Animal performance and rumen parameters were measured in four sheep fed two levels of the toxin-containing legumes *Leucaena leucocephala* (Lam.) de Wit (sun dried stems and leaves) and *Canavalia ensiformis* (L.) DC. seeds in Venezuela.

Method. Four individually penned West African sheep were fed during the leucaena trial a control diet containing 500 g/kg alfalfa; diet 1 containing 250g/kg alfalfa and 250g/kg leucaena and diet 2 containing 500 g/kg leucaena. During the canavalia trial, the control diet contained 400g/kg soya maize (1:1); diet 1 contained 200g/kg canavalia and 200g/kg soya/maize (1:1) and diet 2 contained 400g/kg canavalia. Sheep were fed 500g (DM) diet twice a day. Two weeks of consumption of each diet were allowed before each 5 week experimental period started.

Discussion. *L. leucocephala* did not have any apparent toxic effects on animals such as reduced intake, hair loss, excessive salivation, or weakness, in spite of the presence of the toxic compounds 3,4 DHP and 2,3 DHP in the rumen. There was a small but significant decrease in the body weight (mean weight loss 1.6 kg) as the proportion of leucaena increased in the diets. Dietary supplementation of leucaena did not significantly affect the rumen pH, concentrations of VFAs, microbial counts, rate of rice straw degradation, or the rumen outflow of liquids or solids. However, the concentration of ammonia in the rumen increased and there was an increase in the number of Gram negative rods isolated from the rumen of sheep when leucaena was added to the diet.

When diets were supplemented with *C. ensiformis* the presence of canavanine in the rumen was confirmed. Animals did not show signs of toxicity and there was an increase in the proportions of Gram negative bacteria isolated from the rumen of sheep as the dietary inclusion of canavalia increased.

*In vitro* experiments showed that mimosine had a marked effect on the counts of viable bacteria from the rumen of Venezuelan sheep only when added at high (22.5 umol/ml) concentrations. 2,3 DHP, a bacterial metabolite from mimosine, had a slight inhibitory effect on the bacteria from the rumen of sheep in Aberdeen. This effect was less noticeable in the inoculum from the rumen of sheep previously inoculated with rumen contents from Venezuelan animals. However, 2,3 DHP and the mimosine analogue DOPA enhanced the growth of cultures enriched in 2,3 DHP. These enrichments were inhibited by the DHP analogues 3 OH-pyridine and catechol. An enrichment culture of predominantly Gram negative bacteria was capable of 2,3 DHP degradation.

Several pure cultures (the majority Gram negative) capable of mimosine, 2,3 DHP, and (in fewer cases) 3,4 DHP were isolated. One of them, a Gram variable spore-forming rod, presumably a new species of *Clostridium*, was characterized.

An unidentified aerobic fungus was found to degrade 3,4 DHP. It was isolated from a standard solution of 3,4 DHP left at ambient temperature (18-22°C) for over a week.

The HPLC technique proved useful to determine degradation of mimosine, DHP, and analogue compounds that absorb at UV wavelengths of 274nm.

The inclusion of 3,4 DHP or mimosine in roll tubes containing ferric chloride resulted in an
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efficient method to detect degraders through the formation of black colonies, presumably due to accumulation of ferric sulphide.

The source of 3,4 DHP used was the (acid) hydrolysate of mimosine. The presence of 3,4 DHP was confirmed by HPLC and mass spectrometry. Alanine was identified by mass spectrometry in the mixture of products.

*In vitro* experiments showed that the inclusion of canavanine sulphate in roll tubes did not affect the counts of total viable bacteria from Venezuelan sheep, whether or not the sheep was consuming *Canavalla*. However, there was a better growth on broths containing L-canavanine from sheep consuming *C. ensiformis*. These differences disappeared after two transfers in broths containing L-canavanine. Enrichment cultures of predominantly Gram positive bacteria appeared more sensitive to L-canavanine than Gram negative ones. However, the effect on pure cultures was independent of the Gram staining. When grown on M10 medium, L-canavanine caused inhibition in four out of six tested strains, and ammonia concentrations were depressed in all the cultures. These cultures showed poor degradation of canavanine but degraded arginine extensively. When grown on CH medium (casein hydrolysate) L-canavanine had little effect on growth (only 2 out of the six strains tested were sensitive), ammonia concentrations were not reduced, and canavanine was better degraded but arginine was less degraded than in M10 medium. These results suggest an effect of L-canavanine in steps previous to deamination, i.e peptide hydrolysis.

Finally, the results indicate that presence of bacteria capable of mimosine, 2,3 and 3,4 DHP degrading activity presumably account for the good performance of sheep consuming *L. leucocephala*. The study involving L-canavanine indicate that its effect on rumen bacteria may be acting on peptidolytic steps, which could account for the increased concentration of free and peptide amino acids in the rumen, and for the reduction of rumen ammonia concentrations, which were reproduced *in vitro* on a medium (M10) containing amino acids in free and peptide form.

Further studies are required to understand the mechanisms of microbial toxicity to plant non protein amino acids, and the genetic nature of the degradative capacity. Information in this field would provide new possibilities for the use of tropical legumes in animal feeds.