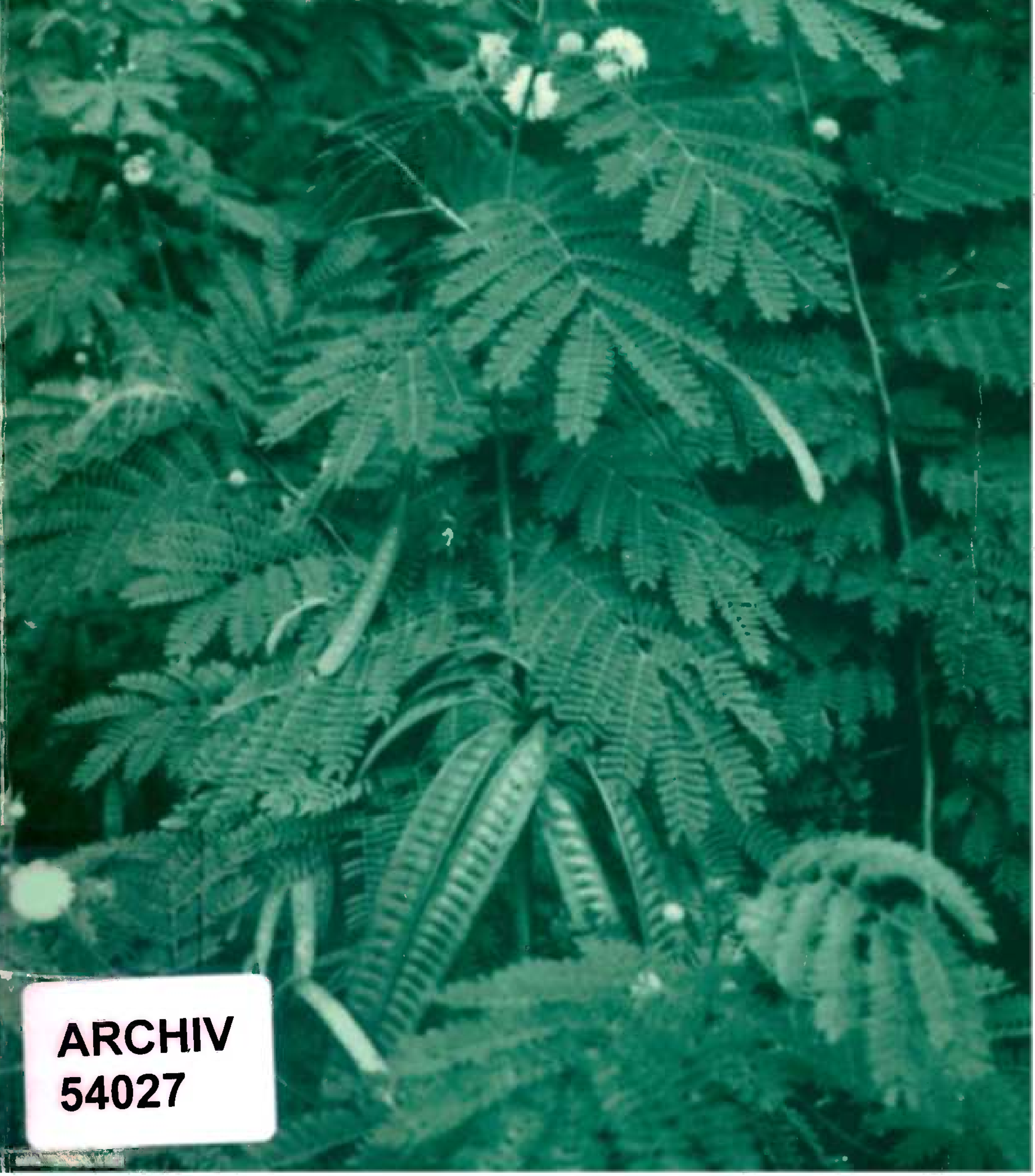
Leucaena Research in the Asian-Pacific Region

Proceedings of a workshop held in Singapore, 23-26 November 1982



IDRC 211e

Leucaena Research in the Asian-Pacific Region

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Leucaena Research in the Asian–Pacific Region

Proceedings of a Workshop Held in Singapore, 23–26 November 1982

Organized by the Nitrogen Fixing Tree Association and the International Development Research Centre

Abstract

Because of *Leucaena leucocephala*'s multiple uses as forage, fuelwood, poles, green manure, etc., this fast-growing, nitrogen-fixing tree has been the subject of much research in the last decade. The results have clarified the capabilities of the plant as well as its limitations. One main constraint to cultivation in vast areas of Latin America and Southeast Asia has been leucaena's inability to survive on acidic, aluminum-saturated soils. At low pH, the aluminum complexes with calcium, which is essential for good growth. Trials have shown that some varieties of *L. diversifolia* can make use of the calcium from the complexes and that crosses between these varieties and *L. leucocephala* perform well on acidic soils. The main constraint to use of leucaena as a forage derives from the plant's content of mimosine, a toxic, nonprotein amino acid. Although leucaena has proved to be a highly nutritious animal feed, the mimosine and its breakdown product, DHP (3-hydroxy-4[1H]pyridone), have caused toxicity among animals fed high levels of leaf meal. Scientists now have evidence that the mimosine is converted into DHP when brought into contact with an enzyme contained in some of the plant's cells harbouring mimosine. This finding needs follow up; it suggests that simple processing, such as chopping fresh leaves, will convert all the mimosine into the less-toxic DHP. Elsewhere, researchers have found that DHP can be metabolized by anaerobic microorganisms that have been found in the guts of ruminants in countries like Indonesia. They have successfully transferred the microorganisms to animals in Australia where toxicity from DHP has deterred graziers from using leucaena as forage. Other research has defined optimal approaches to breeding and genetic improvement of leucaena; characteristics of rhizobia that effectively provide the plant with nitrogen-fixing ability; biomass production under widely different soil conditions; effects on fish, poultry, cattle, goats, and sheep fed leucaena leaf meal; management and cultural practices for both large-scale and smallholder operations; etc. The results are the subject of this publication, which comprises 30 papers from researchers in the Asian–Pacific Region.

Résumé

Leucaena leucocephala a fait l'objet de nombreuses recherches au cours de la dernière décennie, cet arbre légumineux fixateur d'azote et de croissance rapide ayant de nombreux usages comme fourrage, combustible, poteau, engrais vert, etc. Ces études ont permis d'en délimiter les fonctions. L'un des facteurs limitants de sa culture dans de vastes régions de l'Amérique latine et de l'Asie du Sud-Est est l'incapacité de *Leucaena* de survivre dans des sols acides, saturés d'aluminium. Dans le cas d'un faible pH l'aluminium complexe le calcium, essentiel à une croissance régulière. Des essais ont démontré que certaines variétés de *L. diversifolia* peuvent utiliser le calcium présent dans les complexes et que les croisements entre ces variétés et *L. leucocephala* prospèrent dans des sols acides. Le principal obstacle à l'utilisation de *Leucaena* comme fourrage est sa teneur en mimosine, acide aminé non protéique toxique. Bien que ce fourrage soit hautement nutritif, la mimosine et DHP (3-hydroxy-4[1H]pyridone) ont provoqué des cas de toxicité chez les animaux consommant de grandes quantités de farine de feuilles. Les scientifiques ont découvert que la mimosine se décompose en DHP lorsqu'elle entre en contact avec une enzyme contenue dans certaines cellules où elle est présente. Cette découverte a permis de déterminer des moyens simples de neutraliser cette substance toxique, tel que le hachage des feuilles vertes qui décompose la mimosine en DHP moins toxique. Ailleurs, des chercheurs ont trouvé que le DHP peut être métabolisé par des microorganismes anaérobiques présents dans l'intestin des ruminants dans certains pays comme l'Indonésie. Ils ont réussi à transférer ces microorganismes à des animaux en Australie où les pasteurs refusent l'emploi du fourrage de *Leucaena* à cause de la toxicité de DHP. D'autres recherches préconisent une approche optimale: de la sélection et de l'amélioration génétique de *Leucaena*; des caractères des rhizobiums qui assurent la fonction de la fixation d'azote chez la plante-hôte; de la production de bio-masse dans diverses conditions de sols très variés; des effets des rations de farine de feuilles sur les poissons, les volailles, le bétail, les chèvres et les moutons; de la gestion et des pratiques culturales des

opérations des petites et des grandes exploitations, etc. Tous ces résultats sont détaillés dans la présente brochure qui contient trente communications exposées par des chercheurs de la région du Pacifique asiatique.

Resumen

Debido a los múltiples usos de la *Leucaena leucocephala* como forraje, combustible, madera, abono, etc., este árbol, de rápido crecimiento y habilidad para fijar el nitrógeno, ha sido objeto de abundante investigación en la última década. Los resultados han aclarado las capacidades de la planta, así como sus limitaciones. Uno de los problemas para su cultivo en vastas áreas de Latinoamérica y el Sudeste Asiático ha sido su incapacidad para sobrevivir en suelos ácidos, saturados de aluminio. A niveles bajos de pH, el aluminio forma complejos con el calcio que es esencial para un buen crecimiento. Las pruebas han mostrado que algunas variedades de *L. diversifolia* pueden usar el calcio de los complejos y que los cruces entre estas variedades y la *L. leucocephala* se desempeñan bien en suelos ácidos. El principal inconveniente para usar la leucaena como forraje proviene de su contenido de mimosina, un aminoácido tóxico no proteínico. Aunque la leucaena ha probado ser un alimento animal altamente nutritivo, la mimosina y su producto de descomposición, el DHP (3-hydroxy-4[1H]pyridone), han causado toxicidad entre los animales alimentados con altos niveles de harina de follaje. Los científicos tienen ahora evidencia de que la mimosina se convierte en DHP cuando entra en contacto con una enzima que se encuentra en algunas células de la planta que contienen mimosina. Este hallazgo necesita seguimiento, pero sugiere que un simple procesamiento, como picar las hojas frescas, convierte toda la mimosina en el menos tóxico DHP. En otras partes, los investigadores han encontrado que el DHP puede ser metabolizado por microorganismos anaeróbicos que han sido hallados en el intestino de rumiantes en países como Indonesia. Ellos han traspasado con éxito los microorganismos a animales en Australia donde la toxicidad del DHP ha impedido que los ganaderos empleen la leucaena como forraje. Otras investigaciones han definido los enfoques óptimos para el fitomejoramiento de la leucaena, las características de la rizobia que efectivamente dotará a la planta de la habilidad de fijar nitrógeno, la producción de biomasa bajo condiciones edáficas ampliamente distintas, los efectos sobre los peces, las aves, el ganado, las cabras y las ovejas alimentadas con harina de hoja de leucaena, las prácticas culturales y de manejo para las actividades a gran escala o del pequeño agricultor, etc. Los resultados son el tema de esta publicación que abarca 30 trabajos de investigadores en la región Asiopacífica.

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Detoxification of Leucaena by Enzymic or Microbial Processes

J.B. Lowry *Project for Animal Research and Development (Balai Penelitian Ternak), Bogor, Indonesia*

New information about the effects of leucaena in the diets of livestock has become available so rapidly that many researchers are still unaware of the differences in toxicity brought about by mimosine and its degradation product 3-hydroxy-4(1H)pyridone (DHP). Although mimosine is directly toxic, DHP is only indirectly so through its goitrogenic action. Thus, animals that can break down the mimosine to DHP can tolerate higher dietary levels of leucaena than can other animals, and animals that can degrade DHP even further can tolerate higher levels yet, perhaps even diets that are solely leucaena. The enzyme necessary to convert mimosine to DHP is contained in some (but not all) of the leucaena cells that harbour the mimosine. Conversion occurs when the enzyme comes in contact with the substrate, for example, through maceration. The enzyme becomes inactive at pH levels lower than 4, when heated suddenly to temperatures higher than 70°C, and when dried. These findings have enormous implications for past and future research. For instance, animals grazing leucaena pastures would initiate conversion of mimosine to DHP by chewing the leaves. The conversion would continue until virtually complete in ruminants but would be halted in monogastric animals by the acid in the stomach. Commercial leaf meal that has been sun dried during clear conditions or rapidly air dried would have the mimosine virtually intact, but the enzyme would be denatured. In contrast, leaf meal from leucaena that had deteriorated, for example in a plastic bag in the sun or lying in a heap during wet conditions, would have much of its mimosine converted to DHP. The conversions that take place under specific circumstances could explain many of the conflicting reports about toxicity from leucaena fed to both ruminants and nonruminants.

Une nouvelle documentation concernant le rôle du Leucaena *dans l'alimentation des bestiaux s'est à ce point développée que beaucoup de chercheurs n'ont pas encore pris connaissance des différences dans la toxicité imputée à la mimosine et à son produit de dégradation 3-hydroxy-4 [1H] pyridone (DHP). Bien que la mimosine soit directement toxique, le DHP ne l'est qu'indirectement par ses propriétés goitrogènes. C'est pourquoi les animaux capables de décomposer la mimosine en DHP peuvent tolérer dans leur régime des quantités plus considérables de* Leucaena *que d'autres animaux, et ceux qui peuvent décomposer DHP plus encore, en toléreront des quantités encore supérieures et s'accommoderont même d'un régime entièrement à base de* Leucaena*. L'enzyme nécessaire pour convertir la mimosine en DHP se retrouve dans certaines des cellules (mais non dans toutes) de* Leucaena *qui contiennent la mimosine. La conversion s'effectue lorsque cet enzyme entre en contact avec le substrat, par exemple, par la macération. L'enzyme devient inactif devant un pH inférieur à 4, lorsqu'on le chauffe brusquement à plus de 70 °C, et à l'état sec. Ces constatations offrent d'immenses implications pour les études passées et à venir. Par exemple, les animaux paissant dans des pâturages de* Leucaena *commenceraient cette conversion de la mimosine en DHP en mâchant les feuilles. Le processus se poursuivrait jusqu'à son parachèvement presque intégral chez les ruminants, mais serait interrompu par les acides stomacaux chez les animaux monogastriques. La farine commerciale de feuilles séchées au soleil, ou rapidement à l'air, laisserait la mimosine à peu près intacte, mais l'enzyme serait dénaturé. Par contre, la farine de feuilles de* Leucaena *avariée pour être restée exposée au soleil dans des sacs en plastique, ou en tas dans des conditions humides, verrait une grosse partie de sa mimosine convertie en DHP. Ces conversions se produisant dans des circonstances bien particulières pourraient expliquer certains rapports contradictoires concernant la toxicité de* Leucaena *dans les régimes des ruminants et des monogastriques.*

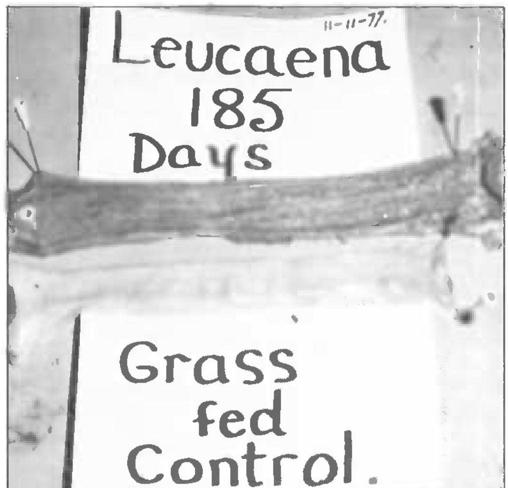
La nueva información sobre efectos de la leucaena en las dietas del ganado ha aparecido tan rápidamente que muchos investigadores aún desconocen las diferencias en toxicidad producidas por la mimosina y su producto de degradación 3-hydroxy-4(1H)pyridone (DHP). Aunque la mimosina es directamente tóxica, el DHP lo es solo indirectamente mediante su acción bociogénica. Así, los animales que pueden descomponer la mimosina en DHP pueden tolerar niveles más altos de leucaena en la dieta que otros animales, y los animales que pueden degradar aún más el DHP pueden tolerar también mayores niveles, quizás dietas solamente de leucaena. La enzima necesaria para convertir la mimosina en DHP se encuentra en algunas (no todas) de las células de leucaena que contienen la mimosina. La conversión ocurre cuando la enzima entra en contacto con el sustrato, por ejemplo, mediante maceración. La enzima se

toma inactiva a niveles de pH menores de 4, cuando se calienta repentinamente a temperaturas por encima de 70°C o cuando se seca. Estos hallazgos tienen serias implicaciones sobre la investigación pasada y futura. Por ejemplo, los animales que pastan leucaena iniciarían la conversión de mimosina en DHP mediante la masticación de las hojas. La conversión continuaría hasta ser virtualmente completa en rumiantes, pero sería detenida en los monogástricos por el ácido del estómago. La harina de hoja comercial que ha sido secada al sol en condiciones claras o secada rápidamente al aire tendría la mimosina prácticamente intacta, pero la enzima estaría desnaturalizada. En contraste, la harina de hoja de leucaena que se hubiere deteriorado, digamos en una bolsa plástica al sol o amontonada en condiciones de humedad, tendría mucha de la mimosina convertida en DHP. Las conversiones que se producen bajo diversas circunstancias específicas podrían explicar los numerosos informes contradictorios sobre la toxicidad de la leucaena que se da tanto a rumiantes como a no rumiantes.

The main constraint on the use of leucaena in animal feed is the toxic, nonprotein amino acid mimosine, which, under certain conditions, is readily converted to another toxic compound, 3-hydroxy-4(1H)pyridone (DHP). Mimosine and DHP have quite different effects, the knowledge of which is developing quite fast and being disseminated in a rather uneven way. For this reason, I think it useful to summarize research on the toxic properties of leucaena in terms of three periods: up to 1976, 1976–79, 1979 to date.

Developments to Date

In 1937, there were classic studies on the isolation and structural determination of mimosine and, later, on its cytotoxic and depilatory effects. After an early diversion



Jones elucidated the effects of leucaena on livestock, documenting, for example, ulceration of the esophagus.

concerning selenium, virtually all the adverse effects of leucaena were attributed to mimosine. The value of the crop in particular animal-production systems was recognized; however, large differences in response to leucaena diets emerged. Ruminants were observed to convert mimosine to DHP, which was regarded as essentially nontoxic. Nevertheless, toxicity was still a problem. This period is well covered in the National Academy of Sciences (NAS 1977) book on leucaena, and, because of the publication's wide distribution, most workers with leucaena are aware of the basic findings up to 1976.

The separate toxic effects of mimosine and DHP became apparent, between 1976 and 1979, and there were definitive studies on the goitrogenic effects of DHP. These suggested that, when leucaena is fed to ruminants at high levels, there is an intractable problem caused by the antithyroid effects of DHP. Breeding plants with low mimosine content and limiting the supply of leucaena in the animals' diets were seen as the only ways to avoid toxicity. Despite the rather daunting chronic toxicity revealed in some studies, the nutritive value of leucaena was recognized, and, with proper management, leucaena could contribute very usefully to increased animal production. The work on the goitrogenic activity of DHP is well published but scattered. The advances were gathered together by R.J. Jones (1979). Jones' article was timely and was directed to the right people. It is still making an impact. A surprising number of scientists interested in leucaena are not yet aware of the different toxic properties of mimosine and DHP.

From 1979 to the present has been a period of rapid and stimulating change. No sooner did the definitive picture seem to be emerging (defined largely by Jones and Hegarty) than the foundations were shaken once again. Having observed utilization of leucaena in different countries, Jones became convinced that there were qualitative differences — that the apparent absence of chronic effects in some areas was a reflection not of low-mimosine leucaena, low intake, or poor observation of animals but of different metabolism of the feed. In 1979, in Hawaii, he monitored goats in a village system and found that DHP was being rapidly broken down and that complete detoxification of leucaena apparently took place (Jones 1981). In 1980, the same phenomenon was observed at Ciawi in Indonesia. The general conclusion was that leucaena can be fed, without toxic effects, to animals that have the necessary microorganisms in their rumen to degrade DHP, although there may be other nutritional problems caused by leucaena's very low sodium content. These latest advances have not been covered by any major publication, and the proceedings of this workshop will clearly be important in this respect.

Ruminal Degradation of DHP

Empirical evidence obtained at Balai Penelitian Ternak (BPT), Ciawi, Indonesia, indicates that virtually complete breakdown of DHP can occur in Indonesian ruminants. The first attempt at studying mimosine–DHP metabolism was with four goats bred within the animal complex at Ciawi and raised on a standard diet that did not include leucaena. When they were given fresh leucaena as the sole feed, they exhibited DHP concentrations of 0.3–0.8% in urine for 4 weeks. Although there was fluctuation, there was no reason to believe that any large changes would occur, and the experiment was discontinued with the conclusion that the animals were *not* able to break down DHP.

My colleagues and I at BPT were on the watch for villages where leucaena was being fed to livestock at high levels without apparent chronic effects. We found one such case: goats were being fed a sole diet of leucaena, obtained from trees on a tea estate. Two of these goats were brought to BPT and were found to have urinary DHP concentrations less than 0.1%. Clearly, these animals *were* breaking down DHP.

About this time (June 1980), we commenced a major 6-month growth study in collaboration with the Agency for Development and Application of Technology. Ongole cattle were fed local Hawaiian-type leucaena at graded levels with a local grass (mostly *Isachne globosa*) (Wahyuni et al., in press). Although primarily a growth trial, the study included measurements of mimosine and DHP in feed and urine and monitoring of serum thyroxine. Unfortunately, full-balance studies were not done until later in the trial.

Urinary DHP was initially generally high among animals on a high-leucaena diet but became much lower after 4 months, with no simple transition being observed. What was clear was that, after 4 months, there was effective breakdown of DHP.

The mean daily data for six animals being fed 100% leucaena over a 7-day period were recorded. Intake of dry matter was 6.32 kg, mimosine 66.2 g, and DHP 12.9 g. Output of feces (dry matter) was 3.26 kg including 1.37 g mimosine and 1.36 g DHP. Urine output was 5.32 L, with mimosine content being 0.08 g and DHP 0.05 g.

As the amounts of mimosine and DHP in the urine were much less than those in the feces (which probably represents a level too low for effective absorption), the amounts circulating were very small indeed.

Thyroxine concentration did not vary significantly between treatments. There was no evidence of any toxic effects of leucaena, and there was ample evidence that DHP was broken down harmlessly.

Early in 1981, a series of growth experiments with sheep and goats were undertaken after Neville Yates arrived at BPT. Where input and output of mimosine–DHP were monitored, it was found that, virtually from the outset, about 90% of the DHP equivalent was broken down. There was no apparent difference between sheep and goats. The leucaena feed was so free from toxic effects that it became hard to justify further work on mimosine and DHP. However, we did demonstrate in vitro that a ruminal microbial process was responsible for the lowered DHP levels (B. Tangenjaja, unpublished thesis).

When animals that had not been in contact with leucaena for some generations were fed plants from new experimental plantings to which no animals had access, ruminal breakdown of DHP did not occur. It is uncertain how long this situation would have persisted if we hadn't introduced animals from a village where DHP breakdown did occur and then brought in many tonnes of leucaena from the surrounding district to supply feed for a large-scale experiment with cattle. Either of these events could have dispersed the microorganisms responsible for DHP degradation, and, from then on, adaptation occurred spontaneously.

We have, thus, inadvertently and in a lamentably uncontrolled way, arrived at some indications of how easily the ability to detoxify leucaena might be acquired by, and transferred between, animals when the necessary microorganisms are in the surrounding environment.

The major finding overall is that effective breakdown of DHP can occur in Indonesian ruminants and that there is unlikely to be chronic toxicity in any practical situation.

The disjunct distribution of this process around the tropics is, of course, of major interest and importance.

Autolysis of Mimosine to DHP

From the present perspective, it seems that the foregut fermentation of ruminant metabolism is uniquely suited to deal with the toxins of leucaena, either mimosine or DHP. Presumably, this applies also to other food plants that have water-soluble toxins, susceptible to reductive or microbial alteration. Monogastric animals and herbivores with hindgut fermentation absorb water-soluble toxins more readily than do the ruminants.

Because the effects of mimosine and DHP are distinct, one needs to know which of the two is causing toxicity in a given situation. This might seem obvious, but researchers have known for some time that heating, drying, and wilting of leucaena cause some conversion of mimosine to DHP but they haven't produced any literature on how rapid or complete this change can be.

Leucaena seedlings contain an enzyme that catalyzes the hydrolysis of mimosine to DHP, pyruvic acid, and ammonia (Smith and Fowden 1966). We have found the same enzyme in the mature plant, and it can be very active indeed (Lowry et al., in press). The enzyme is present in the leaflets but not in the petiole, rachis, and rachillae; in the lamina of the green pod but not in the raised marginal strands. It is not present in stem or mature seeds. All of these tissues, however, contain mimosine.

Enzyme and substrate occur together in the same cells. This has been shown by autolysis of mimosine under treatments that break cell membranes without grossly disrupting the tissue. For example, simply freezing and thawing the tissues prompts rapid conversion of mimosine at room temperature. Although frosting of leucaena is rare in the field, there have been feeding experiments in which fresh leucaena was stored frozen. Such material would contain very little mimosine when fed to the livestock.

Effect of Elevated Temperatures

When intact leucaena leaves are subjected to temperatures up to 45°C without drying, very little autolysis occurs while the cells are living. At temperatures higher than 70°C, the enzyme is denatured so that, for example, the mimosine in leaves plunged into boiling water would undergo almost no change. The interesting effects occur in the range 55–70°C, high enough to cause plasmolysis in the cell but not high enough to denature the enzyme. Under these conditions, autolysis is very rapid.

Thus, quite simple treatments, such as dipping leaves in water at 60°C for 3 min would change most of the mimosine in the leaflets (but not the stem tissue) to DHP. Fortunately, as the leaflets detach readily and are separated from the stems for production of leaf meal, the distribution of activity favours the production of low-mimosine leaf meal.

Effect of Tissue Disruption

The most obvious way to bring about autolysis without temperature manipulation is to put enzyme and substrate in contact by maceration of the tissue. Under optimum conditions (maceration at 40°C with an equal weight of buffer at pH 7–8), mimosine is largely hydrolyzed within 2 min; by macerating leaves and stems together, one can convert virtually all the mimosine — even that in cells without the enzyme — to DHP.

These findings indicate that any mechanical damage in harvesting and handling leucaena will affect the mimosine and DHP levels. Researchers should be aware of this if feeding animals leucaena as fresh chopped material. Likewise, in the processing of fresh leucaena — for example as protein concentrate — it is unlikely that there is much mimosine present unless enzyme activity has been prevented.

When fresh leucaena is ingested, conditions — maceration, temperature, addition of slightly alkaline saliva — favour rapid autolysis of mimosine. In fact, when we fed fresh, intact leucaena to a goat fitted with an esophageal fistula, we found that 30% conversion of mimosine occurred before the ingesta reached the rumen. There is nothing in the rumen (unlike acid in a monogastric stomach) to stop the autolysis. Thus, microbial activity is not necessary for the conversion of mimosine to DHP when fresh leucaena is used as feed. In other words, ingestion of mimosine from fresh leucaena is always accompanied by ingestion of DHP. It may be possible in nonruminants for the mimosine levels to be subacute and the DHP to exert chronic effects.

Inhibition

Along with sudden heating to temperatures higher than 70°C and contact with acid (the enzyme is virtually inactive below pH 4), drying prevents autolysis. When leucaena is vacuum dried or rapidly air dried, the mimosine is largely unaffected and there is almost no activity on rehydration. Sun drying under clear conditions, as is usually the case in commercial production of leaf meal, leaves the mimosine intact. However, if conditions are humid, temperature elevated, and drying delayed, autolysis can occur. A leucaena sample in a plastic bag in the sun, or a heap of fresh leucaena heating up by its own respiration, will undergo rapid change.

Relevance to Human Nutrition

Leucaena pods and young leaves are used as food in some countries, although problems with mimosine are known. Given the ease of autolysis to DHP and the prevalence of goitre, the possibility that DHP is acting as a previously unknown human goitrogen seems quite real.

Leaf-Meal Production

In some countries, leucaena leaf meal is produced commercially for inclusion in poultry and pig feeds but is usually added at low levels because of the toxic effects associated with mimosine. However, it should be relatively easy to prepare leaf meals with most of the mimosine converted to DHP. This would allow higher rates of inclusion without direct toxicity. It remains to be seen how this works in practice — whether, for instance, the autolysis adversely affects protein quality. The gross composition would, of course, be unaltered, unlike the case with a treatment such as water leaching.

Another way to avoid the adverse effects of mimosine in leaf meal is to use a mixture of species. Most antinutrients, like mimosine and DHP, are secondary compounds, found in one species but not in another. In most cases, there would be no interaction between them. Thus, in a mixture of species, one could have each secondary compound diluted to below the threshold for growth depression. The mixed leaf meal could be used at a higher level than any one of the species in it. In fact, we encountered one leaf-meal dealer who was already doing this in Indonesia, claiming to be blending *Calliandra calothyrsus*, *Sesbania grandiflora*, and *Acacia villosa* with leucaena.

Although we have not yet evaluated mixed-leaf meals, we recognize that their production would be appropriate to the biological resources of tropical countries where there are several species of fast-growing tree legumes available. Mixing species would be technologically simple, and the additional handling and storage costs would be offset by the larger market that would result. Quality control would be a problem, but leaf-meal composition can be monitored through the same secondary compounds that constitute a utilization problem. The flavonol glycosides have proved to be good indicators in this respect.