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Australian goats detoxify the goitrogen 3-hydroxy-4(1H) pyridone (DHP) after rumen infusion from an Indonesian goat

R.J. Jones and J.B. Lowry

CSIRO Division of Tropical Crops and Pastures, Davies Laboratory, P.M.B., P.O., Atikenvale, Townsville, Queensland (Australia, 4814) and Research Institute for Animal Production, P.O. Box 123, Bogor, W. Java (Indonesia), 4 August 1983

Summary. The failure of Australian goats fed *Leucaena leucocephala* (leucaena) to degrade 3-hydroxy-4(1H) pyridone (DHP), the goitrogenic metabolite of mimosine, was overcome when they were infused with rumen fluid from an Indonesian goat. The leucaena toxicity problem in Australia may well be solved by transfer of specific bacteria capable of degrading DHP anaerobically.

Key words. Leucaena toxicity; goat; 3-hydroxy-4(1H) pyridone; goitrogenic agent; rumen fluid; mimosine.

Leucaena leucocephala (leucaena) produces high yields of protein rich forage in a range of tropical and sub-tropical environments, and is regarded as a promising tropical forage species[2]. However, toxicity associated with hypothyroidism has often been reported in animals fed a leucaena diet[3]. The goitrogen is 3-hydroxy-4(1H) pyridone (DHP)[4], the product of autolytic[5] or microbial[6] breakdown of the toxic amino acid mimosine which occurs in the plant. This toxicity, which prevents intensive use of leucaena as a forage in Australia[3], New Guinea[7] and probably Africa[8,9] has not been reported from much of Asia or tropical America. Absence of chronic toxicity for local ruminants in Hawaii[10] and Indonesia[11] is probably due to extensive ruminal degradation of DHP because less than 5% of the DHP from ingested leucaena is detected in the urine. The toxicity of leucaena to ruminants in northern Australia has been attributed to the absence of rumen microorganisms which will degrade DHP[10]. This hypothesis could not be tested in either Australia or Hawaii because of quarantine restrictions. Consequently, a rather unusual approach of conducting the experiment in a different country with imported animals and feed was used.

Materials and methods. 4 wether goats (Angora × Saanen) were individually fed on leucaena at the CSIRO Lansdown Research Station, Woodstock, via Townsville, Queensland, Australia, for a period of 5 weeks. The feed was gradually changed from fresh to dried leucaena leaf which was to be their sole diet for a subsequent study in Indonesia. All goats were excreting large quantities of the goitrogen DHP in their urine – a characteristic of ruminants eating leucaena in Australia[3]. Goats and feed were flown from Australia to Indonesia. The goats were housed in individual metabolism cages at Balai Penelitian Ternak (Research Institute for Animal Production), Ciawi, near Bogor, West Java, where they were offered 400 g day^{-1} of Australian grown leucaena leaf containing 2% mimosine. They were allocated at random to receive one of 2 treatments – a control and a rumen infusion treatment. Control goats were housed in a separate building from the goats receiving the infusion treatment.

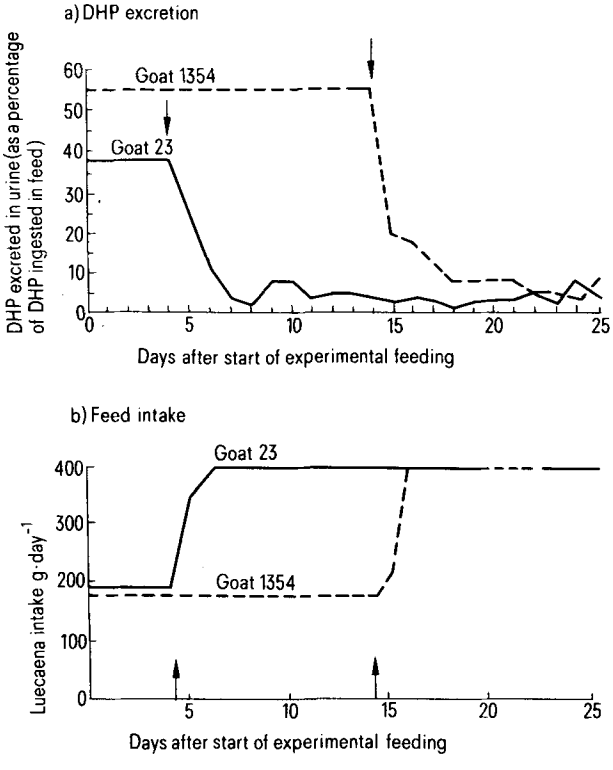
On day 4 of experimental feeding in Indonesia, the 2 treated goats (No. 23 and No. 1353) were infused with approximately 350 ml of rumen fluid from an Indonesian goat fed fresh leucaena and known, by the low ($< 0.04\%$) DHP concentration in its urine, to be degrading DHP. On day 14, 1 of the control goats (No. 1354) received via stomach tube strained rumen fluid (150 ml) from the treated Australian goat No. 23.

Feed intake and urine and feces output were measured daily. Small aliquots (20 ml) of the daily urine output were taken for analysis and also a small sub-sample (10 g) of the feces. The remainder of the urine and feces were separately bulked over a 10-day period following commencement of treatments for each animal. Mimosine and DHP in feed, feces and urine samples were measured (after hydrolysis in HCl in a boiling water bath for 1 h) by high performance liquid chromatography[11]. Degradation of DHP (1 mg in 9 ml rumen fluid medium) was measured in anaerobic in vitro studies[13,14] using fresh, frozen and autoclaved strained rumen fluid from an adapted goat.

Results. Before treatments were imposed, both pairs of goats had identical mean urinary DHP concentrations of 0.28%. Mean feed intake was somewhat higher for the treated group – 350 vs 290 g·day^{-1}.

Following infusion, there was a dramatic reduction in the excretion of DHP in the urine of both treated goats. Mean concentration of DHP in the bulked urine samples was only 0.08% compared with 0.35% for the urine of control goats. Daily excretion of urinary DHP (expressed as a percentage of the DHP ingested as mimosine) is plotted in the figure for goat 23 and goat 1354 which received an infusion from goat 23. Values declined to low levels within 5 days after infusion and remained low. At day 25 all treated goats were excreting less than 5% of their daily intake of DHP. Concentration of mimosine and DHP in the feces of all goats was low and accounted for only a small proportion of the total mimosine ingested. Results are not reported here.

Daily feed intake increased sharply to the maximum offered in 2 days after infusion and remained at this level for the dura-



Effect of infusing Australian goats with rumen fluid from goats adapted to degrade DHP. *a* Urinary DHP excretion as a percentage of the DHP equivalent of mimosine ingested. *b* Leucaena intake, g dry leaf per day. Maximum offered daily was 400 g. Values prior to infusion are the mean daily output of DHP and the mean daily feed intakes respectively. Arrows ↑↓ indicate date of infusion from a goat able to degrade DHP.

tion of the experiment (fig.). Whereas before infusion goats were lethargic and ate their feed over most of the 24 h, after infusion they were alert and consumed all their feed in less than 5 h. The change in disposition and the increase in feed intake following infusion was similar to the responses in leucaena-fed steers which were changed to a diet of cowpea hay[15]. The in vitro study showed that fresh or frozen rumen fluid resulted in total degradation of DHP in 48 h whereas no degradation occurred if the rumen fluid was autoclaved.

Discussion. The dramatic increases in feed intake following infusion, together with the equally dramatic decline in DHP excretion which was maintained for 21 days until the experiment terminated, strongly implies a permanent change in the rumen microbial population of the infused goats – at least while eating leucaena. The fact that this ability could also be successfully transferred from a treated Australian goat to one of the control goats only 10 days after receiving an infusion from an Indonesian goat, is further evidence that transfer of DHP degrading ability had occurred. The increased feed intake appears to be associated with a reduction in circulating DHP, since feed intake of steers also increases dramatically when leucaena is replaced by cowpea hay in the diet[15].

The in vitro study clearly shows that the degradation of DHP is caused by microorganisms, most probably bacteria, since removing the protozoa failed to prevent degradation of DHP. It is generally assumed that rumen microorganisms are ubiquitous and that the feeding regimen and the host physiology are the major determinants of the composition of the rumen microbial populations[13]. The implication from our recent studies[10,11] that microorganisms essential for the successful metabolism of a particular toxin were not available over large areas, including all of tropical Australia, has met with considerable resistance where these results have been discussed. Differences in DHP metabolism between countries have been ascribed instead to differences in animal species, genotype, or management, to environment or leucaena composition. These possibilities are eliminated in the present experiment by transporting both animals and feed from Australia.

The results obtained in this study are believed to be the first demonstration of a persistent nutritional benefit from microbial introduction into the rumen of animals on the same feed as that of the donor animal. The situation can be compared to the absence of effective *Rhizobium* populations for nodulation of some introduced tropical pasture legumes. Introduction of *Rhizobium* into Australia from overseas has been necessary before effective symbiosis could occur[16]. In the light of these results with leucaena, the possibility should be explored that other introduced pasture plants that are toxic to ruminants in a particular country may not be toxic in their country of origin.

For intensive use of leucaena as a ruminant feed in Australia and possibly elsewhere, it appears to be necessary to introduce the specific rumen microorganisms to degrade DHP. Importing ruminants from areas known to be free of leucaena toxicity is a simple practical solution to the problem. However, in countries such as Australia, where quarantine regulations prohibit the direct importation of ruminants, the solution will be more difficult. It may necessitate the isolation and culture of the organisms in vitro as a prerequisite to their importation and release.

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Experimental alveolar echinococcosis. Suitability of a murine model of intrahepatic infection by *Echinococcus multilocularis* for immunological studies[1]

M. Liance, D.A. Vuitton, S. Guerret-Stocker, J.P. Carbillet, J.A. Grimaud and R. Houin[2]

Laboratoire de Parasitologie, Faculté de Médecine, F-94010 Créteil (France); Services d'Hépatologie et d'Anatomie pathologique, C.H.U., F-25030 Besançon (France); Laboratoire de Pathologie Cellulaire du Foie, E.R.A. 819 CNRS, Institut Pasteur, F-69007 Lyon (France), 23 December 1983

Summary. An experimental model of human alveolar echinococcosis was developed, using intrahepatic injection of *E. multilocularis* larvae in mice differing by their sensitivity to this parasite; it seems to be suitable for studying the relationship between cell-mediated immunity and a) growth of the parasite, b) development of fibrosis.
Key words. Alveolar echinococcosis; *Echinococcus multilocularis;* cell-mediated immunity; inbred mice; experimental model.

Alveolar echinococcosis (AE) is a rare and fatal parasitic disease related to the proliferation in the liver of the larval form of the cestode *Echinococcus multilocularis (E.m.)*. Rodents are natural intermediate hosts for this larval development, and foxes, dogs and cats can serve as hosts for the adult form[3].

Human AE is endemic in USSR, Japan, Canada and Alaska and, in Europe, it is particularly encountered in the eastern