Prevalence of DHP toxicity and detection of *S. jonesii* in ruminants consuming *Leucaena leucocephala* in eastern Indonesia

Michael J Halliday [A], Tanda Panjaitain [B], Jacob Nulik [C], Dahlanudin [D], Jagadish Padmanabha [E], Chris S. McSweeney [E], Sulaiman Depamede [D], Debora Kana Hau [C], Kurniawan [B], Muhammad Fauzan [B], Sutartha [B], Baiq Tutik Yuliana [B], Charles Pakereng [C], Putri Ara [C], Dessy Liubana [C], Resti G Edison [C] and H Max Shelton [A]

[A] School of Agriculture and Food Sciences, The University of Queensland, St Lucia 4072, Australia
[B] Assessment Institute for Agricultural Technology: NTB, Mataram, Lombok, NTB 83371, Indonesia
[C] Assessment Institute for Agricultural Technology: NTT, Kupang, Timor, NTT 85362, Indonesia
[D] Faculty of Animal Science, The University of Mataram, Mataram, Lombok, NTB 83125, Indonesia
[E] CSIRO Animal, Food & Health Sciences, Queensland Biosciences Precinct, St Lucia 4067, Australia
Contact email: m.halliday@uq.edu.au

Keywords: *Synergistes jonesii*, dihydroxypyridine, mimosine, urine, rumen fluid, PCR.

Introduction

Leucaena leucocephala (leucaena) is a productive forage tree legume widely used in eastern Indonesia. While highly nutritious, it possesses the toxin mimosine which adversely affects animal production. In ruminants, mimosine is readily converted to the two isomers of dihydroxypyridine (3,4-DHP and 2,3-DHP) known to cause goitre, suppress appetite, and cause severe mineral deficiencies. These adverse symptoms may be partially responsible for the reluctance of some farmers to feed leucaena.

A bacterium capable of complete degradation of DHP, *Synergistes jonesii*, originally discovered in Hawaii in goats consuming leucaena (Jones and Megarrity 1986), was later found in Indonesia which led to the assumption that all Indonesian ruminants were protected from leucaena toxicity even on 100% leucaena diets.

The objective of this study, conducted during October-November 2011, was to confirm this hypothesis via an extensive survey of the toxicity status of ruminants consuming leucaena in eastern Indonesia.

Methods

Cattle, goats and buffalos from 5 villages in each of the islands of Lombok, Sumbawa, Sumba, and Timor were selected from existing leucaena-based fattening systems (Table 1). Urine samples were collected from up to 10 animals within each village; discussion with farmers revealed that leucaena ranged between 30-100% of diet. Urine samples were preserved and analysed for DHP by HPLC using the method described in Graham *et al.* (2013). Rumen fluid was collected from 3 animals in each village for PCR detection of *S. jonesii* at CSIRO Animal, Food & Health Sciences lab in Brisbane, Australia using a modified method described in Graham *et al.* (2013). No rumen samples were able to be collected in Sumbawa.

Results and Discussion

On Lombok island, where leucaena is often fed to goats, villages less than 40 km apart (in some cases <1 km) differed completely in their toxicity status; goats in the village of Bayan had a mean DHP excretion approaching 1000 mg/L, whereas goats sampled in the village of Pemenang had no urinary DHP excretion on the same diet of leucaena. PCR analysis of rumen fluid collected confirmed the presence of *S. jonesii* in Pemenang, but not in Bayan.

On the isolated island of Sumba, farmers have often reported an aversion of cattle towards the consumption of leucaena (Jacob Nulik, personal communication). However, goats and buffalos were located consuming high leucaena diets without adverse effects. Ten buffalos consuming up to 100% leucaena from 3 districts were sampled; all were excreting low levels (<150 mg/L) of DHP. *Synergistes jonesii*, identical to the type strain (strain 78-1, ATCC 49833), was detected in 5 of 7 samples of rumen fluid.

The 5 villages sampled in Sumbawa showed high variability both between and within villages. In the village of Rhee Jatisari, which had a highly productive leucaena-based cattle-fattening system, farmers regularly bought in new bulls naïve to leucaena. Data showed a high variability between old and new (<5-6 weeks on leucaena) animals, which showed low and high urinary DHP respectively. This was expected as it can take up to 5 weeks to acquire *S. jonesii* from nearby stock (Jones *et al.* 1985).

Ruminants consuming leucaena on the island of Timor also showed high variability in DHP excretion; villages in Amarasi (Jones 1983) had DHP excretions ranging between 0 to >1000 mg/L DHP. Regular stock movement from cattle trading and purchases is likely to be the cause of the variability, and like Sumbawa, both areas are productive leucaena-based cattle fattening regions.

Table 1. Village urinary total DHP (3,4-DHP + 2,3-DHP) excretion range and detection of *S. jonesii.*

Island	Village	Animal (#)	Mean urinary DHP (mg/L) (± SE)	Urinary DHP range (mg/L)	*S. jonesii* detected
Lombok	Bayan	goats (10) & cattle (9)	420 (±142)	0-2000	N
	Pemenang	goats (8)	5 (±1)	0-10	Y
	Pringgabaya	goats (9)	481 (±143)	0-1200	Y
	Sekotong	goats (9) & cattle (9)	276 (±124)	0-2000	Y
	Rambitan	goats (7)	105 (±67)	0-400	Y
Sumbawa	Poto Tano	cattle (7)	811 (±383)	0-2000	N/A
	Baturea	cattle (6)	765 (±352)	0-2000	N/A
	Rhee Jatisari	cattle (9)	421 (±125)	0-1000	N/A
	Penyenger	cattle (9)	818 (±359)	0-2800	N/A
	Labangka I	cattle (8)	816 (±247)	0-1500	N/A
Sumba	Kamalaputi	goats (6)	4 (±2)	0-10	Y
	Kambaniru	goats (5)	22 (±15)	0-100	Y
	Melolo	buffalo (4)	82 (±21)	0-150	Y
	Wanga	buffalo (1)	25 (-)	0-25	Y
	Kakaha	cattle (2) & buffalo (2)	45 (±32)	0-150	Y
Timor	Ponain, Amarasi	cattle (6)	216 (±138)	0-700	Y
	Tesbatan II, Amarasi	cattle (5)	227 (±117)	0-500	Y
	Oelbeba	cattle (4)	532 (±149)	100-900	N
	Lili	cattle (5)	27 (±9)	0-60	Y
	Sumlili	goats (4) & cattle (4)	830 (±276)	0-2000	Y

Conclusions

In summary, though PCR results indicate that *S. jonesii* is widespread throughout sampled villages in Lombok, Sumba, and Timor where leucaena diets have been employed for long periods. Contrary to the initial hypothesis, capacity to degrade DHP does not appear throughout the entire region, and is often isolated within certain farms or certain villages; significant numbers of animals were found to be excreting high levels of urinary DHP even though *S. jonesii* had been detected. Animals not fully protected will be underperforming and subject to adverse health concerns from undiagnosed DHP toxicity. As such, it is important to educate local extension officers about methods to detect and manage DHP toxicity, to overcome a possible barrier to the adoption of leucaena.

Acknowledgements

This study was funded by ACIAR. A sincere thanks is extended to the many farmers and helpers in Indonesia, without whom this would not be possible.

References

Graham SR, Dalzell SA, Nguyen TN, Davis CK, Greenway D, McSweeney CS, Shelton HM (2013) Efficacy, persistence and presence of *Synergistes jonesii* inoculum in cattle grazing leucaena in Queensland: On-farm observations pre- and post-inoculation. *Animal Production Science* **53**, (in press).

Jones PH (1983) Lamtoro and The Amarasi Model from Timor. *Bulletin of Indonesian Economic Studies* **19**, 106-112.

Jones RJ, Lowry JB, Megarrity RG (1985) Transfer of DHP-degrading bacteria from adapted to unadapted ruminants. *Leucaena Research Reports* **6**, 5-6.

Jones RJ, Megarrity RG (1986) Successful transfer of DHP-degrading bacteria from Hawaiian goats to Australian ruminants to overcome the toxicity of Leucaena. *Australian Veterinary Journal* **63**, 259-262.