Urinary Excretion of Mimosine Metabolites by Hair Sheep Fed Foliage of *Leucaena leucocephala*

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The 22nd International Grassland Congress (Revitalising Grasslands to Sustain Our Communities) took place in Sydney, Australia from September 15 through September 19, 2013.

Proceedings Editors: David L. Michalk, Geoffrey D. Millar, Warwick B. Badgery, and Kim M. Broadfoot

Publisher: New South Wales Department of Primary Industry, Kite St., Orange New South Wales, Australia

Presenter Information

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Keywords: Rumen liquor, inoculation, urine testing, intake, subclinical toxicity.

Introduction

Leucaena leucocephala is an adapted legume widely distributed in the tropical regions of Mexico. The high crude protein content of leucaena leaves renders it appropriate for ruminant feeding under commercial conditions. However, the foliage contains the non-protein amino acid mimosine, which, if consumed in high amounts, may induce toxicity in animals which have not previously consumed the legume or without microorganisms capable of degrading mimosine and its derivatives 2,3-DHP (dihydroxypyridine) and 3,4-DHP (Hammond 1995, Palmer *et al*. 2010, Dalzell *et al*. 2012). Barros-Rodríguez *et al*. (2012) found that dry matter intake and weight gain were reduced when sheep grazed paddocks with 55,000 plants of leucaena per hectare. Early work in Australia led to the isolation of *Synergistes jonesii*, an anaerobic bacterium able to degrade 3,4-DHP and 2,3-DHP to non-toxic compounds (Allison *et al*. 1992). In Mexico, the presence of this microorganism in the rumen has not yet been confirmed. Inoculation of non-accustomed animals with rumen liquor of ruminants adapted to the consumption of leucaena can reduce the impact of mimosine and its metabolites on animal health (Ghosh *et al*. 2009; Palmer *et al*. 2010). The aim of the present work was to evaluate the effects of transferring rumen liquor of cows adapted to the consumption of *L. leucocephala* to sheep without experience of consumption, on urinary excretion of 3.4-DHP and 2.3-DHP by means of a colorimetric technique.

Materials and methods

The experiment was carried out at the Faculty of Veterinary Medicine and Animal Science, University of Yucatan, Mexico. Two crossbred cows adapted to the consumption of *L. leucocephala* were used as donors of rumen liquor. Twelve Pelibuey lambs of 20 kg live weight housed in metabolic crates were allocated to 3 groups and fed a ration of *Brachiaria brizantha* hay (49%), wheat bran (19%), soybean meal (25%), cane molasses (5%) and minerals (2%) (control); or an experimental ration of *L. leucocephala* meal (50%), *B. brizantha* hay (23%), cracked corn (20%), cane molasses (5%) and minerals (2%). Treatments were: T1: control ration, without inoculation with rumen liquor from donor cows; T2: experimental ration, with inoculation of 250 ml of rumen liquor by a nasoesophageal tube for 3 consecutive days; T3: experimental ration, without inoculation with rumen liquor. All urine excreted was collected and measured every 24 hours. The urine was processed by means of a kit consisting of Milex MCA Millipore filters in acetate cellulose of 0.45 µm Minisart ® and a column Maxi-Clean™ SPE 300 mg C18 c/100 p 25. Changes in urine colour were interpreted in the following way: 1: no apparent change in colour (absence of 3,4-DHP); 2: change in apparent urine colour to blue (2,3-DHP present); and 3: change in urine colour with greater intensity to purple/brown (3,4-DHP present), according to the procedures described by Hammond (1995), Rincón *et al.* (2000) and Palmer *et al.* (2010). Results were analysed as a completely randomised design with 3 treatments and 4 replicates (lambs) with the GLM procedure of SAS. When differences were observed in treatment means, these were compared using the Tukey test.

Results and Discussion

Dry matter (DM) intake showed significant differences ($P<0.05$) between treatments (Table 1). Adding leucaena to the base ration increased intake after 5 days when rumen inoculum was supplied, but not until day 20 in the absence of inoculum ($P<0.05$). It must be kept in mind that percent incorporation of *L. leucocephala* in the ration was 50% on a DM basis, well above the 30% recommended by Hammond (1995), to avoid toxicity in ruminants. Despite these intake increases, colour analyses of urine indicated that mimosine derivatives such as 3,4-DHP and 2,3-DHP were present in the urine of the lambs fed leucaena (Table 2). Excretion of mimosine metabolites 3,4-DHP and 2,3-DHP in the urine of Pelibuey sheep in groups receiving leucaena during the first 10 days of the experiment was relatively high, indicating an absence in the rumen of microorganisms capable of degrading mimosine. Subsequently the intensity of the colour change reduced as a result of inoculation of lambs with rumen liquor from donor cows adapted to the consumption of leucaena. The change in the colour of urine to blue suggests a partial degradation of the metabolite 3,4-DHP to a less toxic compound such as 2,3-DHP. The increase in intensity of

Table 1. Voluntary intake (g DM) of Pelibuey lambs fed *L. leucocephala*, inoculated with rumen liquor from cows adapted to the consumption of leucaena. Means within rows with a different superscript differ (P<0.05).

Days	Without leucaena	With leucaena	
	T1 without inoculation	T2 with inoculation	T3 without inoculation
1	750 a	786 a	708 b
2	716 a	721 a	490 a
3	760 a	789 a	542 a
5	792 b	910 a	811ab
10	864 a	1062 a	907 a
15	799 b	1039 a	900 b
20	866 b	1053 a	1017 a

Table 2. Colorimetric analysis of the urine of Pelibuey lambs fed *L. leucocephala*, inoculated with rumen liquor of cows adapted to the consumption of leucaena. Means within rows with a different superscript differ (P<0.05).

Days	Without leucaena	With leucaena	
	T1 without inoculation	T2 with inoculation	T3 without inoculation
1	1.0 b	2.7 a	2.8 a
2	1.0 b	2.6 a	2.9 a
3	1.0 b	2.6 a	2.9 a
5	1.0 b	2.6 a	2.9 a
10	1.0 c	2.0 b	3.0 a
15	1.0 c	2.0 b	3.0 a
20	1.0 c	2.0 b	3.0 a

the change in colour (to purple) in lambs, which were not inoculated with rumen liquor from donor cows, suggests the presence of mimosine metabolites (3,4-DHP and 2,3-DHP) in the urine. We do not know at this stage if *Synergistes jonesii* carried out the isomerisation reaction of 3,4-DHP to 2,3-DHP (Dalzell *et al.* 2006) or if other bacteria in the rumen ecosystem possessed such capacity (Palmer *et al*. 2006). Evaluation of the colour of urine by this method is a cheap and feasible means of assessing first hand the possible occurrence of sub-clinical toxicity in ruminants fed high levels of foliage of *L. leucocephala*. Inoculation with rumen liquor from ruminants previously adapted to the consumption of this legume seems to be an option to protect ruminants from mimosine toxicity (Graham 2007; Ghosh *et al*. 2009; Palmer *et al*. 2010).

Conclusions

The use of *L. leucocephala* in the feeding of ruminants can improve animal performance. Transfer of rumen liquor from animals adapted to the consumption of leucaena to non-accustomed animals may be an option to reduce the possible toxic effects of mimosine in rations, when the percent incorporation of leucaena is high (*i.e.* > 30% of ration DM). The presence of microorganisms capable of degrading mimosine to non-toxic metabolites may decrease the risk of toxicity. Change of colour in the urine with the technique described here, may be a quick way to assess the degree of protection against toxicity, where the change of colour suggests the possible occurrence of sub-clinical toxicity in ruminants fed foliage of *L. leucocephala*. It is of interest that urine colour in animals fed leucaena without infusion with rumen liquor after 20 days indicated the presence of 3,4-DHP, yet DM intake equalled that of the infused animals.

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