

**CHARACTERISTICS AND PROTEIN BINDING AFFINITY OF CONDENSED
TANNINS IN *LEUCAENA* SPECIES**

By

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**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
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DEDICATION

To my parents, Khun Poh Chy and Khun Mae Paa Khamseekhiew

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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Four experiments were conducted to evaluate the role and metabolism of phenolic compounds particularly condensed tannins (CT) in selected *Leucaena* species [*Leucaena*-hybrid Bahru; *LLB*, *Leucaena*-hybrid Rendang; *LLR*, *Leucaena leucocephala*-local; *LLL* and *Leucaena leucocephala*-Subang; *LLS*] using *in vitro* and *in vivo* studies. The occurrence of phenolic compounds in the above *Leucaena* and their correlations with *in vitro* gas production and degradability were investigated in the first experiment. The results showed that hybrid *LLR* had the highest CT content, resulting in the lowest *in vitro* gas production and degradability among the four species tested. Within shoot of similar age, the *in vitro* gas production and N degradability were negatively correlated to CT content, but such relationship were not detected if shoot samples of different age groups were pooled. The above findings indicated that concentration of tannins may not be the sole factor affecting nutrient utilisation.

Results of the second experiment showed that there were no significant differences in the total phenolics and extractable CT contents, gas production and DM degradability

for the samples dried at temperature of 45° C as compared to the standard freeze drying procedure. The present results indicated that where facilities for freeze drying are not available, drying at 45° C would serve as an option for measurement of the content of tannins as well as the effects on the *in vitro*. Drying at 60 °C and the addition of polyethylene glycol (PEG) reduced the adverse effect of CT. When treated at 60° C, the *in vitro* gas production and N degradability increased by about 24.7 and 26.2 %, respectively. With the addition of PEG, the corresponding values were 42.6 and 22.6%, respectively. Once again, DM degradability was highest in the local LLL (63.3%), followed by the hybrids LLB (58.9%) and LLR (55.1%), suggesting the depression of DM degradability of the hybrids as the results of their higher tannin contents.

In the third experiment, CT were isolated and purified from three *Leucaena* species to evaluate differences in their biological binding affinity (astringency) and molecular weight. The study showed that the phenolics precipitation protein (PPP) value for LLR and LLB were significantly higher than that of LLL. Similarly, LLR and LLB also exhibited higher binding affinity than LLL, with the values of 0.13, 0.72 and 1.32 mg CT mg⁻¹ bovine serum albumin (BSA), respectively. The above findings provide further explanation for the stronger adverse effect of CT of the hybrid species as compared to the local variety. Although majority of CT purified from the hybrid species was made of larger molecules than those of the local *Leucaena*, thus once again support the hypothesis that CT of stronger binding affinity are of larger molecular weight. The conclusion based on the current results is not conclusive and need further studies.

The last Chapter consisted of two in vivo experiments. The results showed that inclusion of PEG in diets improved DM and N digestibilities, N retention, rumen ammonia (NH₃) as well as microbial N supply in sheep, with no significant differences on animal performance when the two types of *Leucaena* species (hybrid and local) were compared.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**WATAK DAN AFINITI PROTEIN GABUNGAN TANNIN KENTAL DALAM
SPESIS *LEUCAENA***

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Empat eksperimen telah dijalankan untuk menilai peranan dan metabolisme sebatian finolik terutamanya tanin kental (CT) dalam spesis *Leucaena* yang terpilih [*Leucaena-hybrid* Bahru; *LLB*, *Leucaena-hybrid* Rendang; *LLR*, *Leucaena leucocephala*-local; *LLL* dan *L. Leucocephala*-Subang; *LLS*] dengan menggunakan kajian *in vitro* dan *in vivo*. Kemunculan sebatian finolik dalam *Leucaena* dan kolerasi mereka dengan produksi gas *in vitro* dan degradasi dikaji diselidik dalam eksperimen pertama. Keputusan menunjukkan hibrid *LLR* mempunyai kandungan CT paling tinggi yang menyebabkan, produksi gas *in vitro* dan degradasi N adalah yang paling rendah di antara empat tumbuhan yang diuji. Paada tunas yang sama umur, produksi gas *in vitro* dan degradasi N adalah berkolerasi negatif kepada kandungan CT, tetapi hubungan tersebut tidak ditemui jika sampel tunas yang berlainan umur dikumpulkan. Keputusan di atas menunjukkan bahawa kepekatan tannin berkemungkinan bukan faktor tunggal yang mempengaruhi penggunaan nutrien.

Eksperimen kedua menunjukkan bahawa tiada perbezaan bererti dalam jumlah finolik, kandungan CT yang boleh diekstrak, produksi gas dan degradasi sampel yang dikeringkan pada 45°C berbanding dengan prosedur pengeringan beku biasa. Keputusan kini menunjukkan bahawa apabila tiada kemudahan untuk pengeringan beku, pengeringan pada 45°C akan menjadi satu pilihan untuk mengukur kandungan tannin dan pengaruhnya terhadap *in vitro*. Pengeringan pada suhu 60°C dan penambahan polietilin glikol (PEG) merendahkan kesan berlawanan CT. Apabila dirawat pada 60°C, produksi gas *in vitro* dan degradasi N masing-masing meningkat kira-kira 24.7 dan 22.6%. Dengan penambahan PEG, nilai-nilai bersamaan adalah 42.6 dan 22.6% masing-masing. Sekali lagi, degradasi DM adalah tertinggi untuk sampel LLL tempatan (63.3%), diikuti dengan LLB (58.9%) dan LLR (55.1%), mencadangkan penurunan penghadaman DM hibrid adalah akibat daripada kandungan tannin yang lebih tinggi.

Dalam eksperimen ketiga, CT diasingkan dan dibersihkan dari tiga spesis *Leucaena* untuk menilai perbezaan dalam daya gabungan biologikal (astringensi) dan berat molekul. Kajian menunjukkan bahawa nilai pengendapan protein finolik (PPP) LLR dan LLB adalah lebih tinggi secara bererti daripada LLL. Bersamaan ini, CT yang dibersihkan untuk LLR dan LLB juga mempamerkan afiniti gabungan yang lebih tinggi daripada LLL, dengan nilai 0.13, 0.72 dan 1.32 mg CT mg⁻¹ albumin serum sapi (BSA), masing-masing. Keputusan di atas membekalkan penerangan lanjut bagi kesan berlawanan CT daripada spesis hibrid berbanding dengan variasi tempatan. Walaupun kebanyakan CT yang dibersihkan daripada spesis hibrid terbentuk daripada molekul yang lebih besar berbanding *Leucaena* tempatan, hipotesis bahawa CT yang

mempunyai daya gabungan yang lebih kuat mempunyai berat molekul yang lebih tinggi. Kesimpulan berdasarkan keputusan terkini adalah tidak muktamad dan memerlukan kajian yang lebih lanjut.

Bab yang terakhir terdiri daripada dua eksperimen *in vivo*. Keputusan menunjukkan bahawa penambahan PEG dalam diet memanfaatkan penghadaman DM dan N, penyimpanan N, ammonia rumen (NH₃) dan bekalan mikrobial N dalam biri-biri, dengan tiada perbezaan bererti pada persembahan haiwan apabila dua jenis spesis *Leucaena* (hibrid dan tempatan) dibandingkan. Spesis LLL tempatan adalah yang paling diminati oleh biri-biri.

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I certify that an Examination Committee has met on 16th March 2006 to conduct the final examination of Bodee Khamseekhiew on his Doctor of Philosophy thesis entitled “Characteristics and Protein Binding Affinity of Condensed Tannins in *Leucaena* Species” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

BODEE KHAMSEEKHEW

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