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

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Introduction

Leucaena leucocephala (leucaena) is a productive forage tree legume widely used in eastern Indonesia. While highly nutritious, it contains the toxin mimosine, which adversely affects animal production. In ruminants, mimosine is readily converted to the 2 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 test 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 and 100% of the diet. Urine samples were preserved and analyzed 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 laboratory in Brisbane, Australia, using a modified method described in Graham et al. (2013). No rumen samples were collected in Sumbawa.

Results and Discussion

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

On the isolated island of Sumba, farmers have often reported that cattle are reluctant to eat leucaena (Jacob Nulik, unpublished data). 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.

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Table 1. Urinary total DHP (3,4-DHP + 2,3-DHP) excretion ranges and detection of *S. jonesii* in ruminants in villages in eastern Indonesia.

Island	Village	Animal (#)	Mean urinary DHP (mg/L) (±s.e.)	Urinary DHP range (mg/L)	*S. jonesii* detected
Lombok	Bayan	goats (10) & cattle (9)	420 (±142)	0−2,000	N
	Pemenang	goats (8)	5 (±1)	0−10	Y
	Pringgabaya	goats (9)	481 (±143)	0−1,200	Y
	Sekotong	goats (9) & cattle (9)	276 (±124)	0-2,000	Y
	Rambitan	goats (7)	105 (±67)	0−400	Y
Sumbawa	Poto Tano	cattle (7)	811 (±383)	0−2,000	N/A
	Baturea	cattle (6)	765 (±352)	0−2,000	N/A
	Rhee Jatisari	cattle (9)	421 (±125)	0−1,000	N/A
	Penyenger	cattle (9)	818 (±359)	0−2,800	N/A
	Labangka I	cattle (8)	816 (±247)	0−1,500	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 (-)	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)	0−2,000	Y

Animals in 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; DHP excretion rates in villages in Amarasi (Jones 1983) ranged between 0 and >1,000 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.

Conclusions

In summary, PCR results indicate that *S. jonesii* is widespread throughout ruminants sampled in 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, suggesting animals were experiencing subclinical DHP toxicity. *Synergistes jonesii* was detected in many of these animals; however, different strains of the bacterium may have different DHP-degrading capacity. Animals not fully protected will be underperforming and subject to adverse health concerns from untreated DHP toxicity. As such, it is important to educate local extension officers about methods to detect and manage DHP toxicity, including improved inoculation protocols, to overcome a possible barrier to the further adoption of leucaena.

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