Urinary excretion of mimosine derivatives by cows with and without experience in consumption of *Leucaena leucocephala*

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Introduction

Leucaena leucocephala is a leguminous tree widely distributed in the tropical regions of the world. In Mexico, it has been incorporated into silvopastoral systems and is highly regarded, owing to its high content of crude protein. Nonetheless, *L. leucocephala* contains secondary metabolites, such as mimosine, a non-protein free amino acid, which may induce toxic effects in unadapted ruminants that consume the forage (Hammond 1995). Although *Synergistes jonesii*, an anaerobic bacterium, has the ability to degrade 3,4-DHP and 2,3-DHP to non-toxic compounds (Allison *et al*. 1992), in Mexico its presence has not yet been confirmed. Recent work has suggested the occurrence of sub-clinical toxicity to 3,4-DHP and 2,3-DHP in cattle grazing *L. leucocephala* in Australia and Thailand (Graham 2007; Dalzell *et al*. 2012; Phaikaew *et al*. 2012). Several options such as the transfer of rumen liquor and the adaptation of ruminants to the intake of *L. leucocephala* have been studied in an attempt to reduce the excretion of mimosine and its metabolites (Palmer *et al*. 2010).

The aim of the present work was to evaluate the effect of the experience of consumption of *L. leucocephala* on excretion of mimosine derivatives (3,4-DHP and 2,3-DHP) in the urine of cattle.

Materials and methods

The experiment was carried out at the Faculty of Veterinary Medicine and Animal Science, University of Yucatan, Mexico. Two groups of 4 female cattle were used. The first group had been consuming *L. leucocephala* and the second group had no previous experience. Average live weights of cattle were 413 and 251 kg, respectively. During the experimental phase, which lasted for 42 days, all animals were fed different proportions of *L. leucocephala*: *Pennisetum purpureum* and a concentrated supplement. Proportions of the feed provided are given in Table 1. Voluntary feed intake was measured daily.

Urine sampling was carried out at 12, 24, 36, 48, 60 and 72 h after offering the experimental ration. Urine was processed by means of kits consisting of Milex MCA Millipore in cellulose acetate of 0.45 µm Minisart ® and a column of Maxi-Clean™ SPE 300 mg C18 c/100 p 25. Changes in colour of urine were interpreted as: 1: without apparent change in colour (absence of 3,4-DHP and 2,3-DHP); 2: apparent change in colour to blue (2,3-DHP present); and 3: higher intensity in change of colour (purple/brown) (3,4-DHP present); as described by Hammond (1995), Rincón *et al.* (2000) and Palmer *et al.* (2010). Data on total DM intake (g/kgLW$^{0.75}$), and intake of *L. leucocephala* (g/kgLW$^{0.75}$) were analysed based on a 2 x 4 factorial arrangement of treatments with 2 factors (with and without experience) and 4 levels of incorporation of *L. leucocephala* (0%, 15%, 30% and 45% of ration DM). After this, a multiple comparison test (Tukey) was carried out to identify statistical differences between treatments.

Results and Discussion

Total DM intake between both groups of cattle was different ($P<0.05$), being higher in cows without experience of consumption of the legume, while DM intake of leucaena was higher ($P<0.05$) in the group with experience in consumption of leucaena (Table 2). Levels of consumption of leucaena in the rations did not show significant differences ($P>0.05$) from the rations offered,

Table 1. Percent incorporation of *L. leucocephala* in the ration of cows in relation to total dry matter (DM) intake.

Animals	Experienced in consuming *L. leucocephala*		No experience with *L. leucocephala*		
	L. leucocephala	*P. purpureum*	*L. leucocephala*	*P. purpureum*	Concentrate
1	0	100	0	70	30
2	15	85	15	55	30
3	30	70	30	40	30
4	45	55	45	25	30

Table 2. Intake of total DM and *L. leucocephala* (g/kgLW$^{0.75}$) of cattle with different levels of inclusion.

Level of Incorporation (%)	With experience	Without experience	Mean
Total DM intake (%)			
0	71.2	86.71	78.95
15	66.4	96.09	81.24
30	83.49	84.49	83.99
45	95.92	89.83	92.87
Mean	79.25 b	89.28 a	
Intake of leucaena (DM)			
0	0	0	0
15	17.02	16.24	16.63 a
30	30.16	25.63	27.89 b
45	48.74	40.56	44.65 c
Mean	31.97 a	27.47 b	

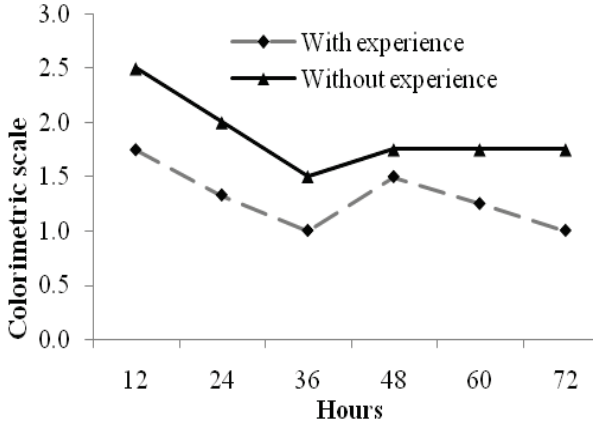

Figure 1. Colorimetric scale of urine of cattle fed leucaena with and without experience in consumption of *L. leucocephala* with different levels of inclusion.

which differs from the data of Ghosh *et al*. (2009), who reported that intake of *L. leucocephala* decreased as a higher amount was offered, when animals were unfamiliar with the legume. Colorimetric analysis of urine excreted showed no significant differences (P>0.05) between groups (Figure 1), which suggest that although cattle may be experienced in eating *L. leucocephala*, subclinical toxicity can still occur as it has been shown by other authors (Palmer *et al*. 2010; Dalzell *et al*. 2012; Phaikaew *et al*. 2012).

Conclusions

Intake of a ration containing *L. leucocephala* by cattle might not be affected by previous experience with the legume. The presence of mimosine derivatives in the urine of both experienced and non-accustomed cattle suggests that subclinical toxicity could occur in cattle with experience in consumption of *L. leucocephala*. Further studies are needed to clarify the liveweight performance on the different rations.

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