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DEGRADATION OF MIMOSINE AND 3-HYDROXY-4(IH)-PYRIDONE (DHP) BY INDONESIAN GOATS

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When leucaena was fed to a goat the concentration of DHP in the urine was 0.7 g/100 ml on the first day of feeding but declined to 0.2 g/100 ml one day later and to 0.03 g/100 ml after 20 days. Mimosine concentration in the urine was about 0.03 g/100 ml throughout the 3 weeks. After feeding on leucaena for 6 weeks only 5–15% of total mimosine ingested was recovered in the urine and faeces. Rumen microorganisms degraded DHP rapidly in vitro; the rate was faster with microbes from the goat fed leucaena than from the goat not fed leucaena. Defaunation of the rumen content with Teric GN–9 did not reduce the ability of microorganisms to degrade DHP.

Key words: Goat, leucaena, mimosine, DHP

Leucaena Leucocephala (Lam) de Wit is a tropical forage legume with characteristics, productivity and composition that make it desirable for use in animal feeds. Its negative attribute is the presence of the toxic amino acid mimosine. The clinical signs of toxicity range from loss of hair in pigs, rabbits, rats and donkeys to depressed serum thyroxine (t4) levels, enlarged thyroid gland, goitrous offspring and even death in ruminants.

The diverse results from feeding leucaena were apparently explained by the elucidation of the quite different toxic properties of mimosine and its degradation product 3-hydroxy-4(IH)-pyridone (DHP) and the intraruminal conversion of mimosine to DHP. Mimosine has acute cytotoxic effects, while DHP has low general toxicity but is a chronic goitrogen (Hegarty et al 1976). It thus appeared that although acute mimosine toxicity would be rare in ruminants there was still a problem with DHP toxicity; but because of the low general toxicity and rapid urinary excretion of DHP the problem could be managed by controlling the intake of mimosine.

In a study with goats in Hawaii, Jones (1981) showed that extensive degradation on DHP occurred in vivo and that leucaena could constitute the sole diet with no apparent ill effect. We now report on the metabolism of mimosine and DHP by goats fed leucaena in Indonesia.

Materials and Methods

Feeding and sampling of goats: One Indonesian Ettawah goat (20 kg body weight) with a fistulated rumen was fed a diet of freshly chopped *Leucaena leucocephala* cv Cunningham for 6 weeks. It was then housed in a metabolic cage and offered 3 kg fresh leucaena daily for 3 weeks with free access to

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water. Feed intake, urine and faeces output were measured daily and sub-samples of leucaena, urine and faeces were collected and analysed for mimosine and DHP by high-pressure liquid chromatography using Hewlett Packard model 1084B connected to LC terminal model 79850B, Boblingen, West Germany. The column was Zypax SCX 50 x 0.4 cm (Dupont Co., Wilmington). The eluting solvent was a mixture of 940 ml citric acid 0.1 M and 65 ml Na_2HPO_4 0.2 M at pH 2.4 and the detector was UV absorbance at 280 mm.

Another fistulated goat (23 kg body weight) was fed leucaena for 6 months then housed in a metabolic cage and fed for 2 months a non-leucaena diet consisting of 50% elephant grass, 40% concentrate and 10% fish meal at the rate of 400 g DM/d. After an overnight fast, rumen liquor (500 ml) was withdrawn for incubation studies. The goat was then fed freshly chopped leucaena (3 kg/day) with urine samples collected daily for 3 weeks, and analysed for mimosine and DHP. After 3 weeks of leucaena feeding, rumen liquor (500 ml) was again withdrawn after an overnight fast. Leucaena feeding was continued and after a further 5 weeks the rumen was defaunated by infusing in the rumen 17.25 g nonyl phenol ethoxylate (Teric GN-9, ICI Australia) in 140 ml of water (Bird et al 1979) on each of three consecutive days. The animal continued to receive leucaena (3kg/d) and after 5 days rumen liquor (500 ml) was again collected. Microscopic examination showed that the rumen liquor was free of protozoa.

Incubation of DHP with rumen contents: Rumen liquor was fractionated into microorganisms and supernatant fractions according to the method of Tangendjaja et al (1983). The microbial fractions were suspended in a phosphate buffer and adjusted to the original concentration. 1 ml DHP solution (0.9 g/100 ml) and *Calliandra* sp leaf meal (250 mg) (added as a source of nutrients) were placed in a digestion tube with the prepared fractions (25 ml) and incubated according to the first stage of the Tilley and Terry (1963) digestion for 0-48 hr. Incubation was stopped by the addition of HCl to give a final concentration of 0.1 N and the mixtures were then analysed for DHP. The amount of DHP added gave a concentration of 33 mg/100 ml rumen fluid which was calculated to be of a similar concentration to DHP present in the rumen of animals fed leucaena (5.5 g DHP/d) (based on a rumen concent of 3.8 litres and rumen flow of 11.9 litres/d; Weston and Hogan 1967).

Results and Discussion

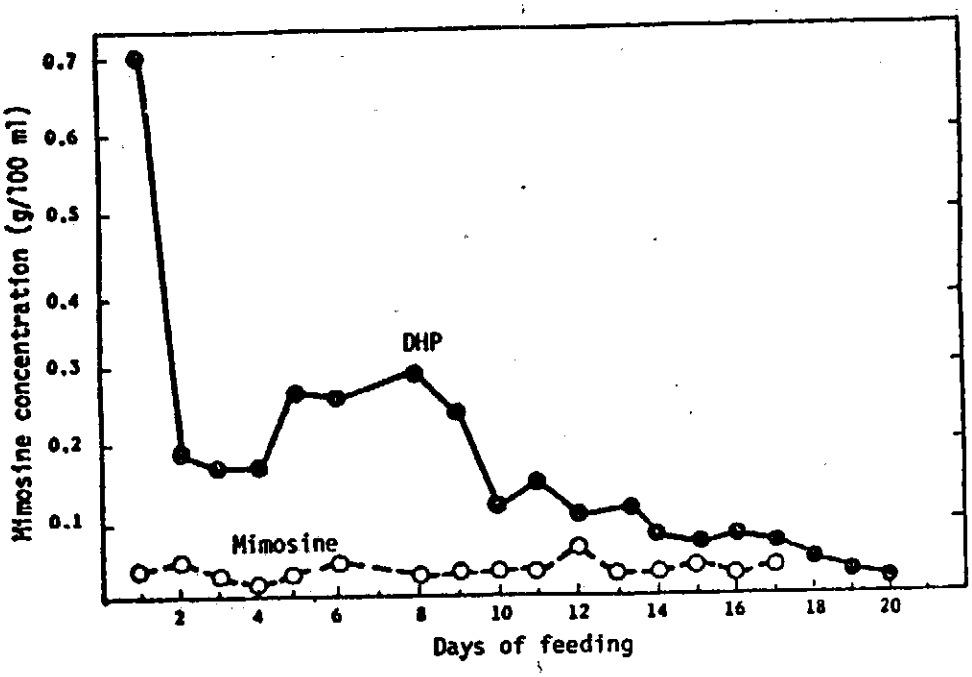
Urinary and faecan mimosine and DHP: The levels of mimosine and DHP in the urine after leucaena feeding recommenced are presented in Figure 1. Introduction of leucaena resulted in a high concentration of urinary DHP on the first day (0.7 g/100 ml) but the concentration fell to 0.2 g/100 ml on the following day and decreased to 0.03 g/100 ml after 20 days. Mimosine concentration in the urine was always lower than that of DHP and was present at 0.03 g/100 ml throughout the three weeks.

Measurements on the second goat after 6-9 weeks of leucaena feeding showed a similarly low concentration of mimosine and DHP in the urine (Table 1). Comparison with data on faeces and feed, also given in Table 1, show that DHP concentration is generally higher than mimosine concentration both in faeces and urine, although mimosine concentration in the feed is higher than that of DHP, indicating major conversion of mimosine to DHP. The presence of some DHP in the feed shows that some mimosine had already broken

down during feed preparation (Lowry et al 1983). The total daily mimosine and DHP input/output, calculated as mimosine equivalents, shows an average input of 6.2 g daily (range 3-11 g) and output of 0.5 g (range 0.2-1.0 g). Thus 90% (85-95%) of mimosine ingested was not accounted for in the urine and faeces. The total mimosine equivalent recovered in the faeces was 4.4% of mimosine intake and 5.5% in the urine.

Figuere 1:

DHP and mimosine concentration in the urine of an indonesian goat during 20 days of feeding with 100% fresh leucaena



The level of urinary DHP, after adaptation to the introduction of leucaena, of 0.02 g/100 ml is lower than the level of DHP found in the urine of the leucaena-intolerant Australiam goats (0.3 g/100 ml) (Jones and Megarrity 1981).

Incubation of DHP by rumen fluid: Figure 2 shows the residual DHP after incubation, in both the microbial and supernatant fractions of rumen liquor obtained from a goat fed leucaena or the non-leucaena diet. DHP levels were markedly reduced when it was incubated in the microorganism fractions obtained either from a leucaena- or non-leucaena-fed goat but the rate of loss of DHP was faster in the liquor from the former. The supernatant fraction of the rumen liquor did not reduce DHP levels irrespective of the basal diet of the animal.

Table 1:

Mimosine and DHP levels in urine, faeces and feed of an Indonesian goat fed leucaena

	Amount present[1]	
	Mimosine	DHP
Urine (g/100 ml)	0.015 (0.002-0.035)	0.027 (0.008-0.107)
Faeces (g/100 g wet weight)	0.024 (0.018-0.031)	0.045 (0.023-0.065)
Feed (g/100 g dry weight)	1.221 (0.887-1.841)[2]	0.284 (0.172-0.644)

[1]Values are average and range over 3 weeks. Measurement was carried out daily 6-9 weeks after commencement of feeding.

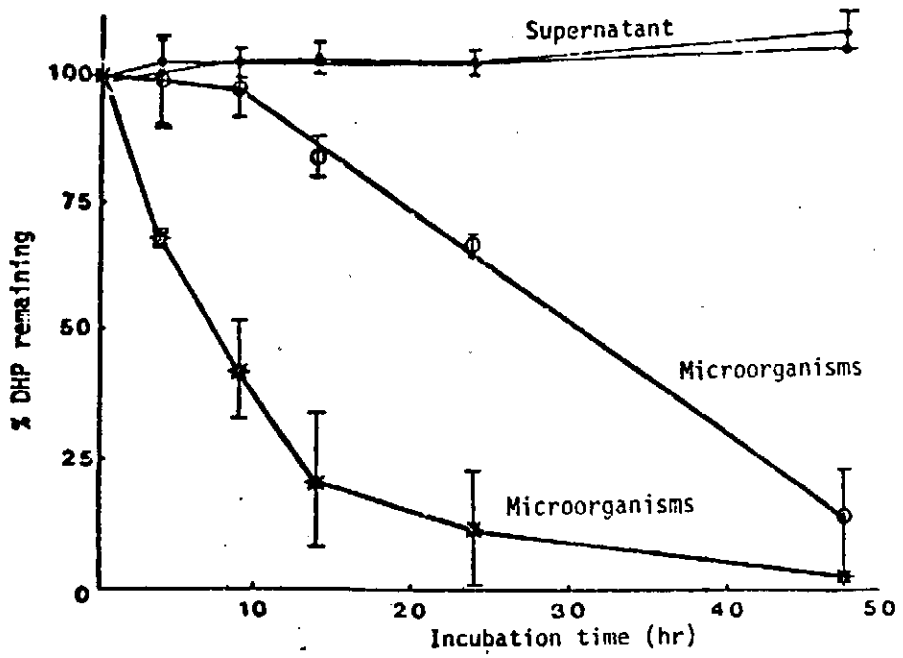
[2]Dry matter of feed was 35.5 g/100g.

These results indicate that DHP was degraded by enzyme (s) contained on, or in, microorganisms rather than by enzyme(s) excreted by microorganisms by sheep rumen microorganisms (Tangendjaja et al 1983).

The increase in the rate of DHP degradation after adaptation to leucaena presumably is due to increasing numbers of microorganisms which are able to degrade DHP.

Figure 2:

DHP levels during in vitro incubation with the microorganism and supernatant fractions obtained from an Indonesian goat fed a non-leucaena diet (0——0) for about 2 months followed by a leucaena diet (——*) for 3 weeks. Vertical line is SD from 3 replicates*



Incubation of the microorganism fractions of defaunated rumen liquor with DHP resulted in a very rapid loss of DHP with 87% DHP lost in 9 hr and total loss in 14 hr incubation. These values are higher than those obtained from the faunated microorganism fractions (57% and 80% losses respectively). The supernatant fraction did not degrade the DHP in agreement with previous findings. The increase in DHP degrading activity in the rumen liquor of the goat after defaunation and subsequent feeding of lecaena for five days is presumably due to the increased numbers of bacteria growing in the absence of protozoa. All rumen ciliates engulf bacteria to some extent (Coleman and Samford 1979) and the killing of protozoa by Teric GN-9 would favour growth of a greater bacterial population.

Elimination of DHP toxicity is thus a ruminal microbial process and due to bacteria rather than protozoa. From these results it would appear that Indonesian goats are able to tolerate high levels of leucaena in their diet without suffering toxicity.

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