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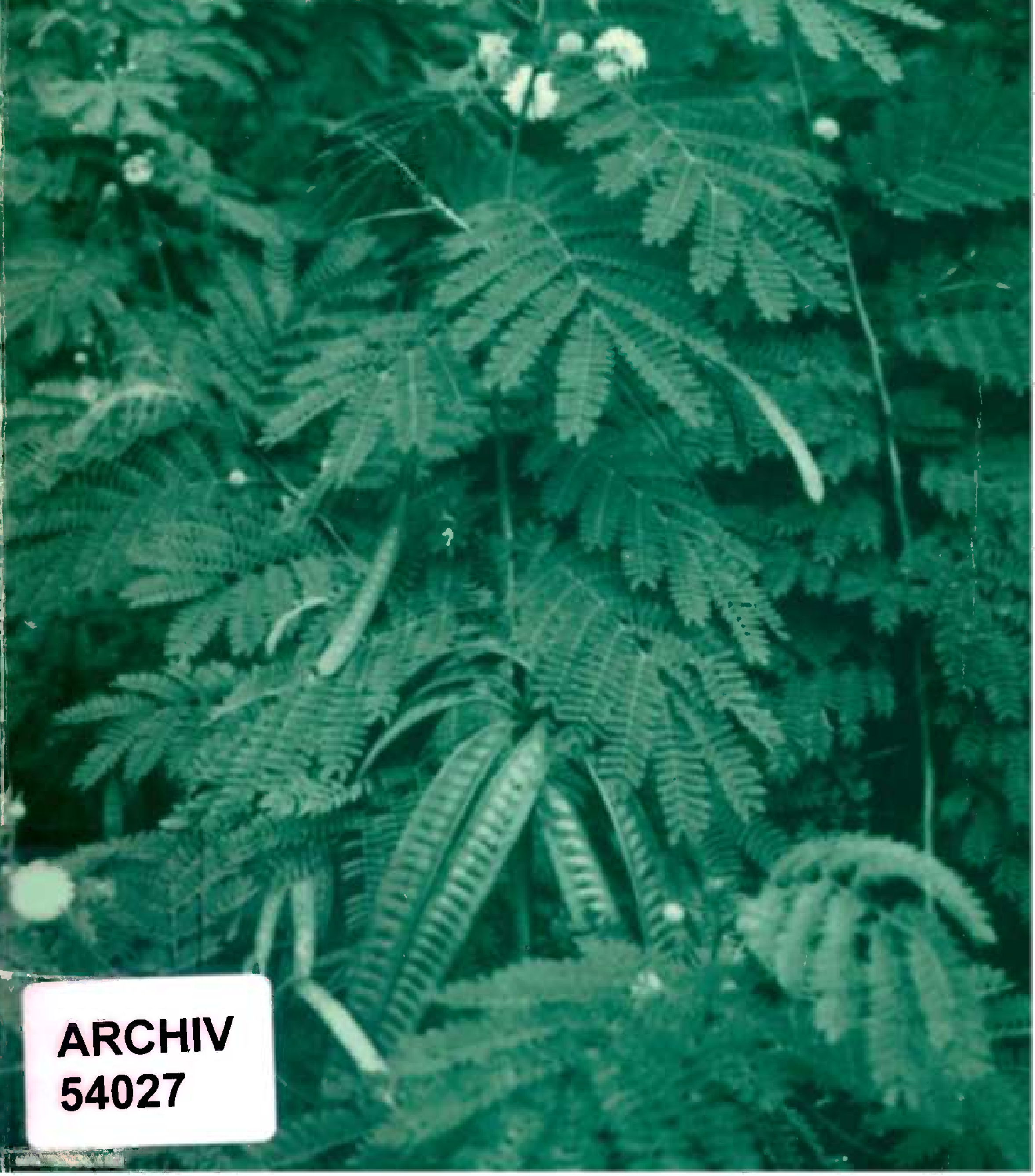
Leucaena Research in the Asian-Pacific Region

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



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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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Contents

Effects of Leucaena Leaf Meal in Feed for Japanese Quail

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Toxicity of leucaena forage is a subject of much research. The problem has almost been solved for the ruminants but not for poultry. Although mimosine is the main toxin, tannin has been proposed as partially responsible for the poor performance of poultry fed leucaena. This possibility was studied in 10-day-old Japanese quail fed leucaena containing different levels of mimosine and tannin for 4 weeks. Their progress, as measured by weight gain, feed-conversion efficiency, and digestibility coefficient, was significantly below that of chicks fed control diets; the diet with the highest level of mimosine produced the lowest weight gains, whereas the adverse effects from the leucaena with the highest tannin were similar to those for the other diets with comparable mimosine contents.

La toxicité de Leucaena *fait l'objet de nombreuses études. Le problème a été presque résolu en ce qui concerne les ruminants, mais non pour la volaille. Bien que la mimosine constitue la toxine principale, on a allégué que le tanin contribuait, pour sa part, aux piètres résultats obtenus de la volaille nourrie avec* Leucaena. *Cette possibilité a été étudiée sur des cailles japonaises âgées de 10 jours, et nourries durant 4 semaines avec du* Leucaena *contenant différentes teneurs de mimosine et de tanin. Leur développement, évalué d'après les gains de poids, la conversion alimentaire et le coefficient de digestibilité a été notoirement inférieur à celui des poussins recevant des régimes-témoins. La ration contenant la plus forte dose de mimosine a donné les gains pondéraux les plus faibles, et les effets défavorables de* Leucaena *ayant le plus fort contenu de tanin ont été identiques à ceux obtenus des autres régimes ayant une teneur en mimosine comparable.*

La toxicidad del forraje de leucaena es un tema de mucha investigación. El problema ha sido casi solucionado para los rumiantes pero no para las aves. Aunque la mimosina es la principal toxina, el tanino ha sido propuesto como responsable parcial del mal desempeño de las aves alimentadas con leucaena. Esta posibilidad fue estudiada en codornices japonesas de 10 días de edad alimentadas con leucaena a diversos niveles de mimosina y tanino por 4 semanas. Su progreso —medido por la ganancia de peso, la eficiencia de conversión alimenticia, y el coeficiente de digestibilidad— fue significativamente más bajo que el de pollos con dietas de control; la dieta con el mayor nivel de mimosina produjo menores ganancias de peso, mientras que los efectos adversos de la leucaena con mayor contenido de tanino fueron similares a los de otras dietas con contenidos comparables de mimosina.

Padgett and Ivey (1959) and Wilson et al. (1961) have indicated that the Japanese quail (*Coturnix japonica*) is suitable for pilot studies on poultry because of its short life cycle and low feed requirements. The physiological systems of Japanese quail are similar to those of poultry.

Studies on leucaena-supplemented rations have shown promise for the crop's use in poultry feed, with increases in the weight of chicks (Dingayan and Fronda 1950) and improved egg colour (Sadoval 1954). Palafox (1948) recommended feeding poultry only 2–3 g fresh leaves/day, from age 14 days onward. Diets containing more than 10–15% leucaena have been shown to depress the growth and maturity of birds. Several researchers have reported mimosine as the cause of the poor growth and have found that ferrous sulfate reduces toxicity (Ross and Springhall 1963).

In an experiment conducted in Hawaii, Japanese quail receiving 20% leucaena showed depressed growth, poor feeding efficiency, undersized sex organs, delay in sexual maturity, and high mortality. However, the histological studies did not indicate any adverse effects of leucaena on the liver, spleen, intestine, or thyroid. When the ration containing 20% leucaena was supplemented with methionine (0.1%) and corn oil (6%), the birds showed significant improvement. Mimosine was traced in the excreta after ingestion of leucaena, although a major portion was metabolized (Thanjan 1967). Ross et al. (1980) reported a drop in egg production from the 7th day after quails started consuming a diet with 20% leucaena, whereas a group receiving 1% pure mimosine performed normally till 14 days. This study indicated toxicity not only from mimosine but also from some other compounds present in leucaena.

D'Mello and Fraser (in press) reported the presence of 2.0–3.36% tannin in leucaena forage and suggested that tannin was responsible for toxicity. Tannins adversely affect the digestibility of the feed and utilization of the nutrients. A diet containing tannic acid depressed the growth of chicks, although the effects were reversed when chemicals, such as sodium carbonate and calcium hydroxide (at 0.022%), or nonionic polymers, such as polyvinyl pyrrolidone (PVP) or Tween 80, were added. These chemicals bind tannic acid and prevent it from precipitating proteins (Rayudu et al. 1970).

Recently, we conducted a feeding trial with Japanese quail at the University of Hawaii for 4 weeks, starting with 10-day-old birds, and using a randomized, complete block design. Six different dietary treatments (Table 1) were evaluated, with three replications, and each treatment unit had seven birds. The birds had free access to feed and water.

The quail of each unit were separated according to sex and collectively weighed at the end of each week when other measurements such as feed consumption, water intake, weight of the fecal matter were also recorded.

At the end of the trial, a few birds receiving the positive-control and the K8 tender-leaf-meal diets were killed, and the thyroid, liver, kidney, and sexual organs examined.

Mimosine in the feed was analyzed by the colorimetric method (Meggarity 1978), which records the total for mimosine and DHP content. Tannin analysis of the leaf meals was carried out by the Vanillin–HCl method and expressed in percent catechin equivalent.

Results

At the end of the feeding trial, both female and male quail on the K8 tender-leaf meal

Table 1. Composition of six rations fed to Japanese quail for 4 weeks. [a]

	Diet					
	Positive control	Negative control (alm)	K8 tlm	K8 mlm	K156 lm	K8 mlm + PVP
Ingredients (%)						
Choline	0.160	0.160	0.160	0.160	0.160	0.158
$CaCO_3$	3.000	3.000	3.000	3.000	3.000	2.970
Def. phosphorus	3.500	3.500	3.500	3.500	3.500	3.465
Methionine	0.100	0.100	0.100	0.100	0.100	0.099
Meat, bone meal	5.000	5.000	5.000	5.000	5.000	4.950
Tuna meal	5.000	5.000	5.000	5.000	5.000	4.950
Salt	0.300	0.300	0.300	0.300	0.300	0.297
Vitamin mix	0.038	0.038	0.038	0.038	0.038	0.038
Corn	39.5	31.1	32.8	32.2	31.9	31.878
Soy meal	37.4	33.8	32.1	32.7	33.0	32.373
Tallow	0.69	3.0	2.76	2.84	2.97	2.812
Cellulose	2.31	—	0.24	0.16	0.03	0.158
Alfalfa meal	3.00	15.00	—	—	—	—
Leucaena meal	—	—	15.00	15.00	15.00	14.850
Polyvinyl pyrrolidone (PVP)	—	—	—	—	—	1.00
Nutritive value						
Crude protein (%)	28.83	29.27	29.63	29.26	29.37	29.41
Ether extract (%)	3.78	6.30	5.90	6.78	6.56	6.55
Ash (%)	12.30	13.08	12.20	12.83	13.01	12.70
Dry matter (%)	89.06	89.30	89.43	89.93	89.74	89.80
Total energy[b] (cal/kg)	2600.00	2600.00	2600.00	2600.00	2600.00	2574.00
Mimosine (%)	—	—	1.10	0.44	0.30	0.43
Tannin (% catechin equivalent)	0.0189	0.0945	0.225	0.645	0.1725	0.639

[a] tlm = tender-leaf meal; mlm = mature-leaf meal; lm = leaf meal; and alm = alfalfa leaf meal.
[b] Total energy was computed, based on the feeding values.

Table 2. Growth of 10-day-old Japanese quail fed leucaena for 4 weeks. [a]

	Weight gain (g/bird)		Average feed-conversion efficiency (g/g weight gain)	Average digestibility coefficient of feed	Average water consumption (mL/bird)
	Female	Male			
Positive control	106.7a	86.5a	3.54a	0.6320a	738
Negative control	101.3ab	97.7a	3.63a	0.5609c	819
K8 tlm	60.3c	66.6c	3.93b	0.5720bc	659
K8 mlm	81.6b	77.3b	4.04b	0.5704bc	735
K156 lm	83.2b	87.2b	4.30c	0.5735bc	860
K8 mlm + PVP	83.5b	77.7b	4.41c	0.5970b	904

[a] In vertical columns, figures followed by the same letters do not differ significantly ($P<0.05$).

Table 3. Nitrogen and mimosine contents in fecal matter of Japanese quail maintained on six different diets.

	Nitrogen (%)		Mimosine (%)	
	Week 2	Week 4	Week 2	Week 4
Positive control	4.70	6.73	—	—
Negative control	3.89	5.80	—	—
K8 tlm	5.47	6.52	1.37	1.43
K8 mlm	4.49	6.74	0.77	0.67
K156 lm	5.77	7.32	0.45	0.35
K8 mlm + PVP	5.51	6.16	0.77	0.60

showed extremely poor weight gain, which was highly significant (Table 2). Among the other treatments, birds maintained on both the positive control and negative control diets had significantly higher weight gains than all the other groups among which there was no significant difference. This trend had been set within 1 week of the initiation of the experiment.

Female birds were heavier than the males in all except two of the treatments. Birds receiving the K8 tender-leaf meal were very small, with thin, loose skin. None of the groups lost their feathers.

The average feed-conversion efficiency was high for both the positive and negative control diets, intermediate for both the K8 tender- and mature-leaf diets, and low for the K156 and K8–PVP diets, the differences between the three groups being significant. Feed-conversion efficiency was high in the 1st week and progressively declined as the birds gained weight.

There was a small wastage of feed for all four groups receiving leucaena leaf meal, which was noticeable in the form of green dust in the collection trays. Such wastage was high in the K156 units. In the K8–PVP treatment units, the birds were often found with a thick coating of feed hardened on their beaks. In some cases, they needed help to shed these feed coats.

The positive control had the highest digestibility coefficient, which was significantly ($P<0.05$) higher than all the others. The negative control diet had the lowest, although it did not differ significantly from the leucaena leaf-meal diets. The quail on the K8–PVP diet consumed the most water, but the difference was not significant. Nitrogen contents were highest in the fecal samples of the K156 group and lowest in the negative control (Table 3).

The fecal samples of the K8 tender-leaf-meal group had the highest mimosine content (Table 3). Electrophoresis indicated that almost 75% of the total content was in the form of mimosine, with the rest being DHP. When the thyroid, liver, kidney, and sexual organs of the control and leucaena-fed birds were examined, a general correlation was observed between size of organs and body weight, although there was no visible difference in the size of the thyroids. Several yolks, ranging from 2 mm to 10 mm, were found in the females of the control treatments, whereas no yolks were visible in the birds fed K8 tender-leaf meal.

Discussion

The nutritive value of all the treatments in terms of protein, energy, fat, and ash was almost equal. The variation among these feeds was in their mimosine and tannin contents. K8 tender-leaf meal, which was responsible for poorest weight gain, had the highest mimosine content (1.10%) and low tannin content (0.225%). The other leucaena treatments had mimosine concentrations ranging from 0.30% to 0.44%, whereas they varied widely in their tannin content. Therefore, the poor performance in the group consuming leucaena leaf meal could be attributed to the toxicity of mimosine (Springhall and Ross 1965a; Thanjan 1967; Labadan 1969; D'Mello and Thomas 1978).

The mimosine content of the fecal samples was proportional to the mimosine present in the feed. As a major portion of the mimosine was excreted in the form of mimosine (not DHP), the thyroids were not affected. Mimosine suppressed the growth of the quails and, as a result, may delay maturity. The specific role of mimosine in suppressing growth is not known.

Between the K8 mature-leaf-meal diet and the K156 diet, there was no statistical difference in growth rate, digestibility coefficient, or N contents of the fecal samples, although the tannin contents were 0.6450% and 0.1725% respectively. Tannin affects the digestibility of the feed by precipitating proteins. One might expect, therefore, that high tannin contents would cause large amounts of nitrogen to be excreted in the feces and be accompanied by protein deficiencies in the birds. However, in this trial, there was no evidence to confirm the suppression of growth by tannin, as the N contents in the fecal samples of the high-tannin diet group (K8) were lower than those for the low-tannin group (K156).

Feed-conversion efficiency in this trial was probably affected by the wastage of feed. It may have been advisable to use small pellets of leaf meal instead of 20-mesh powder.

Use of PVP as suggested by Rayudu et al. (1970) was effective in binding the external source of tannin, but it is doubtful about its role internally, before the tannin–protein interaction. The presence of PVP caused the formation of a hard coat of feed around the beaks of the birds, which in turn might have affected the feed efficiency.